Local drug administration : an experimental study on its possibilities and limitations

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LOCAL DRUG ADMINISTRATION
– AN EXPERIMENTAL STUDY ON ITS POSSIBILITIES
AND LIMITATIONS –

Local drug administration — an experimental study on its possibilities and limitations —

Proefschrift

ter verkrijging van de graad van doctor in de geneeskunde
aan de Rijksuniversiteit Limburg te Maastricht,
op gezag van de Rector Magnificus, prof. dr. F.I.M. Bonke,
volgens het besluit van het College van Dekanen,
in het openbaar te verdedigen op vrijdag 20 maart 1987
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*Voor Trudie en Rob
Voor mijn ouders*

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1. INTRODUCTION

1.1 GENERAL INTRODUCTION

For a drug to produce a specific pharmacological response it must gain access to its primary site of action. The therapeutic efficacy of drugs is often limited by their inability to gain access to the site of action at an appropriate dosage, and/or by their ability to reach non-desired sites of action and producing unwanted side-effects. The use of effective drug delivery systems might enhance the efficacy of drugs by establishing a more selective presence of the drug at its primary site of action. To do so, particular attention must be given to (1) the desired site of action, (2) the site of action of the drug that has to be avoided because of toxicity, (3) the route of administration of the drug.

The last decades have seen the emergence of a great number of approaches to the problem of controlled drug delivery. Recent technological developments, such as polymeric sustained release systems and osmotic pumps (Blackshear et al, 1972; Urquhart et al, 1984; Struyker Boudier, 1986) have made rate controlled drug delivery possible (Poznansky and Juliano, 1984). These drug delivery systems are used to control drug delivery in time while less attention has been paid to their uses in the spatial control of drug delivery, in which applications they are conveniently termed drug targeting systems.

1.2 DRUG TARGETING SYSTEMS

A number of drug targeting systems have been described in the literature with varying degrees of scientific testing to validate the promise they seem to offer. A brief overview follows:

1. Liposomal drug delivery systems (reviewed by Juliano and Layton, 1980; Gregoriadis et al, 1982a). A great variety of drugs can easily be incorporated in liposomes or phospholipid vesicles. Liposomes interact with target cells via endocytosis and/or fusion

(Pagano and Weinstein, 1978). Primary sites of uptake are the reticulo-endothelial cells in the liver and the spleen. A few years ago, liposomes were seen as a major new approach to cancer chemotherapy, but this is now seen as an unlikely eventuality, although it is shown that liposomes can to some extent be taken up by solid tumors (Kaye, 1981). A liposome, injected into the circulation, must cross several barriers, be effectively taken up by tissues or pathological tumors and meanwhile evade the capture by cells of the reticulo-endothelial system. Use of liposomes as sustained release systems is another promising approach, although alternative technologies are available.

2. Cellular drug carriers. For this purpose, erythrocytes (Humpreys and Ihler, 1982), fibroblasts and leucocytes (Dean et al, 1981; Hobbs, 1983), or encapsulated cells (Chang, 1972) loaded with enzymes or drugs can act as a carrier to target the encapsulated substance. The use of intact cells for drug delivery represents a highly experimental approach.
3. Microspheres and macromolecules as drug carriers. Here, denaturated albumin microspheres, capable of entrapping certain drugs and delivering them in vivo are used (Kato et al, 1980). Additionally they may contain ultrafine Fe_3O_4 particles, making them susceptible to the effect of an externally applied magnetic field (Widder and Senyei, 1983). This system allows a high degree of target site specificity. Soluble macromolecules, like albumin, fibrinogen and polyethylene glycol (Poznansky et al, 1982; Trouet et al, 1982) may gain access to regions not available to larger insoluble particles and have the capacity to avoid rapid clearance by the reticulo-endothelial system, which makes them advantageous over liposomes. Although they offer important advantages, the question of drug targeting beyond the vascular space remains open.
4. Antibodies as drug carriers. Antibodies, linked to drugs, can be used as targeting agents to introduce drugs to specific tissues. This technique has been applied especially in the area of tumor therapy (Levy and Miller, 1983; Towbridge and Domingo, 1983). Toxicity and specificity are limitations of this means of drug delivery.

5. Pro-drug delivery systems. A pro-drug is an agent which must undergo biotransformation prior to exerting its pharmacological effect (Stella et al, 1980; Notari, 1981; Bodor, 1982). Pro-drugs are used in experimental research (Smits and Struyker Boudier, 1985) on the treatment of hypertension and for a site-specific release of drugs like dopamine to the brain (Bodor and Simpkins, 1983). Taking advantage of the unique characteristics of cells and/or tissues, it has to be possible to design pro-drugs as cell or organ specific entities.
6. (Implantable) infusion pumps used for instance for intra-arterial or intrathecal drug administration (Sandouk et al, 1986). The most elementary drug targeting system is the infusion pump. A number of commercial as well as experimental versions of the intra-arterial infusion pump are now available. A major advantage of these systems is that they allow not only spatial, but also time-control of drug delivery.

All above described targeting systems have not proceeded to the stage of extensive published clinical experience. The infusion pumps have been used in phase III clinical trials; extensive clinical testing is proceeding.

1.3 RATIONALE FOR LOCAL DRUG DELIVERY

In clinical practice as well as in experimental research there is a great need to deliver a drug directly to a specific target site. In fact localized drug delivery has acquired a place in daily medical routine in for example the topical treatment of certain dermatological diseases, the use of nasal decongestants, the use of aerosols to treat bronchial asthma, and the local application of ear- and eyedrops. An example of the latter is the use of the beta-adrenergic blocking drug timolol in the treatment of glaucoma. In literature, effects of systemic spill-over of timolol eye drops, i.e. bradycardia and hypotension, are reported (Kosman, 1979; Chiou, 1981). In all these applica-

tions, the respective target tissue can be reached easily. When the target tissue is not that easy to reach, other routes for the local administration of drugs have to be used. In the following paragraph examples will be given of one approach of local drug delivery i.e. the delivery of drugs directly into the blood vessel supplying the target organ.

The use of local drug delivery is mainly based on the hope that the drug, when delivered locally, will be confined to the target site, thus avoiding any systemic effects, and/or that higher drug concentrations will be reached at the target site. However the lack of a theoretical basis for these interventions is striking. In the present chapter applications of intra-arterial drug administration also called local drug administration, in several areas of clinical practice and experimental research will be described. Pharmacokinetic guidelines will be provided which clarify the potentials and limitations of local drug administration. In the last part of this chapter, the practical applications will be analyzed on the basis of these pharmacokinetic principles.

1.4 CLINICAL APPLICATIONS OF LOCAL DRUG ADMINISTRATION

1.4.1 Regional cancer chemotherapy

The liver

Local drug administration has found wide application in cancer chemotherapy. It is used, for example, to treat hepatic metastases of colon carcinoma. The liver is the most frequent localization of metastases of colon carcinoma. The presence of hepatic metastases has a major influence on the prognosis (Jaffe et al, 1968; Wagner et al, 1984). The survival of untreated patients with hepatic metastases of colon carcinoma is short and varies from 1-22 months (Pettavel and Morgenthaler, 1978; Bengtsson et al, 1981). Control of hepatic metastases could therefore provide a substantial improvement of the therapy. However, the results of traditional therapy of hepatic metastases (systemic chemotherapy, surgery, radiation) are unsatisfac-

tory (Kemeny, 1983a; Stagg et al, 1984).

Malignant lesions in the liver derive their blood supply predominantly from the hepatic artery (Breedis and Young, 1954; Ackermann et al, 1969), which favors the hepatic artery as the route of administration for cytotoxic drugs. Since the infusion of cytotoxic drugs directly into the hepatic artery could expose the tumor to higher drug concentrations and/or reduce the systemic side-effects (Ensminger et al, 1978), the latter often limiting chronic systemic chemotherapy, local i.e. intra-arterial drug delivery was seen to be of likely benefit to treat hepatic metastases.

5-Fluorouracil (5-FU) and 5-fluoro-2'-deoxyuridine (5-FUdr) are the drugs mostly used for continuous intrahepatic infusion. In table 1.1, examples of clinical studies applying the intra-arterial infusion of 5-FU and 5-FUdr are given.

Table 1.1: Studies applying the intra-arterial infusion of 5-FU and 5-FUdr.

Author	Drug dosage	Number of patients	Response (%)
5-Fluorouracil			
Grage et al, 1979 ^o	20 mg/kg/d for 14 d; then 10 mg/kg/d for 7 d	30/31	34
Berger et al, 1981*	20 mg/kg/d for 14 d; then 15 mg/kg/d for 7 d	30	57
5-Fluoro-2-deoxyuridine			
Sullivan et al, 1964*	0.1-0.5 mg/kg/hr for for 21-41 d	16	75
Reed et al, 1981*	0.3-0.5 mg/kg/d with a 5-7 d interval period	77	83

Ensminger et al, 1983*	0.3 mg/kg/d 14 d, alternated with saline 14 d	60	83
Kemeny et al, 1983b*	0.3 mg/kg/d 14 d, alternated with saline 14 d	54	50
Balch et al, 1983*	0.3 mg/kg/d 14 d, alternated with saline 14 d	81	88
Weiss et al, 1983*	0.3 mg/kg/d 14 d, alternated with saline 14 d	21	29
Niederhuber et al,* 1984	0.3 mg/kg/d 14 d, alternated with saline 14 d	93	79
Schwartz et al, 1985*	0.3 mg/kg/d 14 d alternated with saline 14 d	20	15
Shepard et al, 1985*	0.3 mg/kg 14 d alternated with saline + mitomycin i.v. 15 mg/m ² in 1 d	53	32
Cohen et al, 1986*	0.3 mg/kg/d 14 d alternated with saline, mitomycin 15 mg/m ² and BCNU 150 mg/m ² in 1 hr every 8 weeks	36	70
Kemeny et al, ^o 1985a, 1986	0.3 mg/kg/d for 14 d alternated with saline 14 d	12/10	A trend toward better survival
Hodgson et al, 1986*	0.3 mg/kg/d 14 d alternated with saline 14 d (A) + resection (B) + arterial embolization (C) + portal vein branch ligation (D)	A: 6 B: 11 C: 4 D: 6	The more radical the intervention, the better the results

^ocomparative with i.v. treated patients

*non comparative

Most studies use historical control as the reference. Response rate is defined differently in almost every study. It may vary from a subjective clinical benefit, improvements of liver-function tests (Sullivan et al, 1964) to response on liver scans (Niederhuber et al, 1984). Although a comparison between the studies is difficult because of non-

uniformity in the definitions of response rates and in criteria for patient selection, some conclusions may be drawn: (1) A reduction in systemic concentration can be achieved by the intrahepatic administration of 5-FU as well as 5-FUdr (Ensminger et al, 1978); (2) Systemic side-effects are less than during the classic intravenous drug administration (Ensminger and Gyves, 1983); (3) Although the response rate with local drug administration is enhanced as compared to systemic therapy (34-88% vs. 15-20%, respectively), a prolonged survival of the patients has not yet been reported (Schwartz et al, 1985; Dy et al, 1986; Huberman, 1983); (4) Regional side-effects especially fibrosis of the extrahepatic biliary system (17%; Hohn et al, 1985; Haq et al, 1986), chemical hepatitis (20-50%; Cohen et al, 1986) and cholecystitis (13%; Kemeny et al, 1985b) are relatively frequent; (5) In general, the therapeutic effects of regional chemotherapy for the treatment of hepatic metastases of colorectal carcinoma are not increased as compared to systemic chemotherapy (Foster, 1984; Schwartz et al, 1985; Dy et al, 1986; Hodgson et al, 1986); (6) To date, there is no experimental or clinical pharmacokinetic study demonstrating an increase of drug concentration at the tumor site during local as compared to intravenous drug administration of the same dose.

Recent studies describe the effects of continuous hepatic arterial infusion therapy with 5-FU or 5-FUdr in combination with other cytotoxic drugs (Shepard et al, 1985; Cohen et al, 1986), with surgical resection of hepatic metastases (Kemeny et al, 1985, 1986; Hodgson et al, 1986) and/or with hepatic arterial and portal vein embolization (Hodgson et al, 1986) to improve the survival rate of patients with hepatic metastases from colorectal origin. Embolization is performed to disrupt tumor cells from any blood supply.

The brain

The brain is another region to which cytotoxic drugs have been administered directly. Patients with brain tumors have a poor prognosis (Wilson, 1979). The median survival of patients with brain metastases from extra-cranial primary tumors is about 2-3 months; patients with malignant astrocytoma survive about 1 year following radiation therapy and systemic chemotherapy. The regional, i.e. the

Table 1.2: Examples of clinical studies on the direct delivery of cytotoxic drugs to the brain.

Author	Tumor	Infused artery	Drug dosage	Number of patients	Side-effects (regional and/or systemic)	Response (%)
Klopp et al, 1950	Epidermoid carcinoma of the submaxillary glands	External carotid	HN ₂ 1.3-3.9 mg every 8-12 h for 7-10 d	7	bone marrow suppression nausea, vomiting	Pain relief in all cases
Madajewicz et al, 1981	Brain metastases of lung cancer	Internal carotid or vertebral artery	BCNU 100 mg/m ² every 4 weeks	31	Eye (100%) Seizures (15%)	40
Cascino et al, 1983	Brain metastases of lung, breast, genito-urinary, colon cancer and melanoma	Internal carotid or vertebral	BCNU 100 mg/m ² every 5-6 weeks	31	No	20
Greenberg et al, 1984	Astrocytoma	Internal carotid	BCNU 200 mg/m ² every 4 weeks	31	Eye 50% (vasculitis, blindness, decreased vision) white matter necrosis (20%)	25
Feun et al, 1984	Astrocytoma Gr III, IV	Internal carotid	Cis-platinum 60-120 mg/m ² every 4 weeks	35	Nausea, vomiting (15%) CNS (23%)	50
Greenberg et al, 1986	Astrocytoma	Internal carotid or vertebral	Diaziquone ² 10-30 ng/m ² every 4 weeks	21	Bone marrow suppression (100%)	10

intracarotid or intravertebral arterial administration of cytotoxic drugs, such as BCNU (1,3-bis-(chloro ethyl)-1-nitrosurea), has been used for the treatment of malignant brain tumors. This application of target organ directed drug delivery is also based on the assumption that the drug availability to the tumor region is enhanced while the systemic drug availability is decreased as compared to systemic chemotherapy (Kleinschmidt-DeMasters, 1986). Table 1.2 describes examples of clinical studies in which the delivery of cytotoxic drugs to the brain has been reported. As may be concluded from this table, there is a wide variety of drugs in use and regional (i.e. central nervous system) toxicity is an important limiting factor.

Response rates of 10-50% during regional cytotoxic drug therapy indicate that this method might be useful to treat brain tumors. Again however, there is yet no clinical study, indicating a significant increase in survival after the regional drug treatment of brain tumors, as compared to systemic therapy.

Other regions

Other regions into which cytotoxic drugs are administered directly are the bronchus, the maxillofacial region, the pelvis, the bladder and the extremities (table 1.3). Regional chemotherapy by perfusion (Muchmore et al, 1985; Krementz, 1986) is often applied to treat tumors like melanoma and soft-tissue sarcoma of the limb. As may be obvious the systemic toxicity is greatly reduced.

As may be clear thus far, almost every part of the body has been catheterized for regional chemotherapy of a tumor in that specific area. However, there seems to be no consistency in the use of the drugs at the different regions. The actual therapeutic benefits of equi-dose regional over systemic chemotherapy have not been clarified yet.

1.4.2 Thrombolytic therapy

The intra-arterial administration of thrombolytic drugs, like streptokinase, into occluded vessels is a second important area of localized drug administration. Streptokinase was discovered in 1933 when Tillet and Gauner observed that a filtrate of group C beta hemo-

Table 1.3: Examples of clinical studies on the direct delivery of cytotoxic drugs to several regions.

Author	Tumor	Infused artery	Drug dosage	Number of patients	Side-effects (regional and/or systemic)	Response (%)
Uyama et al, 1983	Bladder	Common iliac	Adriamycin 20-30 mg/d at 3-4 week intervals	20	No compression (10): 100% stomatitis 100% alopecia	90
Schulz et al, 1984	Bronchial carcinoma (Squamous cell)	Bronchial	Mitomycin 20 mg Adriamycin 30 mg in 15 min	21	No	31
Szabo, 1985	Squamous cell Carcinoma of temporal, the maxillo-facial region	Superficial	Bleomycin 15 mg repeated 3-5 times	288	Thrombosis of common carotid artery	Not described
Oberfield, 1985	Pelvis	Hypogastric, Internal iliac, Common iliac	Methotrexate 50 mg/d 5-FU 5.0-7.5 mg/kg/d 5-FU dr 0.1-0.3 mg/kg/d	55	Bleeding at the catheter side (2/55)	32
Muchmore et al, 1985	Malignant melanoma of the limb	Iliac, Axillar (regional perfusion)	Melphalan 0.45-1.5 mg/kg for 45-120 min	1000	Minor	>50 stage IIIB >70 stage I

lytic streptococci lysed a human plasma clot (Tillet and Gauner, 1933). Streptokinase produces fibrinolysis by activating the body's natural fibrinolytic system by converting the inactive pro-enzyme plasminogen to the active enzyme plasmin, which lyses fibrin clots (Sharma et al, 1982). In table 1.4, examples of studies applying the intra-arterial administration of streptokinase are described.

Table 1.4: Studies applying the intra-arterial infusion of streptokinase

Author	Infusion rate	Num- ber of patients	Reperfusion*	Systemic side- effects
Intrarenal administration of streptokinase				
Fisher et al, 1981	25.000 U/hr for 25 h	1	100%	No
Cronan and Dorfman, 1983	5000 U/hr for 24-36 hr	3	100%	No
Brandjes et al, 1985	5000 U/hr for 19 hr	1	100%	No
Intracoronary administration of streptokinase				
Anderson et al, 1983	5000 U/min until reperfusion 3000 U/min for 30-60 min	24	78%	Minor
Khaja et al, 1983	15.000 U/min for 2 min 5000 u/min to a total of 250.000	20	60%	No
Kennedy et al, 1983	4000 U/min for 60 min	134	68%	5.2%
Rogers et al, 1983	240.000 U	25	80%	4%

Simoons et al, 1985	250.000 U (preceded by 500.00 U i.v. in 117 patients)	264/269	85%	16% (Verheugt et al, 1985)

Intravenous administration of streptokinase (myocardial infarction)

ECSCG 1979	250.000 U in 20 min 100.000 U/hr for 12 hr	155	not determined	25%
Schröder, 1983 (review)	250.000 U loading dose 100.000/h for 12-24 hr	2467	78%	6%
GISSI 1986	500.000 U/hr (in total 1.500.000 U), vs no therapy	5905/ 5901	18% decrease in 3 weeks hospital mortality (time- dependent)	

* Reperfusion is defined as the re-establishment of flow in the occluded vessel

Streptokinase has been administered also to occluded vessels other than renal and coronary arteries. Examples are the administration of streptokinase to the superior mesenteric artery, the abdominal aorta, the superficial femoral artery (Hess et al, 1982), a distal calf artery and a palmar artery (Marder and Francis, 1984). In literature, however, only case reports are available.

In general, it can be said that the dose of intracoronary administered streptokinase is reduced more than 5-fold as compared to systemic administration (+ 250.000 U vs. + 1.500.000 U at local and systemic therapy). Reperfusion is reached in about 80% at local vs. 70% at intravenous therapy and seems strongly dependent on the time at which thrombolytic therapy is started. Systemic bleeding effects during local therapy are observed in approximately 5% of the patients and in 25% during systemic therapy (a minority of which is fatal). Thrombolytic therapy at myocardial infarction seems to be especially

advantageous when admitted within the first hours of symptoms to patients with large infarction. (Vermeer et al, 1986; Serruys et al, 1986; Simoons et al, 1986)

A major drawback of intracoronary thrombolytic therapy, however, is the necessity to introduce the catheter. Since the facilities (equipment and trained people) are not uniformly available, only a minority of all patients receive the local therapy. Another disadvantage is that the introduction of an intra-arterial catheter prolongs the time before the treatment can be started (DeWood, 1985).

1.5 EXPERIMENTAL APPLICATIONS OF LOCAL DRUG ADMINISTRATION

In comparison with the above described clinical applications, local drug administration is used in a wide variety in experimental research. The kidney may serve as an example: to study the renal effects of drugs or to elucidate the role of the kidney in the pathogenesis of hypertension. Examples of the former are the intrarenal vs. the systemic administration of atriopeptin II to investigate the renal vasodilatory effects of this substance (Smits et al, 1986) and the beneficial effects of intrarenal over systemic administration of prednisolone to prolong the survival time of rat renal transplants (Ruers et al, 1986). Circumventing the brisk first-pass effect of the lung, bradykinin was given intrarenally instead of intravenously, to study the effects on rat renal nerves (Smits and Brody, 1984). Local (intrarenal) administration of noradrenalin has been used to investigate the role of renal neurogenic activity in the development and maintenance of hypertension (Kleinjans et al, 1983).

The brain is another target for local drug administration in experimental research. Injections of drugs into the vertebral artery to achieve higher drug concentrations in the brain as compared to systemic delivery were performed by Bock and Van Zwieten (1971) in experiments on the central mechanism of drugs like clonidine and alpha-methyldopa (Henning and Van Zwieten, 1968; cf. review Van Zwieten, 1975). However, there are great species differences in the brain regions that are supplied by the vertebral arteries (Reneman et al,

1974; Wellens et al 1975), indicating the need for caution in distinguishing local vs. systemic effect when drugs are infused intravertebrally.

In experimental cancer research the technique of local drug administration is also applied (Verbeek, 1983). Hepatic tumors in rats were treated either by intrahepatic arterial, intravenous or oral administration of the cytotoxic agent 5-FU by Cotino and Zwaveling (1976): intra-arterially administered 5-FU was found to be more effective than intravenously or orally administered 5-FU. Intrarenally administered vincristine resulted in 2.8 times higher drug concentrations in sarcoma transplants situated in the kidney as compared to systemic delivery of the same drug (Tvete et al, 1984). This beneficial effect could even be enhanced by clamping the renal artery. A tumor transplanted into the hind limb of a rabbit was successfully treated by the intra-arterial administration of adriamycin. Intravenous infusion was not effective (Swistel et al, 1984).

The above-described examples of applications of local drug administration in clinical practice and experimental research may present a rather confusing picture: on the one hand, it seems as if any drug is thought to be suitable for intra-arterial drug administration. On the other hand most properly controlled clinical studies suggest that systemic side-effects of intra-arterial drug administration are not avoided and that the therapeutic effect is little increased, if at all. The use of target organ directed drug administration without any considerations of its theoretical base might explain the discrepancy between the enthusiastic use and the poor results. Therefore, this theoretical basis will be described below. Since the administration of a drug directly into the supplying artery of the target organ is the most often used variant, the theoretical considerations will be restricted to the administration of drugs into the supplying artery of the target organ. Other forms of target organ directed drug delivery such as the use of pro-drugs (Smits and Struyker Boudier, 1985) or the intracerebroventricular administration (Levin and Landahl, 1985) will not be discussed. Also drug targeting

by liposomes (Gregoriadis and Allison, 1982, Jori et al, 1986; Ranney, 1986), lactosaminated albumin (Fiume et al, 1986), or by monoclonal antibodies (Zalcberg, 1985) will not be further discussed here.

1.6 THEORETICAL CONSIDERATIONS OF LOCAL DRUG ADMINISTRATION

1.6.1 The physiological pharmacokinetic approach

In general, three mathematical approaches have been taken to describe and interpret pharmacokinetic data. The first and most widely used approach is to describe the fate of drugs in the body by the so called open compartment models. In this approach the mathematical analysis is abstracted from a physiological description of the body as a set of tissues and organs. Instead, the body is described as a set of compartments, each based upon a number of unique kinetic parameters for each given drug. An example of this approach is the so-called two-compartment model with a central and peripheral distribution compartment. The mathematical descriptions of the kinetic pattern of the drug in the compartments are based on the concentration time relation of the drug in the plasma. Analyzing the time course of the drug concentrations in plasma, values of an imaginary distribution volume, half-life time of elimination and total body clearance can be calculated (Gibaldi et al, 1969; Greenblatt and Koch-Weser, 1975).

An implication of dynamical systems theory offers a physiological approach to the subject (Cutler, 1979; Van Rossum et al, 1986). In this approach the body is considered as a dynamical system, not only reacting to the input from outside but also self-organizing and controlling to some extent its input. The pharmacokinetic behavior of tissues and organs is characterized by parameters as transit and residence times, blood flow, clearance and distribution volume. The body transport function, fixed for a given individual and drug, is found from measurement of the output concentrations, applying a known input function. Statistical moments are used to describe the profile of these functions (Van Rossum, 1985).

A third approach to describe and interpret pharmacokinetic data is physiological modelling, emphasizing on physiological and anatomo-

mical parameters (Rowland, 1984a, 1985). Measurements of the concentration of the drug in tissues are used to analyze and describe pharmacokinetics mathematically. It has its origins in 1937, when Teorell formulated a general model with circulating blood and tissue compartments, some of which are sites of administration and others are sites of elimination, to describe drug kinetics (Rowland, 1985). Ever since, the physiological approach has been developed by many authors (Bischoff and Dedrick, 1968; Weiss and Förster, 1979; Gerlowski and Jain, 1983; Tsuji et al, 1985; King et al, 1986).

Fig. 1.1 shows a physiologically based perfusion model. In this model, the pharmacokinetic processes in each organ, tissue or group of kinetically related tissues are described in terms of parameters relating to physiology, anatomy and biochemistry (Rowland, 1985). Examples

PHYSIOLOGICAL PHARMACOKINETIC MODEL

Fig. 1.1: Scheme of a flow-dependent physiological pharmacokinetic model. Q is the flow to the respective organs.

of these parameters are tissue size, vascular perfusion, permeability of the tissue membrane to a drug, binding and/or partition of the drug between blood and tissue compartments, and elimination processes.

With this physiological approach it is possible to describe and even predict the pharmacokinetic behavior of a given drug in a given tissue, provided that physiological parameters as tissue blood flow and volume, drug distribution coefficient between organ tissue and plasma, protein binding, and tissue clearance are known.

The rate of presentation of a drug to a tissue is the product of blood flow, Q , and the arterial blood concentration of the drug, C_A (fig. 1.2)

$$\text{Rate of presentation} = Q \cdot C_A \quad (1)$$

The rate at which the drug leaves on the venous side is $Q \cdot C_V$, where C_V is the blood concentration in the returning venous blood. The difference between these rates is the rate of drug extraction by the organ.

$$\text{Rate of extraction} = Q \cdot (C_A - C_V) \quad (2)$$

By dividing (2) into (1), a useful parameter, the extraction ratio, E , is found

$$E = \frac{\text{Rate of extraction}}{\text{Rate of presentation}} = \frac{C_A - C_V}{C_A} = 1 - \frac{C_V}{C_A} \quad (3)$$

The value of the extraction ratio is anywhere between zero and one. Relating the rate of drug extraction to the incoming concentration another useful parameter clearance, Cl , is found:

MASS BALANCE

Fig. 1.2: The extraction of a drug by an eliminating organ in relation to the mass balance: the extraction accounted for its rates of entry and leaving: A. normalized to the rate of entry, which provides the fraction extracted: the extraction ratio. B. normalized to the entering concentration allowing to account for the drug in terms of clearance and flow.

$$Cl = \frac{\text{Rate of extraction}}{\text{Arterial blood concentration}} = Q \cdot \frac{(C_A - C_V)}{C_A} \quad (4)$$

By substituting (3) into (4)

$$Cl = Q.E \quad (5)$$

The dependency of E on the intrinsic capability of an organ to eliminate (i.e. its intrinsic clearance) a drug was analyzed by Wilkinson and Shand (1975):

$$E = \frac{Cl_{intr}}{Q+Cl_{intr}} = \frac{1}{\frac{Q}{Cl_{intr}} + 1} \quad (6)$$

hence

$$Cl = Q \cdot \frac{Cl_{intr}}{Q+Cl_{intr}} \quad (7)$$

This approach shows that for a drug with a high extraction ratio (>0.7) changes in blood flow produce corresponding changes in clearance but not in extraction ratio (table 1.5). In other words, the clearance of a high extraction drug is flow dependent. On the other hand for a drug with a low extraction ratio (<0.3) changes in blood flow produce inversely related changes in extraction ratio but not in clearance: the clearance of low extraction drugs is flow independent.

Table 1.5: Changes in clearance and extraction ratio with changes in blood flow.

Drug with a	Blood flow	Extraction ratio	Clearance
high extraction ratio	↑ ↓	—	↑ ↓
low extraction ratio	↑ ↓	↓ ↑	— —

↑: increase, ↓: decrease, —: little or no change
(according to Rowland and Tozer, 1980)

Taking the drug's plasma protein binding in the blood into account (equation 8) it is indicated that

$$E = \frac{f_u \cdot Cl'_{intr}}{Q + f_u \cdot Cl'_{intr}} \quad (8)$$

where Cl' is the intrinsic clearance of unbound drug and f_u the fraction of drug, unbound in the plasma. From equation 8 it can be derived that the higher a drug's intrinsic free clearance the less sensitive is its extraction and clearance to changes in binding (Jansen, 1981). On the other hand, for drugs with a low intrinsic free clearance, and extraction ratio, an almost linear relationship exists between clearance and binding:

$$Cl \approx f_u \cdot Cl'_{intr} \quad (9)$$

The physiological pharmacokinetic approach also forms the basis of theoretical considerations of the advantages of local over systemic drug delivery. In the following section mathematical expressions will be derived for the advantages of local over systemic drug delivery using the above described physiological model.

In general, the rate equation for a given drug in a given tissue is given by:

$$\begin{aligned} &\text{Rate of change of mass of the drug in the tissue} = \\ &\text{Rate of entry} - \text{Rate of exit} - \text{Rate of elimination} + \\ &\text{Rate of synthesis} \end{aligned} \quad (10)$$

A simplified physiological pharmacokinetic model in which a drug can be given directly to a tissue or systemically, is depicted in fig. 1.3. In this figure, a target organ with a given volume V_T , a blood flow Q_T and an extraction ratio E_T is described. All remaining organs are lumped into one compartment with a flow (Q_S) and a clearance for the drug (Cl_S). Cl_S is defined by the total body clearance of the drug minus the clearance of the drug by the target organ.

Fig. 1.3: Scheme of a two-compartment physiological pharmacokinetic model, suited for local drug administration. C_s is the systemic plasma concentration, Q_s the systemic plasma flow. C_t and Q_t the concentration and the plasma flow, respectively, in the artery supplying the target organ. V_t is the volume of the target organ. E_t the extraction ratio of a drug in the target organ. Other organs are lumped together (rest). Cl_s is the systemic (i.e. non-target organ) clearance.

More or less non-physiological assumptions of the model are that the drug in an organ is well-mixed and that the drug concentration in an organ is proportional to the drug concentration in the venous blood (Wagner, 1985; Roberts and Rowland, 1985; Terasaki et al, 1985).

The mass balance of a drug in organ T is:

$$V_T \frac{dC_T}{dt} = Q_T \cdot \left(C_p - \frac{C_T}{R_T} \right) - Cl_{T'} \cdot \frac{C_T}{R_T} \quad (11)$$

where V_T is the volume of the organ, C_T and C_p the organ and afferent blood (plasma) concentration, respectively; Q_T the blood flow, R_T the distribution coefficient of the drug between organ tissue and plasma at equilibrium; $Cl_{T'}$ is the clearance constant in the organ.

For the drug in the blood (plasma) compartment the mass balance is

$$V_p \cdot \frac{dC_p}{dt} = Q_T \cdot \frac{C_T}{R_T} - Q \cdot C_p - Cl_p \cdot C_p \quad (12)$$

where Q represents the total blood flow i.e. cardiac output.

For steady state, expressions of C_T and C_p for intra-arterial (i.a.) and intravenous (i.v.) infusion can be derived from equations (11) and (12).

I.v. infusion:

C_T

$$Q_T \cdot C_p - \frac{C_T}{R_T} - Cl_T \cdot \frac{C_T}{R_T} = 0 \quad (13)$$

$$C_T = \frac{Q_T \cdot R_T}{Q_T + Cl_T} \cdot C_p = C_p \cdot R_T \cdot \frac{1}{1 + E_T} \quad (14)$$

C_p

$$Q_T \cdot \frac{C_T}{R_T} - Q_T \cdot C_p - Cl_S \cdot C_p + k_o = 0 \quad (15)$$

where Cl_S represents the total body clearance of the drug outside the target organ and k_o the constant rate infusion input.

$$C_p (Q_T + Cl_S) = Q_T \cdot \frac{C_T}{R_T} + k_o \quad (16)$$

Substitution of (14) into (16) leads to

$$C_p (Q_T + Cl_S) = Q_T \cdot \frac{Q_T \cdot R_T \cdot C_p}{(Q_T + R_T) \cdot R_T} + k_o \quad (17)$$

By rearrangement, this leads to

$$C_p = k_o / Cl_S + \frac{Q_T \cdot Cl_T}{Q_T + Cl_T} = k_o / Cl_S + \frac{Cl_T}{1 + E_T} \quad (18)$$

I.a. infusion C_T

$$Q_T \cdot \left(C_p - \frac{C_T}{R_T} \right) - Cl_T \cdot \frac{C_T}{R_T} + k_o = 0 \quad (19)$$

$$(k_o + Q_T \cdot C_p) \cdot R_T / Q_T + Cl_T = 0 \quad (20)$$

 C_p

$$Q_T \cdot \frac{C_T}{R_T} - Q_T \cdot C_p - Cl_S \cdot C_p = 0 \quad (21)$$

Substitution of equation (20) into (21) leads to

$$Q_T \cdot \frac{(k_o + Q_T \cdot C_p)}{Q_T + Cl_T} - Q_T \cdot C_p - Cl_S \cdot C_p = 0 \quad (22)$$

Rearrangement of equation (22) leads to

$$C_p = \frac{Q_T \cdot k_o}{Q_T + Cl_T} \Bigg/ Cl_S + \frac{Q_T \cdot Cl_T}{Q_T + Cl_T} \quad (23)$$

$$C_T = \frac{k_o}{Q_T} + C_p \quad (24)$$

1.6.2 Regional and systemic advantages of intra-arterial over systemic drug administration

Assuming that local and systemic drug effects are concentration related, the ratio of the concentrations (local or systemic) at local and systemic drug administration are used as parameters of advantage (regional or systemic respectively) of local over systemic drug administration. Hence at steady state the regional advantage, R_D , defined

as the gain in drug concentration in the artery supplying the target organ (C_{TA}) following local over systemic drug administration, is given by

$$R_D = C_{TAia}/C_{TAiv} \quad (25)$$

Substitution of equation (23) into (24) and division by (18) leads to

$$R_D = 1 + Cl_S/Q_T \quad (26)$$

The reduction in the systemic concentration following local over systemic drug administration, the so-called systemic advantage, is given by:

$$C_{pia}/C_{piv} \quad (27)$$

C_{pia} , the systemic plasma concentration at intra-arterial drug administration, equals

$$(1 - E_T) \cdot k_o / Cl_S \quad (28)$$

where E_T represents the extraction ratio of the drug by the target organ. C_{piv} is defined by:

$$k_o / Cl_S \quad (29)$$

Division of (28) by (29) leads to

$$C_{pia}/C_{piv} = 1 - E_T \quad (30)$$

The above-described relationships are valid at steady-state conditions following constant rate infusion, but represent also the total amounts of drug delivered to the target organ following bolus injections (Eckman et al, 1974; Chen and Gross, 1979; Weiss, 1985).

Any advantage of local over systemic drug administration can only be achieved under special conditions: 1) the infused drug is extracted efficiently by the target organ or 2) the drug has a high systemic (i.e. non-target organ) clearance (Cl_s) in relation to the blood flow of the target organ (Q_T). The local infusion of a drug with a high extraction ratio leads to a reduced systemic concentration as compared to systemic administration of the same drug at the same constant rate of infusion. Hence, systemic side-effects are reduced, assuming these to be dose dependent (Collins, 1984). On the other hand local infusion of a drug with a low extraction ratio does not reduce the systemic concentration significantly as compared to systemic administration. In relation to the second option it can be seen that equation (25) and (26) approach one, indicating no advantage, when the drug clearance outside the target organ is small with respect to the blood flow through the target organ. A regional advantage achieved by local drug administration can be applied to lower the dose and thus the systemic side-effects, during local as compared to systemic drug administration, assuming a defined therapeutic steady state drug concentration in the arterial bed supplying the target organ.

1.7 THEORY AND PRACTICE : ANALYSIS OF THE APPLICATIONS OF LOCAL DRUG ADMINISTRATION

From the above, it is clear that advantages of intra-arterial over systemic drug administration are determined by three parameters: (1) the extraction ratio of the drug by the target organ; (2) the blood flow of the target organ; (3) related to two, the systemic clearance of the drug.

To reduce the systemic drug availability, the drug has to be cleared efficiently before it reaches the systemic circulation. Is such a drug not found or not effective then a drug that is not cleared by the target organ, but that has a high non-target organ clearance,

has to be infused into a target organ with a relatively low blood flow.

In the following paragraph, the applications of local drug administration described earlier in this chapter will be analyzed according to the above theoretical requisites for advantageous local drug administration.

1.7.1 The drug is efficiently extracted by the target organ

The cytotoxic agents 5-FU and 5-FUdr are used to treat hepatic metastases of colorectal carcinoma by infusing them into the hepatic artery. The hepatic extraction ratio of 5-FU is 0.4 and of 5-FUdr 0.9 (Ensminger and Gyves, 1984). Some authors have suggested that 5-FUdr is a pro-drug from which 5-FU is generated, which would make predictions of advantages based on the extraction ratio of 5-FUdr questionable (Armstrong and Diasio, 1980; Ishitsuka et al, 1980). When infused locally, both drugs should be expected to result in lowered systemic concentrations as compared to their systemic delivery. However, no one has yet done controlled clinical comparative pharmacokinetic studies on the actual concentrations of these drugs in the venous blood and in the tumor and normal liver tissue during local and systemic administration. Therefore the advantage of hepatic arterial over systemic chemotherapy with 5-FU and 5-FUdr remains unverified. In this respect a disturbing observation was made by Boublil et al (1985) measuring systemic plasma concentrations of 5-FU during a continuous 5-day regional chemotherapy of 500-1650 mg/m²/d in patients with hepatic metastases from colorectal origin. They showed increased plasma concentrations and a decline in the hepatic extraction ratio in the last 2 days of a 5-day infusion period. These data suggest saturation of hepatic elimination mechanisms and/or hepatic toxicity during long-term continuous infusion. This may lead to a reduction in the systemic advantage of intrahepatic arterial drug administration and to increased systemic toxic effects of the drug.

As described in paragraph 1.4.1, the long-term intra-arterial infusion of cytotoxic drugs for the treatment of cancer of the head is another important area of regional cancer therapy. BCNU (1.3-bis-(2-chloroethyl)-1-nitrosurea) is the most effective single agent in brain

chemotherapy (Walker et al, 1980). A reduction in the systemic concentration is not to be expected since the brain and/or the brain tumor have no extraction capacity for the drug (Russo et al, 1981). For achieving an advantage of local over systemic drug administration in such a non-clearance-organ, the ratio of the systemic clearance of the infused drug and the blood flow through the target organ has to be considered.

1.7.2 The blood flow to the target organ

Low flow organs: A low blood flow of a target organ facilitates a high regional advantage by local administration of the proper drug. One approach therefore has been to reduce flow by embolizing the local vascular bed before infusing the active agent. Another case to consider is local vs systemic thrombolysis. As may be obvious, only small amounts of blood, if any, pass through embolized vessels. The local administration of thrombolytic agents to embolized vessels therefore yields high regional drug concentrations as compared to systemic thrombolytic therapy. According to the theory, the locally infused dose is reduced (in fact about one fifth) as compared to systemic thrombolytic therapy, thereby reducing the systemic side-effects (<25%, Sharma et al, 1982). However, there are competitors in the field: the fibrin-selective agent, Tissue-Type Plasminogen Activator (t-PA) which has a clot specific thrombolytic action (Collen, 1986), can be given intravenously and may in future end the necessity of the application of intra-arterial drug administration in thrombolytic therapy.

High flow organs: The kidney and the liver are often used target organs. If the drug of choice is not extracted by either target organ, then a regional advantage of local drug administration can only be achieved if the blood flow is reduced. A low flow does not hold for the kidney nor for the liver, which receive about 10 and 25% of the cardiac output respectively. They are classical examples of high flow organs, and in theory unsuited for achieving a regional advantage by local drug administration.

However, under special circumstances a regional advantage in these both organs can still be achieved. An example is the intrarenal

infusion of a vasoconstrictor agent like noradrenalin (Kleijnans et al, 1983) which will enhance the regional drug concentration as compared to systemic administration since the blood flow to the kidney is reduced.

Another example of an approach to reduce the blood flow to the target organ can be found in regional chemotherapy especially in the regional therapy of hepatic metastases. Since the degree of regional advantage of local over systemic drug administration is inversely related to the flow through the target organ a decrease in the flow of the hepatic artery should enhance the drug concentration in the hepatic tumor: decreasing the blood flow by 90% should increase the drug concentration in the hepatic artery about 10-fold. Therefore, in clinical practice several methods have been applied to decrease hepatic arterial blood flow (Ensminger and Gyves, 1984) such as:

1. Ligation of the hepatic artery (Carlsson et al, 1981; Al-Jurf et al, 1984; Petrelli et al, 1984). Important drawbacks of this method are rapid collateralization as well as thrombosis in and around the catheter and the irreversibility of the manoeuvre (Civalleri et al, 1985).
2. The use of a balloon catheter (Watkins et al, 1960).
3. The simultaneous infusion of vasoconstrictor agents as epinephrine, vasopressin and angiotensin (Iwaki et al, 1978; Hafström et al, 1980).
4. The use of microparticles or microspheres. Biodegradable starch microspheres injected concurrently with the cytotoxic agent block the hepatic arterial flow for about 30 min, thus allowing more time for the tumor to be exposed to high drug concentrations (Erichsen et al, 1985). It is shown that a delayed systemic drug distribution (Lindell et al, 1978) and a high regional advantage (Dakhil et al, 1982; Gyves et al, 1983) can be achieved by injecting starch microspheres into the hepatic artery. Conclusive reports indicating an enhanced survival rate as compared to "normal" intra-arterial chemotherapy are lacking (Ensminger et al, 1985; Fujimoto et al, 1985).

Arterial chemo-embolization with ethylcellulose microcapsules containing the cytotoxic agent is another method for enhancing the drug

concentration to the tumor (Kato et al, 1978; Okamoto et al, 1986).

1.7.3 A high systemic clearance in relation to the blood flow of the target organ

BCNU is the drug of choice in brain chemotherapy. In humans, the plasma clearance of BCNU is about 1200 ml/min (Russo et al, 1981). A reduction in the systemic concentration is not to be expected since the brain and the brain tumor have no extraction capacity for the drug. The regional drug concentrations should be about 5-6 times those after intravenous drug administration (Greenberg et al, 1984), assuming a carotid blood flow of 250 ml/min. In experiments in monkeys, Levin et al (1978) showed that the brain tissue levels at intracarotid infusion were twice those at intravenous administration of ^{14}C -BCNU, while Oldendorf (1975) described a regional advantage of 4.6 in rats. Since encephalopathy is a major complication of intravenous therapy with high doses (1500-2000 mg; Burger et al, 1981), the dose during local therapy has to be reduced at least 5-6 times, to reach equivalent concentrations and toxicity in the target region during local therapy. Indeed, in a study on the regional toxicity of BCNU during its intracarotid infusion a dose of 400 mg was used, while the regional (i.e. central nervous system) toxicity was comparable to that seen during systemic therapy with high dosages. In summary, pharmacokinetically BCNU does seem to be a drug of choice for regional therapy (high systemic clearance and low blood flow to the target organ). However, because of its important regional toxicity, which cannot be reduced by applying the technique of local drug administration, local administration does not serve to reduce its limiting toxicity.

Diaziquone (aziridinyl benzoquinone or AZQ), a drug with a plasma clearance of about 460 ml/min, is active against human brain tumors when given intravenously. Its most important side-effect is myelosuppression. In theory, intracarotid delivery might triple the concentration to the target tissue or might enable to reduce the infused dose to about one third. Therefore, this drug seems to be an alternative for BCNU, because the reduced dose at local drug administration should result in decreased systemic side-effects. However, Egorin et al (1984) were unable to detect any significant regional advantage to intracarotid infusion of AZQ in tumor bearing puppies. As may be

obvious, a reduction of the carotid blood flow might enhance the concentration in the target tissue and the regional advantage.

The total body clearances of 5-FU and 5-FUdr are 2-5 and 5-15 l/min, respectively (Ensminger and Gyves, 1984). Since hepatic metastases of 3-5 cm receive 95% of their blood supply via the hepatic artery (Ackermann et al, 1969) the hepatic arterial blood flow and not the total hepatic blood flow is used for calculating the regional advantage. For 5-FU, this advantage would be about 6-13, for 5-FUdr 13-38, assuming a hepatic arterial flow of 0.4 l/min. The reduction in systemic concentration should in theory be about 50% for 5-FU and 80% for 5-FUdr, assuming steady-state conditions and linear kinetics, and could be even enhanced by reducing the dose. As before, the suggested generation of 5-FU from 5-FUdr is a complicating factor, which may decrease the advantage of local administration of 5-FUdr.

In conclusion, (1) examples can be found of the correct application of the theoretical guidelines; (2) as many examples can be found in which theory is neglected; (3) a kinetic evaluation of the actual advantages of local over systemic drug administration is rare.

1.8. THIS THESIS

The introduction of intra-arterial infusions of cytotoxic drugs in cancer chemotherapy by Klopp et al (1950) excited interest in this means of drug delivery. Clinical studies were performed to prove its superiority over systemic drug administration, but they lacked theoretical and/or experimental justification.

The theoretical basis for intra-arterial drug administration was provided for in the 70s and 80s (Eckman, 1974; Chen and Gross, 1979, 1980; Weiss, 1985; Smits and Thijssen, 1986). It was found that only under special circumstances, with the right combination of drug and target organ, could the method be expected to have advantage over systemic administration.

To date, experimental verification of this theoretical basis is still rare. Evaluation of the advantages of intra-arterial over systemic drug administration has to be performed under controlled experi-

mental conditions in which important physiological and pharmacological parameters such as the blood flow to the target organ, the extraction ratio of the drug by the target organ, the concentration of the drug in the target artery and/or the target organ, the systemic concentration, and the systemic clearance of the drug are known or can be calculated.

In this thesis, techniques are described that enable the measurement of these parameters in a small experimental animal, the rat, providing a means for the experimental evaluation of the advantages of regional over systemic drug administration. These techniques include the evaluation of indocyanine green as an indicator of rat liver blood flow (chapter 3), the introduction of the electromagnetic flow method to assess rat hepatic arterial and portal venous blood flow (chapter 4), and the liver biopsy technique for repeated liver tissue sampling in one animal (chapter 5).

Drug administration directed to the kidney, heart, liver and testis was compared to the systemic administration of the same drug at the same rate at steady-state conditions (table 1.6). To evaluate the often used method of intrarenal drug administration in the rat, a low renal clearance substance, Chromium-51 Ethylenediaminetetracetate (^{51}Cr EDTA), and a high renal clearance substance, ^{125}I odo hippuric acid (^{125}IHA), were given directly to the left and right kidney (chapter 6). Propranolol, a high hepatic clearance substance, was given into the coronary system of rat cardiac grafts (chapter 8) and intrarenally (chapter 7) to investigate the suitability of the rat cardiac transplant for local drug administration and to investigate the regional advantage during local administration. Acenocoumarol, an oral anticoagulant, was infused intrahepatically (via the hepatic artery) and intravenously to study the effect of the route of administration on the hepatic drug concentration (chapter 9). Acenocoumarol was also given intrarenally and intratesticularly to study the effect of flow on the regional and systemic advantage. Phenobarbital co-treatment was used to study the effect of an enhanced systemic clearance on the regional advantage (chapter 10; table 1.6). The effects of chronic local acenocoumarol administration in the testis on testicular vitamin K dependent enzyme systems and on fertility were studied in chapter

11. In the last chapter, concluding remarks and future lines are presented. In Appendix I the results of a simulation study are presented. The effects of saturable tissue binding sites on the regional and systemic advantages of a locally administered drug were studied. This simulation study is based on data described in chapter 2 and chapter 9.

Table 1.6: An outline of the experiments described in chapter 6-11 of this thesis.

The infused drug is cleared by the "target organ"

High extraction ratio	¹²⁵ IHA in the kidney (ch. 6) Propranolol in the transplanted heart (ch. 8)
Low extraction ratio	⁵¹ Cr EDTA in the kidney (ch. 6) Acenocoumarol in the liver (ch. 9)

The infused drug is not cleared by the "target organ"

	High systemic clearance	Low systemic clearance
High blood flow	Propranolol in the kidney (ch. 7)	Acenocoumarol in the kidney (ch. 10)
Low blood flow	Acenocoumarol in the testis (phenobarbital co-treatment; ch. 10)	Acenocoumarol in the testis (ch. 10, 11)

2. MATERIALS AND METHODS

2.1 INTRODUCTION

From the previous chapter, it may be obvious that several conditions have to be fulfilled before the actual benefits of local over systemic drug administration can be evaluated experimentally. Some of these conditions are described below.

1. Access for drug administration directly to the target organ has to be possible;
2. Determination of the drug concentration has to be performed in systemic blood (plasma) and in the target organ;
3. The blood flow to the target organ has to be measured in absolute terms.

In this chapter, routes for local drug administration to the kidney, the transplanted heart, the liver and the testis of the rat will be described. The drugs used in our experiments will be presented in paragraph 2.3. Special attention will be given to pharmacokinetic aspects of the drugs in relation to the respective target organ. Analytical procedures for the determination of the concentration of the drugs in blood or plasma and target tissue will be described. Methods for flow measurements will be given in paragraph 2.4. Mathematical descriptions for pharmacokinetic analysis of experimental data will be described in the last paragraph of this chapter.

2.2 ROUTES OF LOCAL DRUG ADMINISTRATION

For the direct administration of a drug into the blood stream of a target organ, techniques that allow long-term access to the supplying artery, but do not disturb the organ blood flow, are preferred. In this thesis, target organ directed drug administration is described for several organs: the kidney, the heart, the liver, and the testis. The techniques used to gain access to the respective target organs are described here.

2.2.1 The kidney

Intrarenal infusion in the left or the right kidney (chapter 6,7,10) was performed following the method of Smits et al (1983). Briefly, a stretched poly-ethylene (PE-10) catheter was introduced in a retrograde fashion in the suprarenal artery, which originates from the renal artery. The tip was placed just proximal to the renal artery to prevent flow changes in that artery (fig. 2.1). With this method of renal drug administration, renal function is not affected (Smits et al, 1983).

Fig. 2.1: Schematic drawing of a catheter, placed in the suprarenal artery of the rat, used for intrarenal infusion.

2.2.2 The transplanted heart

Based on the same principle as above, a PE-10 catheter was introduced in a retrograde fashion in the carotid artery for local infusion into the coronary circulation of rat cardiac grafts (chapter 8). Since in this model the arch of the transplanted aorta is situated at a very close distance to the liver of the acceptor animal, the cannulation of the carotid artery was performed before the transplantation (Ruers et al, 1987). As will be described in more detail in chapter 8, the blood flows in a retrograde fashion through the transplanted aorta. A drug infused via the carotid artery reaches the transplanted heart almost immediately (fig. 2.2). The aortic valves are closed. Hence, blood reaching the transplanted heart flows retrogradely through the coronary arteries only.

Fig. 2.2: Schematic drawing of a catheter, placed in the carotid artery, used for local infusion in the rat cardiac graft.

2.2.3 The liver

For intrahepatic arterial drug administration, the tip of a catheter was introduced into the gastroduodenal artery (fig. 2.3) according to Cotino and Zwaveling (1976).

As illustrated in fig. 2.3, the proper hepatic artery and the gastroduodenal artery originate from the common hepatic artery. To gain access to the proper hepatic artery a stretched PE-10 catheter was introduced in a retrograde fashion into the gastroduodenal artery. The tip of the catheter was just proximal to the common hepatic artery to prevent flow changes in the hepatic artery.

Fig. 2.3: Schematic drawing of catheter, placed in the gastroduodenal artery, used for intrahepatic infusion.

2.2.4 The testis

The method of cannulation of a side-branch of the target artery to gain access to the target artery itself could not be applied in experiments on the effects of local infusion of acenocoumarol in the rat testis (chapter 10,11). The spermatic arteries of the rat originated, in most of the animals investigated for this purpose, directly from the aorta and had no branches to be cannulated. Investigation of several strains of rats (Sprague-Dawley, Wistar, Wistar-Kyoto and Brown-Norway) indicated that this phenomenon should be regarded as the normal rat anatomy.

The following procedure was developed for intratesticular drug administration. Under light ether anesthesia, the lower part of the abdomen was opened by a midline incision of about 2 cm and the left testis retracted into the abdomen. A silastic tube (internal diameter 0.6 mm), filled with the infusion solution, was placed directly into the testis and fixed with a suture. The testis including the catheter was pushed back gently into the scrotum and the abdomen was closed.

2.3 SUBSTANCES USED FOR PHARMACOKINETIC ANALYSIS

In all studies but one, the infused substances were chosen not because of their specific effects but because of their pharmacokinetic behavior in a given model. The pharmacokinetics of these substances: indocyanine green, propranolol, ^{125} Iodine labeled hippuric acid (^{125} IHA) and ^{51} Chromium labeled EDTA (^{51} Cr EDTA), will be described briefly below. The fifth, S-acenocoumarol, will be described in more detail, because local administration of S-acenocoumarol in the testis was used to evaluate not only the kinetic advantages but also to study the effects of intratesticular administration of this drug.

2.3.1 Indocyanine green

Indocyanine green (ICG, cardiogreen, Fox Green) is a synthetic dye developed for use in color emulsion. Later, it has been used as an indicator in dye dilution techniques for the measurement of cardiac output (Fox et al, 1957).

It is excreted unchanged and almost completely in bile although recent literature reports suggest the formation of a metabolite without any sign of enterohepatic circulation. ICG is also used to evaluate hepatic function and hepatic blood flow in man and experimental animals (Cherrick et al, 1960; Wiegand et al, 1960; Caesar et al, 1961; Leevy et al, 1962).

Assay

ICG is analyzed in plasma by spectrophotometry at 800 nm using pool plasma as reference. In recent studies (Rappaport and Thiessen, 1982; Thiessen et al, 1984), a more specific HPLC method was reported, yielding different ICG plasma concentrations in a rabbit following an intravenous dose of about 100 mg, pointing to an unidentified ICG metabolite or an impurity of the commercially available product (Heintz et al, 1986). The suggested metabolite has not been found in humans (Svensson et al, 1982).

2.3.2 Propranolol

Propranolol was used in 3 studies (chapter 4, 7, and 8). It was

chosen because of its high hepatic clearance (14-28 ml/min) in the rat. According to the physiologic pharmacokinetic approach, the clearance of propranolol should be flow-dependent (chapter 1). Assuming a linear kinetic pattern local administration of such a high hepatic clearance drug should result in a relatively high systemic advantage in the rat cardiac graft (chapter 8), while the regional and systemic advantages of intrarenal over systemic administration of propranolol should be minimal (chapter 7).

Analytical procedure

Following the addition of metoprolol for internal standardization, samples (plasma or heart homogenates) were prepared for analysis by HPLC. For measurement of propranolol in cardiac tissue, the wet heart tissue was homogenized in 4 times its volume (w/v) saline. Propranolol was extracted by 1.5% pentanol in hexane, after alkalization with NaOH (10 N). Propranolol was re-extracted from the organic phase by 150 μ l of 0.01 N H_2SO_4 . An aliquot of the acid phase was analyzed by HPLC.

For ^3H labeled propranolol, the radioactivity in the acid phase of the organic solvent extract was measured. HPLC analysis established that all of that radioactivity could be attributed to propranolol. Propranolol was measured in a scintillation counter after the addition of a commercial scintillation solution, correcting for quenching by internal standardization with ^3H toluene.

2.3.3 Cr EDTA and iodohippurate

Both substances are often used to evaluate renal hemodynamics (Smart et al, 1981; Wanner et al, 1981). ^{51}Cr EDTA is eliminated by the kidney via glomerular filtration and ^{125}I HA via glomerular filtration and active tubular secretion (Cho and Cafreeny, 1970; Brenner et al, 1972).

Assay and urine concentrations of both substances (chapter 6) were determined in a LKB Wallace Universal gamma-counter with overlap correction.

ACENOCOUMAROL

* centre of asymmetry

Fig. 2.4: Structure formula of acenocoumarol.

2.3.4 Acenocoumarol

Acenocoumarol is a 4-hydroxycoumarin derivative, which, like warfarin and phenprocoumon, is clinically in use as an oral anticoagulant. The compound is in use as a racemate (fig. 2.4). The pharmacokinetics of racemic acenocoumarol in the rat were recently investigated by Thijssen et al (1983a). The drug has a short duration of action. Following a single dose of 2 mg/kg subcutaneously in conscious male Wistar rats, prothrombin time, an indicator of plasma coagulation activity, returns to normal within 24 hr. Its apparent half-life time of elimination in the rat is about 3-4 hr. Elimination is mainly via hepatic metabolism, i.e. hydroxylation of the coumarin part of the molecule with subsequent conjugation. There is stereoselectivity of the pharmacokinetics and pharmacodynamics of acenocoumarol in the rat (Thijssen et al, 1985) as well as in man (Godbillon et al, 1981).

Following the subcutaneous infusion of 1 mg/kg of S(-)-acenocoumarol (AC) in conscious male Wistar rats, the elimination rate of AC was found to be 740 ml/hr/kg (Thijssen et al, 1985). The biological half-life time was about half an hour. R(+)-acenocoumarol was found to have an elimination rate of 183 ml/hr/kg and a half-life time of

elimination of about 90 min. Plasma protein binding of S(-)-acenocoumarol was high: 98.5%, which differed only slightly from the plasma protein binding of the R(+)-enantiomer.

Fig. 2.5: The S-acenocoumarol concentration in blood and liver as a function of the infusion rate (0-15 $\mu\text{g/min}$)

There is an extensive liver uptake of acenocoumarol and an apparent dose-dependent hepatic clearance (Thijssen et al, 1983a, 1985; Daemen et al, 1986a) (fig. 2.5; table 2.1). The elimination from the liver is slower than from the circulation (fig. 2.6). From experiments investigating S(-)-acenocoumarol liver distribution in pentobarbital-anesthetized rats during constant rate infusion, we found a saturable hepatic binding site ($K_D=6.7$ nM; $B_{\text{max}}=3$ nmole/g liver tissue) for S(-)-acenocoumarol which might account for its apparent dose-dependent clearance (fig. 2.7 and Daemen et al, 1986a; see also Appendix I).

Fig. 2.6: The S-acenocoumarol concentration in the arterial blood flow (○) and liver (●) of an individual rat as a function of time. S-acenocoumarol was administered by constant rate infusion (12.5 $\mu\text{g}/\text{min}$) over 120 min.

Fig. 2.7: Scatchard analysis of S-acenocoumarol binding in the liver tissue. The liver concentration was assumed to be equal to the plasma concentration. The line is derived from a least-squares fit of the data below 1.5 $\mu\text{g/g}$ liver.

Mechanism of action

Oral anticoagulants of the coumarin type interrupt the cellular vitamin K cycle by blocking the vitamin K epoxide reductase (Suttie et al, 1974; Vermeer et al, 1984a; fig. 2.8). In the liver cell, the hydroquinone of vitamin K is a necessary cofactor for a carboxylase which converts clotting factor proteins to their calcium binding form and leaves the cofactor as vitamin K epoxide. Vitamin K epoxide is then reduced by vitamin K epoxide reductase to vitamin K and vitamin K is further reduced to its hydroquinone by other reductases. In the liver, vitamin K dependent carboxylase are involved in the synthesis of

VITAMIN K CYCLE

Fig. 2.8: Vitamin K cycle. OA = oral anticoagulants.
 1: vitamin K epoxide; 2: vitamin K; 3: vitamin K hydroquinone; 4: vitamin K epoxide reductase; 5: vitamin K reductase(s); 6: carboxylase

Table 2.1: The steady-state blood and liver S(-)-acenocoumarol concentration and the estimated blood clearance in relation to the infusion rate (mean $\pm$ SEM).

Infusion rate n (ug/min)		C_b , ug/ml ^a	C_l , ug/g ^b	Liver to blood ratio	Cl (ml/min)
0.12	2	0.019	0.70	36.8	6.3
0.15	3	0.023+0.002	0.70+0.06	30.4+3.0	6.5+0.6
0.31	4	0.068+0.07	0.64+0.06	9.4+1.1	4.5+0.4
0.625	8	0.161+0.01	0.87+0.09	5.4+0.6	3.9+0.3
6.25	3	1.8 +0.44	3.63+0.7	2.0+0.4	3.5+0.9
12.5	3	3.5 +0.12	4.9 +0.6	1.4+0.6	3.6+0.12
15.0	3	4.7 +0.13	6.6 +0.3	1.4+0.1	3.2+0.1

^a C_b is the blood concentration

^b C_l is the liver concentration

^cCl is the blood clearance

Source: Daemen et al, J Pharm Sci 75: 238-240, 1986.

the coagulation factors II, VII, IX and X (Stenflo et al, 1974). Vitamin K dependent carboxylase activity is also shown to be present in several non-hepatic tissues, such as the spleen, arterial vessel wall, osteoblast cells, kidney and testis (Vermeer et al, 1982). Vitamin K epoxide reductase is shown to be present in non-hepatic tissues as kidney and testis (Thijssen et al, 1986), while gamma carboxy glutamic acid residues containing proteins are found in bone (osteocalcine) and human sperm (Vermeer, 1984b). The functions of these proteins and/or the extrahepatic physiological vitamin K dependent systems are not clear yet.

Analytical procedures

Acenocoumarol concentrations in biological tissues were analyzed by HPLC as described by Thijssen et al (1983b).

2.4 METHODS FOR THE MEASUREMENT OF BLOOD FLOW IN LARGE VESSELS

As stated before, knowledge of the amount of blood flow to the target organ is essential in order to evaluate the potential benefits of target organ directed drug administration.

There are several methods for the measurement of the blood flow to an organ. These can be divided into two groups: direct and indirect methods (table 2.2; Ohnhaus, 1979).

In our experiments target organ blood flow was measured by electromagnetic flowmeters because this method enables continuous measurement of blood flow. The method has long-term stability and does not require frequent calibration. Blood flow of one or more vessels can be measured selectively (Charbon and Van der Mark, 1981).

In chapter 3 and 4, the suitability of the ICG clearance method to determine liver blood flow in the anesthetized rat is evaluated and compared with the electromagnetic assessment of liver blood flow.

Table 2.2: Flow measurements in large blood vessels

A. Direct methods

- Doppler technique
- Electromagnetic technique
- Heat exchange method
- Drop counts

B. Indirect methods

- clearance/dilution method (e.g. indocyanine green, bromosulphthalein blue)
- inert gas washout technique (H_2 , N_2)
- fractional distribution method (microspheres)

2.5 PHARMACOKINETIC ANALYSIS

All pharmacokinetic studies were performed at steady-state conditions, using constant rate infusions over prolonged time. Under those conditions, the following equations hold.

Systemic plasma clearance at steady state (Cl_S ; ml/min) was calculated by

$$inf/C_S \quad (1)$$

where inf represents the infusion rate (ug/min) and C_S the systemic arterial plasma or blood concentration at steady state (ug/ml). The concentration in the artery supplying the target organ at intravenous drug administration (C_{Tiv}) equalled the systemic concentration (C_{Siv}). The concentration in the artery supplying the target organ, at target organ directed drug administration (C_{Tia} ; ug/ml) was calculated by

$$C_{Tia} = C_{Sia} + inf/Q_T \quad (2)$$

where C_{Sia} (ug/ml) represents the steady-state systemic blood (plasma) concentration at intra-arterial drug administration and Q_T the blood flow through the target organ (ml/min). The clearance of the target organ during target directed drug administration (Cl_T ; ml/min) was defined by

$$Cl_T = inf/C_{Tia} \quad (3)$$

During the intrarenal administration of substances, the renal clearance (Cl ; ml/min) was defined by

$$Cl = U/C_T \quad (4)$$

where U equals the amount of drug excreted in the urine (counts/min). Cl during systemic drug administration was defined by

$$Cl = U/C_S \quad (5)$$

The extraction ratio of the kidney (E) was calculated by

$$E = Cl/Q. \quad (6)$$

As before (chapter 1 and Appendix I), the systemic advantage of local drug administration was defined by the formula

$$C_{Sia}/C_{Siv} = 1 - E_T \quad (7)$$

and the regional advantage by the formula

$$C_{Tia}/C_{Tiv} = 1 + Cl_S/Q_T \quad (8)$$

3. MEASUREMENT OF LIVER BLOOD FLOW IN THE RAT: THE EFFECT OF PENTOBARBITAL ANESTHESIA AND HYPOTHERMIA ON THE HEPATIC CLEARANCE OF INDOCYANINE GREEN

3.1 INTRODUCTION

For evaluation of the advantages of intrahepatic over systemic drug administration in the rat, the total liver blood flow has to be determined. The indocyanine green clearance method is often used for this purpose (Ohnhaus, 1979; Yates et al, 1983). In pharmacokinetic experiments the use of anesthesia is frequently necessary. This study was performed to investigate the effects of anesthesia on the ICG clearance in the rat.

Anesthesia can influence organ flow as well as intrinsic clearance. It is known to depress the autonomic nervous reflex system involved in blood pressure regulation (Cox and Bagshaw, 1979) and it may cause hypothermia (Birnie and Grayson, 1952), which might decrease processes (metabolism, active transport) of drug distribution and of drug elimination (McAllister et al, 1978, 1979; Spurr and Dwyer, 1972). Also a direct inhibitory interaction of the anesthetic with the drug metabolizing system might occur (Vermeulen et al, 1983). An indication of the pitfalls of estimating physiological parameters during anesthesia may be found in the deviating data on hepatic blood flow in rats as estimated by the indocyanine green (ICG) clearance method. Values between 0.9-1.5 ml/min/g liver are reported for anesthetized as well as for conscious rats (Yokota et al, 1976). To evaluate the influence of anesthesia in the rat, we have investigated the effect of pentobarbital anesthesia alone and together with the changes in body temperature, on the hepatic clearance of ICG.

3.2 EXPERIMENTAL SECTION

In this study, male inbred Wistar Kyoto rats, 280-330 g, were used. Two days before the experiment, under light ether anesthesia, rats were cannulated (PE-10) in the left femoral artery for blood

sampling, in the left femoral vein for drug infusion, and in the right femoral vein for the intravenous administration of pentobarbital. The catheters were exteriorized in the neck and were flushed with heparin in isotonic saline (5 U/ml).

3.2.1 Infusion and sampling protocol

ICG infusion was started in the conscious animal at a constant rate of 25 ug/min. Two blood samples (100 ul) were obtained at 60 and 75 min. Pilot experiments showed these times sufficient to reach steady-state conditions. While maintaining infusion of ICG, pentobarbital anesthesia was induced by a bolus injection of 0.2 ml pentobarbital solution (60 mg/ml) intravenously and maintained by administration of 0.1 ml every 60 min. After animals had lost consciousness, their body temperature (measured rectally) was maintained at 37.5°C by a heat lamp and a regulator. Two blood samples were obtained following the time schedule as before.

A separate group of rats was anesthetized with pentobarbital and the body temperature maintained at 37.5°C (as above). Infusion of ICG was started and arterial blood was sampled twice at steady state (see time schedule above). During infusion, the body temperature was either increased to 39.5°C by additional heat or cooled to 32.5°C by stopping the heat and when steady state had been attained, blood samples were obtained according to the previous time schedule. ICG plasma concentrations were determined as described in chapter 2.3.

3.2.2 Statistical analysis

All data are presented as mean $\pm$ SEM. Differences between the groups were determined by Student's t-tests. Differences were considered to be statistically significant if $p < 0.05$.

3.3 RESULTS

Plasma concentrations of ICG in the conscious state and under pentobarbital anesthesia were 5.2 ± 0.7 and 9.6 ± 0.8 ug/ml (mean $\pm$ SEM, $n=8$). The estimated blood clearances are in table 3.1.

Table 3.1: The effect of pentobarbital anesthesia and temperature on the blood clearance of indocyanine green (mean $\pm$ SEM, n=8).

Experimental condition	Blood clearance (ml/min)
Conscious	8.0 $\pm$ 1.7
Anesthesia	4.9 $\pm$ 0.6 ^a
Body temperature	
37.5°C	5.7 $\pm$ 0.8
Anesthesia 39.5°C	5.6 $\pm$ 0.7
32.5°C	0.9 $\pm$ 0.1 ^b

^a p<0.01 vs conscious

^b p<0.001 vs. 37.5°C

Anesthesia decreased the mean ICG clearance to about 45%. No difference in ICG clearance was observed during hyperthermia as compared to normothermia (5.6 $\pm$ 0.7 vs. 5.7 $\pm$ 0.8 ml/min). In hypothermia, the clearance of ICG dropped dramatically to 0.9 ml/min which was only 16% of the normothermic value. The time effect of anesthesia on the clearance of ICG was investigated with rats in the normothermic state (37.5°C). No changes in clearances over the infusion period were observed (fig. 3.1).

Fig. 3.1: Arterial blood concentration (ug/ml) of indocyanine green (ICG; mean $\pm$ SEM; n=4) following continuous systemic infusion of 25 ug/min for 360 min during anesthesia, while body temperature was kept at 37.5°C.

3.4 DISCUSSION

The primary purpose of these experiments was to investigate the effect of anesthesia and the effect of hypothermia induced by it on the hepatic clearance of ICG. In the literature, few authors discuss the effect of anesthesia on drug disposition explicitly. Recently, Vermeulen et al (1983) showed that ether anesthesia almost halved hexobarbital clearance in rats. Yokota et al (1976) observed the depressant effect of pentobarbital on ICG clearance in rats and considered hypothermia as a possible cause.

We found that pentobarbital anesthesia depressed the clearance of ICG despite that the body temperature was maintained at 37.5°C. The hypothermia related to anesthesia further reduced the body clearance to one tenth of the conscious values.

Though it is generally assumed that the ICG clearance is liver flow dependent (Caesar et al, 1961; Leevy et al, 1962), our results clearly indicate that the effect of anesthesia and hypothermia on ICG clearance do not reflect changes in liver blood flow. The observed clearance in the conscious rat (about 24 ml/min/kg) is low compared with liver blood flow (about 80 ml/min/kg; Ohnhaus, 1979). This indicates that the extraction ratio for ICG is only 0.3 which suggests a low rather than a high clearance drug. Hence the effect of pentobarbital anesthesia is to depress the intrinsic capacity of the liver to extract ICG from it.

Saturation of ICG elimination during the experimental condition could be a reason for the low hepatic clearance of the substance. It is well known that ICG elimination is dose-dependent and it has been shown that ICG plasma concentrations above 10 ug/ml progressively tend to decrease its clearance (McDevitt et al, 1977; Stoeckel et al, 1980). However, in our experiments steady-state ICG plasma concentrations conscious rats were about 5 ug/ml and in the anesthetized rats 8 ug/ml, thus making saturation kinetics a less likely explanation. Yokota et al (1976), using the continuous infusion technique, reported a value of 18 ml/min/kg for the ICG clearance in the conscious rat and this is comparable with our results. Because of the low extraction (about 0.3) of ICG and its low clearance it is not justified to inter-

pret ICG clearance in the anesthetized rat using the continuous infusion technique in terms of liver flow.

In summary, our data demonstrate clearly that anesthesia and in association with it hypothermia strongly depresses the intrinsic capacity of the liver to extract ICG from the circulation. To estimate pharmacokinetic constants, the use of conscious animals is to be preferred. If the use of anesthesia is necessary, a precise control of the body temperature during anesthesia is essential.

4. LIVER BLOOD FLOW MEASUREMENT IN THE RAT: THE ELECTROMAGNETIC VS. THE CLEARANCE METHOD

4.1 INTRODUCTION

Knowledge of the absolute liver blood flow is important in the experimental evaluation of the effects of drugs and hormones on the hepatic arterial and portal venous inflow (Schenk et al, 1962) as well as in a physiological pharmacokinetic approach to hepatic drug clearance (Wilkinson and Shand, 1975 and chapter 1). Several methods are available to determine liver blood flow in small experimental animals like rats (Ohnhaus, 1979).

The clearance of ICG is widely applied to estimate hepatic blood flow in man and experimental animals (Bradley et al, 1945; Rowell et al, 1964; Yates et al, 1983; Shepherd et al, 1985; Arends et al, 1986). The use of the ICG clearance technique is based on the assumption that the substance has a high hepatic extraction ratio and that, according to the physiological pharmacokinetic approach (Wilkinson and Shand, 1975), the clearance of high hepatic clearance substances (extraction ratio >0.7) reflects liver flow. Therefore, measurement of the ICG clearance should give insight into the amount of hepatic blood flow, while clearance changes should reflect hepatic blood flow rather than changes in hepatic extraction.

However, conflicting data on the ICG extraction ratio are given in literature. In man, an extraction ratio of 0.6-0.8 is described (Wiegand et al, 1960; Leevy et al, 1962), while it is suggested to be even lower in rats (Daemen et al, 1986b), and cats (Krarup and Larson, 1976): 0.30 and 0.25, respectively. In rabbits and rats, a dose-dependent hepatic clearance has been reported (Stoeckel et al, 1980). Therefore, it is questionable whether changes in ICG clearance really reflect changes in liver blood flow rather than changes in hepatic extraction (Groszmann, 1983).

The purpose of this study was to evaluate the value of the ICG clearance method as an indicator of rat hepatic blood flow by comparing the clearance of ICG and of another high hepatic clearance substance, d-propranolol, with the electromagnetic measurement of the

total hepatic blood flow. For this latter purpose, electromagnetic flowmeters were placed on the portal vein, a technique described previously by Jenkins et al (1984, 1985) and on the hepatic artery of the same animal. To verify the electromagnetic method, the effects of adenosine, a vasodilator, phenylephrine, a vasoconstrictor, and a volume loading on hepatic blood flow were determined.

4.2 EXPERIMENTAL SECTION

Male Wistar rats, weighing 300-350 g, were obtained from Winkelman, Borchten, West-Germany. Before the experiment, the animals were on standard lab food and tap water.

4.2.1 Surgery

Anesthesia was introduced by a bolus injection of pentobarbital intraperitoneally (60 mg/kg). To maintain the anesthetic state, a poly-ethylene (PE-10) catheter was placed in the right femoral vein for constant rate infusion of pentobarbital in isotonic saline (150 ug/min). The infusion volume was set at 50 ul/min to prevent dehydration (Maddox et al, 1977). Two PE-10 catheters were introduced in the left femoral vein for drug infusion and one in the right femoral artery for blood pressure/heart rate recording and blood sampling. The trachea was cannulated to provide a patent airway. Temperature was measured rectally and maintained at 37.5°C by a thermostatically controlled heat lamp.

4.2.2 Electromagnetic measurement of hepatic blood flow

After opening the abdominal cavity via a mid-abdominal incision, the intestines were kept in gauzes wetted with isotonic saline. The bursa omentalis was opened and the hepatic artery localized (fig. 4.1A). The gastroduodenal artery was ligated with a silk tie (6-0). The common hepatic artery was freed from connective tissue and two silk ties (6-0) were passed under the vessel. An electromagnetic flow probe (internal diameter 0.5 mm, Skalar, Delft, The Netherlands) was placed around the common hepatic artery. Zero balancing was performed

Fig. 4.1:

- A. Simplified scheme of the anatomy in the upper right abdomen of the rat: 1. liver; 2. proper hepatic artery; 3. common hepatic artery; 4. gastroduodenal artery; 5. bile duct; 6. portal vein; 7. aorta; 8. caval vein; A. electromagnetic flow probe around the common hepatic artery; B. electromagnetic flow probe around the portal vein; C. portal vein occluder.
- B. Example of a recording. MAP: mean arterial pressure; HR: heart rate; HAF: hepatic arterial flow; PVF: portal venous flow (note that the portal venous flow is out of phase).
- C. Example of a recording of the occlusion of the portal vein for zero balancing.

by occluding the vessel via the distally placed tie.

The proximal part of the portal vein was also freed from surrounding tissue. A special mechanic vessel occluder (Skalar, Delft, The Netherlands), which could be remotely controlled, was placed around the portal vein. A second EM flow probe (internal diameter 1.8 mm) was placed around the portal vein proximally from the occluder. Zero balancing was here performed by closing the occluder. During the whole procedure care was taken to avoid any damage to the bile duct. The intestines were then replaced in the abdomen.

The flow signals (fig. 4.1B) were measured with a sine-wave flowmeter (type MDL 400, Skalar, Delft, The Netherlands). Flow as well as blood pressure and heart rate signals were recorded on a Schwarzer polygraph.

4.2.3 Infusion protocol

After the hepatic blood flow had equilibrated, phenylephrine or adenosine was given intravenously. Adenosine (Sigma, St. Louis, Mo, USA) in isotonic saline (4.0 mg/ml) was given by a constant rate infusion of 20, 40, and 100 ug/min. Phenylephrine (Sigma, St. Louis, Mo, USA) in isotonic saline (0.4 mg/ml) was given by a constant rate infusion of 2, 4, and 10 ug/min. Both drugs were delivered at random in the same animals. The infusion rates for each drug were increased stepwise and lasted 5 min per infusion rate. Steady-state changes in the measured parameters were used for calculation.

Fifteen ml isotonic saline (37.5°C) was infused intravenously in 3 min to study the effect of a rapid volume loading on hepatic blood flow in a second group of animals. Changes in blood pressure and heart rate were measured during the maximal changes in portal venous and hepatic arterial blood flow.

In another series of animals, indocyanine green (Brocacef, Heerlen, The Netherlands, 5 mg/ml) and d-propranolol in isotonic saline (0.2 mg/ml) were given simultaneously at a constant rate of 25 and 1 ug/min, respectively. A bolus of 100 ug (50 ul) of d-propranolol was given at the start of the infusion. The d-enantiomer of propranolol does not affect hepatic blood flow (Branch et al, 1973; Rahn et al, 1974). The infusion was maintained for 180 min. Arterial blood samples

(0.3 ml) were taken at 120, 150, and 180 min and transferred into 30 μ l 0.1 M citrate containing glass vessels. Preliminary experiments applying this infusion scheme had shown that steady-state conditions were achieved after 120 min. The mean of the values of blood pressure, heart rate, portal venous and hepatic arterial flow at the sample times were taken for calculation. Hematocrit was determined before and after each infusion period.

Analysis of ICG and propranolol was performed as described in chapter 2.3.1 and 2.3.2.

4.2.4 Statistical Analysis

All data are presented as mean $\pm$ SEM. Groups were compared using Student's t-test. The 0.05 level of probability was used as the level of significance.

Fig. 4.2: Effects of intravenous phenylephrine and adenosine infusion on mean arterial pressure (MAP), heart rate (HR), electromagnetically measured portal venous flow (PVF) and hepatic arterial flow (HAF). Data are presented as mean $\pm$ SEM; n=7-9. *p<0.05; **p<0.01; ***p<0.001.

4.3 RESULTS

The observed values for portal venous and hepatic arterial blood flow were very well comparable in the 3 experiments (adenosine/phenylephrine infusion, clearance studies and volume loading). Values for portal venous flow varied from 13.5 ± 1.3 to 16.1 ± 2.1 ml/min and for mean hepatic arterial flow from 2.2 ± 0.3 to 2.5 ± 0.6 ml/min, (n=9). Hepatic arterial and portal venous flow, measured simultaneously, was found to be very stable over a period of 3 hr. An interaction between the hepatic artery and portal vein was observed in our experiments. A reduction of portal venous flow, performed by clamping this vessel for zero balancing, was compensated for by an increase of the hepatic arterial flow (fig. 4.1C). A volume loading of 15 ml isotonic saline increased the portal venous flow from 13.5 ± 1.3 to 20.8 ± 1.9 ml/min and the hepatic arterial flow from 2.5 ± 0.2 to 2.9 ± 0.3 ml/min (table 4.1).

4.3.1 Adenosine/phenylephrine infusion

The effects of intravenous adenosine and phenylephrine on mean arterial blood pressure (MAP), heart rate (HR), portal venous and hepatic arterial blood flow are depicted in fig. 4.2. Adenosine decreased blood pressure and heart rate dose-dependently: mean arterial

Table 4.1: Mean $\pm$ SEM (n=7) of the values of all measured parameters during a volume loading of 15 ml saline intravenously.

		Control	Volume loading
Blood pressure	(mmHg)	125 ± 6	124 ± 9
Heart rate	(beats/min)	384 ± 6	384 ± 6
Portal venous blood flow	(ml/min)	13.5 ± 1.3	$20.8 \pm 1.9^{***}$
Hepatic arterial blood flow	(ml/min)	2.5 ± 0.2	$2.9 \pm 0.3^{**}$

**p<0.01; ***p<0.001

blood pressure decreased to about 60 mm Hg and heart rate to approximately 300 beats/ min during the infusion of 100 ug/min of adenosine. Control values of MAP and HR were 115 mm Hg and 356 beats/min. Neither portal venous nor hepatic arterial flow changed: portal venous flow was 15.4 ± 2.7 ml/min during the control period and remained unchanged during infusion of adenosine. Mean hepatic arterial flow values were 2.2 ± 0.3 ml/min in the control period and 2.0 ± 0.3 ml/min at the highest infusion rate of adenosine. A dose-dependent increase in MAP and heart rate was observed during the infusion of phenylephrine (fig 4.2). Portal and hepatic arterial flows remained unchanged.

4.3.2 Clearance studies

Mean arterial pressure and heart rate of the animals in this group (n=7) were 132 ± 4 mm Hg and 375 ± 7 beats/min. Portal venous flow was 15.0 ± 1.4 ml/min and mean hepatic arterial flow 2.2 ± 0.3 ml/min. Steady-state plasma concentrations of ICG and d-propranolol were 8.0 ± 0.7 ug/ml and 82 ± 19 ng/ml plasma. Calculated plasma clearances were 3.3 ± 0.4 and 14 ± 2 ml/min for ICG and d-propranolol, respectively (table 4.2). Hematocrit changes during the experiments were not observed: 0.48 ± 0.005 at the start and 0.49 ± 0.006 at the end of the experiment.

Table 4.2: Mean $\pm$ SEM (n=7) of the values of the measured parameters during continuous intravenous infusion of ICG and d-propranolol.

Blood pressure	(mm Hg)	132 ± 4
Heart rate	(beats/min)	375 ± 7
Portal venous blood flow	(ml/min)	15.0 ± 1.4
Hepatic arterial blood flow	(ml/min)	2.2 ± 0.3
ICG plasma concentration	(ug/ml)	8.0 ± 0.7
d-propranolol plasma concentration	(ng/ml)	82 ± 19
ICG plasma clearance	(ml/min)	3.2 ± 0.3
d-propranolol plasma clearance	(ml/min)	14.1 ± 2.0

4.4 DISCUSSION

Direct assessment of the total hepatic blood flow has been achieved in several species, like man (Schenk et al, 1962), dog (Nxumalo et al, 1978), cat (Greenway and Stark, 1967), and rabbit (Takeuchi et al, 1986). To date, it has not been described in the rat. In this study, simultaneous electromagnetic measurements of hepatic arterial and portal venous blood flow in the rat are reported. The measured values are very well comparable with literature data on rat liver flow measurements on the basis of indirect techniques, such as microspheres. Sikuler and Grosmann (1986) reported a value of 20 ml/min with the microsphere method while a value of 15.9 ml/min for portal venous flow was found by Jenkins et al (1984).

A restriction of the present method is the necessity of anesthesia. The effects of anesthetic agents on the hepatic vascular bed are not well established. After pentobarbital anesthesia, hepatic blood flow was essentially the same as in unanesthetized dogs (Greenway and Stark, 1971). In the rat, pentobarbital anesthesia is known to reduce the clearance of ICG (Daemen et al, 1986b and chapter 3). Seyde et al (1985) using the microsphere technique reported that pentobarbital anesthesia did not affect total hepatic blood flow. Similar results were found by Lee et al (1986).

The effects of vasoactive agents, adenosine and phenylephrine, on the hepatic vascular beds were studied. The alpha-adrenoceptor agonist phenylephrine had no effect on portal venous and on hepatic arterial flow. Although hepatic and portal venous pressures were not measured, the rise in systemic mean arterial pressure without changes in flow suggests increases in the resistances of both vascular beds which is consistent with data in literature. Phenylephrine infused intravenously in dogs had similar effects: an increase in resistance but no changes in flow (Greenway and Stark, 1971). On the other hand, direct intrahepatic arterial or intraportal infusion caused a dose-dependent reduction in hepatic arterial and portal venous flow, respectively (Richardson et al, 1977a,b). Intravenous administration of adenosine did not affect hepatic blood flow either. Few investiga-

tors report the effects of adenosine on hepatic hemodynamics. Scholt-holt et al (1967) observed an increase in portal flow and pressure by intestinal vasodilatation in the dog, while no effects on hepatic arterial blood flow was found. Our data indicate strong autoregulatory responses in the rat since blood pressure changes of approximately 45% had no effect on either hepatic arterial or portal venous flow. The significance of local vasoregulation in the rat liver is not established yet. It is known to exist in the portal venous and even more in the hepatic arterial vascular bed, but the phenomenon has not shown to be consistent in literature reports (review: cf. Richardson and Withrington, 1981). A form of intrinsic regulation of the liver, the so-called hydrodynamic interaction (Richardson and Withrington, 1981) between hepatic artery and portal vein, was also observed in our experiments during clamping of the portal vein (fig. 4.1C).

The clearance of ICG was compared here with the clearance of a known high hepatic clearance drug d-propranolol and with the electromagnetic flow method to clarify the suitability of the often used indocyanine green clearance method as an indicator of hepatic blood flow in the anesthetized rat. The plasma clearance of ICG was 3.3 ± 0.4 ml/min, which is comparable with a previous study from our laboratory. Liver plasma flow in this group, calculated by (liver blood flow $\times$ (1-Hct)) was 9.0 ml/min. The plasma extraction ratio of ICG, the quotient of plasma clearance and flow, was 0.36. According to the physiological pharmacokinetic approach, ICG would be a low rather than a high hepatic cleared drug in pentobarbital-anesthetized rats using the continuous infusion technique. It would therefore be unsuited to estimate liver flow in anesthetized rats since changes in ICG clearance might reflect changes in hepatic flow and extraction capability (Wilkinson and Shand, 1975).

Even more evidence of the unsuitability of ICG as an indicator of liver blood flow in the anesthetized rat is provided by the data in table 4.3. In this table the values of electromagnetically measured portal venous and hepatic arterial blood flow in pentobarbital anesthetized rats at normo- and hypothermia are presented. As can be seen the decrease in temperature did not change liver blood flow significantly.

Table 4.3: The effect of temperature on the portal venous and hepatic arterial blood flow in pentobarbital anesthetized rats (mean $\pm$ SEM, n=5).

Body temperature	Portal venous flow (ml/min)	Hepatic arterial flow (ml/min)
37.5°C	16.6 $\pm$ 1.3	2.0 $\pm$ 0.2
32.5°C	16.0 $\pm$ 1.6	1.9 $\pm$ 0.2

The extraction ratio of propranolol in the rat is known to be high, i.e. 0.97 (Shand et al, 1972). In the rat, clearances of 12.6 (Iwamoto et al, 1985), 14 (Smits and Struyker Boudier, 1979), 19 (Schneck et al, 1977) up to 28 ml/min (Evans et al, 1973) have been reported. Because of this high clearance, the drug should be a reliable indicator of liver blood flow. In our study, a blood (plasma) clearance of 14.1 $\pm$ 2.0 ml/min was found. Simultaneous direct measurement of the hepatic blood flow yielded values of 17 ml/min, which indicates an extraction ratio higher than 0.8. As the plasma clearance of propranolol equals its blood clearance (Bianchetti et al, 1980) and the extrahepatic (i.e. renal) clearance of propranolol in rats (Iwamoto et al, 1985) is insignificant, the blood clearance values of d-propranolol correlate more to the values obtained by the electromagnetic flow measurement than those of indocyanine green.

Summarizing, the electromagnetic measurement of total liver blood flow in the rat is introduced in this study and found to be a reproducible and stable method. The obtained values were consistent with data in literature reports. The value of ICG, administered by constant rate infusion, to indicate hepatic blood flow in the rat is seriously questioned.

5. TISSUE SAMPLING IN THE RAT: THE LIVER BIOPSY

5.1 INTRODUCTION

Not only the determination of the blood flow to the target organ but also the determination of the systemic drug concentration and the drug concentration in the target organ are essential in the experimental evaluation of the physiologic pharmacokinetic approach in general and of the benefits of local over systemic drug administration in particular. In the mathematical description of the regional advantages of local over systemic drug administration the concentrations in the artery supplying the target organ and not in the target tissue were compared (chapter 1). However, drug concentrations in the artery supplying the target organ and in the target organ may change independently from each other. This was shown in a simulation study by Smits and Thijssen (1986) in which they observed dramatic changes in tissue concentrations, as a result of saturation of intracellular elimination mechanisms, with only small reflections in the concentrations in the artery supplying the target organ.

In clinical practice, the liver is often the target organ for drug delivery (see chapter 1) and the organ may play a dominant role in the extraction of xenobiotics from the circulation. For determination of the drug concentration in a target organ, as for example the liver, extirpation of the total organ is often necessary. The use of liver biopsies for this purpose seems logical. It has, however, rarely been used in pharmacological studies in small animals (Boelsterli and Zbinden, 1980), while the use of this technique in humans is common practice (Hegarti and Williams, 1984).

In the present study, we investigate the open-liver biopsy technique and its validity to estimate liver drug concentrations of acenocoumarol in time.

5.2 EXPERIMENTAL SECTION

5.2.1 Materials

The S-enantiomer of acenocoumarol (AC) was used in these experiments. For a description of the pharmacokinetics of AC and the analysis of AC see chapter 2.3.

5.2.2 Animal experiments

Male inbred Wistar-Kyoto rats, weighing 270-330 g, were used. All experiments were performed on unconscious animals. For anesthesia, 60 mg/kg pentobarbital was given intraperitoneally. The anesthetic state was maintained by repeated injections of 6 mg of pentobarbital sodium every 60 min.

A catheter (PE-10) was introduced in the femoral vein and in the femoral artery. A small midabdominal incision (2.5-3 cm) was made to give free access to the liver. The incision was held closed with a clamp. The body temperature was controlled rectally and maintained at 37.5°C by a heat lamp.

5.2.3 Drug administration and sampling

AC, in potassium phosphate buffer (0.1 M, pH 8.0) was administered by constant rate infusion via the venous catheter. Arterial blood samples (100 μ l) were obtained via the arterial catheter. The samples were heparinized and stored at -20°C until assayed. To study AC distribution in the liver and between the liver and blood, blood samples and liver biopsies were taken at 120, 150, 180 and 210 min. The mean of the 4 samples was taken as the respective steady-state level. Control experiments showed that steady state was reached within 100 min of infusion (fig. 5.1).

5.2.4 Liver biopsy

After presenting the liver via an abdominal incision, small pieces of liver tissue (20 mg) were cut out of the edge of a liver lobe with a sharp scissors. The abdomen was closed again with the clamp. The whole procedure lasted less than one minute.

The liver biopsies were rinsed immediately with ice-cold iso-

tonic saline, blotted gently on a soft paper tissue, and transferred to a pre-weighed centrifuge tube. After weighing, 300 ul of acetonitrile and 200 ul of isotonic saline were added. After an overnight store at 4°C, the samples were stored at -20°C until assayed. Prior to assay, the frozen biopsies were grained in the centrifuge tube with a glass rod.

After the experiment (i.e. 210 min), the rat was euthanized by an overdose of pentobarbital i.v. The liver was then removed, rinsed with isotonic saline, and homogenized in isotonic saline (25%, w/v).

Table 5.1: Comparison between the concentration of (S)-acenocoumarol (ug/g) in a biopsy and the total liver homogenate.

	Infusion rate (ug/min)									
Place of biopsy	0.156	0.31	0.625	0.625	0.625	0.625	6.25	6.25	12.5	12.5
Left lateral lobe	0.50	0.72	0.99	1.02	1.10	0.87	3.09	2.62	7.20	5.09
Right lateral lobe	0.52	0.78	1.02	1.07	1.28	0.93	3.09	2.78	7.49	5.00
Median lobe, left central part	0.72	0.79	0.99	-	1.18	0.98	3.33	2.37	6.91	4.75
Caudate lobe	0.59	0.74	0.91	0.73	1.98	0.87	3.33	2.52	7.37	5.30
Biopsy, mean	0.58	0.75	0.98	0.94	1.18	0.91	3.21	2.57	7.25	5.04
Total liver	0.58	0.79	0.99	1.05	1.31	0.90	3.15	2.70	7.37	5.38

The correlation between the concentration in the biopsy and the total liver at different infusion rates is as follows:

- left lateral lobe: $y=0.97x-0.02$, $r=0.9931$ (n=10)
- median lobe (right side): $y=0.98x-0.02$, $r=0.9982$ (n=10)
- median lobe (left side): $y=0.91x-0.07$, $r=0.9965$ (n=9)
- caudate lobe: $y=1.02x-0.14$, $r=0.9984$ (n=10)
- biopsy (mean): $y=0.97x-0.02$, $r=0.9990$ (n=9)

5.3 RESULTS

There was an excellent correlation between drug concentrations in liver biopsies and in total liver tissue (table 5.1). The drug was evenly distributed over the liver lobes. Liver biopsies could be taken frequently without deteriorating drug pharmacokinetics. In fig. 5.1, a typical control experiment is presented which shows that liver content and blood concentrations of S-acenocoumarol remained stable at steady state, although several biopsies were taken.

Fig. 5.1: The S-acenocoumarol concentration in the arterial blood (o) and liver (●) of an individual rat as a function of time after a constant rate of infusion of 0.31 $\mu\text{g/min}$. Arrows indicate the time of biopsies.

5.4 DISCUSSION

In studying drug tissue distribution, it would be of value to have a technique to obtain tissue samples at different time points from one individual animal. Whereas in human medicine the technique to obtain liver or kidney biopsies is common practice, in small labora-

tory animals this technique is hardly developed. Boelsterli and Zbinden (1980) used a liver needle biopsy in the rat for enzyme studies and Anderson et al (1978) the technique of wedge liver biopsies in the isolated liver perfusion system to study propranolol distribution. Our results show that liver biopsies can be taken from different lobes at different times without disturbing the hepatic clearance function significantly.

Liver biopsies of approximately 20 mg sufficed for S-acenocoumarol assay in the concentration range studied (i.e. 0.7-6.6 ug/g). The fact that this method constrains the use of anesthetics may be of some disadvantage because anesthesia negatively influences hepatic clearance. However, provided that body temperature of the rat is kept normal, pentobarbital anesthesia only moderately reduced the clearance of S-acenocoumarol (table 5.2).

Table 5.2.: The effect of pentobarbital anesthesia and temperature on the blood clearance of S-acenocoumarol (mean $\pm$ SEM, n=8).

Experimental condition	Blood clearance (ml/min)
Conscious	4.9 $\pm$ 0.4
Anesthesia	3.4 $\pm$ 0.3 ^a
Anesthesia	
Body temperature 37.5°C	3.8 $\pm$ 0.4
Body temperature 32.5°C	2.5 $\pm$ 0.2 ^b
Body temperature 39.5°C	4.4 $\pm$ 0.4
^a p<0.001 vs. conscious	
^b p<0.01 vs. 37.5°C	

Source: J Pharm Pharmacol 38, 122-125, 1986

In summary, the technique of the open-liver biopsy in the rat has been proven reliable for estimating the liver drug distribution in time for one rat. With this technique, the number of laboratory animals (rats) in pharmacokinetic experiments, including hepatic studies, can be reduced.

6. LOCAL DRUG ADMINISTRATION: KINETIC EVALUATION OF DIRECT INFUSION OF CR EDTA AND IODOHIPPURATE INTO THE LEFT AND RIGHT KIDNEY

6.1 INTRODUCTION

Although the local administration of drugs directly into the kidney is often applied in experimental research (Kleijnans et al, 1983, Smits et al, 1986) experimental support of the pharmacokinetic considerations of the advantages of intrarenal over systemic drug administration has, to our knowledge, not been presented yet. We investigated the disposition of ^{51}Cr EDTA (Cr EDTA) and ^{125}I iodohippurate (IHA) following their constant rate of infusion either intravenously (i.v.) or intra-arterially (i.a.) into the right or left kidney of the Wistar Kyoto rat. Both compounds are eliminated by the kidney predominantly, the former by glomerular filtration, the latter by filtration and tubular excretion (Brenner et al, 1972). The study agreed with the theoretical considerations about local drug administration, but also revealed pitfalls, i.e. non-linearity in pharmacokinetics.

6.2 EXPERIMENTAL SECTION

6.2.1 Methods

Male inbred Wistar Kyoto rats, weighing 300-350 g, were used. All experiments were performed under anesthesia (Inactin^R [5-ethyl-5-1'-methyl-propyl-2-thio-barbituric acid], 100 mg/kg intraperitoneally (i.p). A PE-10 catheter was placed in the right femoral artery, for arterial blood sampling, and the jugular vein, for i.v. infusion. After removal of one kidney, a stretched elongated PE-10 catheter was placed in a retrograde fashion in the suprarenal artery of the other kidney for intra-arterial infusion, as described in chapter 2. Because of marked anatomical differences between the blood supply of the right vs. the left kidney (see later), infusion studies were done in the left as well as in the right kidney.

To provide a patent airway the trachea was cannulated. The body

temperature was measured rectally and maintained at 37.5°C with a thermostatically controlled heat lamp. A PE-10 catheter was placed in the bladder for urine sampling. An electromagnetic flow probe (internal diameter 0.7 mm, Skalar, The Netherlands) was placed around the renal artery of the cannulated kidney (fig. 6.1) for renal blood flow measurements. The flow signals were measured with a sine-wave flow-meter (MDL 400, Skalar, The Netherlands) and recorded on a Grass Polygraph.

Fig. 6.1 Scheme of the experimental set-up, infusion and sampling protocol. Arrows indicate the time of blood sampling.

6.2.2 Experimental protocol

Cr EDTA and IHA (Radiochemical Centre, Amersham, England; product code Cr EDTA: CJ 13P, specific activity 1-2 mCi/mg Cr, and product code IHA: IM.315P, specific activity 10-50 mCi/mg I), dissolved in isotonic saline, were infused simultaneously at a constant rate (50 ul/min) during 120 minutes either into the jugular vein or into the suprarenal artery of the left or right kidney. In preliminary experiments it was found that steady state was achieved within 90 minutes of infusion. Renal blood flow was recorded during the entire experiment. Systemic arterial blood samples (100 ul) were taken at $t=90, 105, 120$ min (steady state). Urine was collected over 10 minutes periods at $t=85-95, 100-110$ and $110-120$ minutes. Blood and urine samples were collected in preweighed vessels. The concentrations of Cr EDTA and IHA in the blood and urine samples were determined in a LKB Wallace Universal Gamma Counter with overlap correction.

6.2.3 Composition of the experimental groups

The pharmacokinetics of both substances were studied during i.v. infusion of Cr EDTA (2.5 nCi/min) and IHA (10 nCi/min) in a left nephrectomized group. In a right nephrectomized group, 2.5 nCi/min of Cr EDTA and 40 nCi/min of IHA were infused intravenously.

Intra-arterial infusions of Cr EDTA (2.5 nCi/min) and IHA (10 nCi/min) were performed in a left and right nephrectomized group (right kidney and left kidney experiments, respectively). As hippuric acid is eliminated via an active tubular process subject to saturation, this substance was also infused at a rate of 40 nCi/min into both kidneys, to study a possible dose-dependency of the IHA clearance.

The mean of the three arterial blood and urine samples of each rat were taken for calculation. Total renal blood flow of each individual rat was calculated as the mean of the flow values measured in each period of urine sampling.

6.2.4 Statistical analysis

All groups were composed of 7-8 animals. Data are presented as mean $\pm$ SEM. Data were evaluated by analysis of variance. Significant

differences between the various groups were determined by a modified t-test. Significance was defined at the $p < 0.05$ level.

6.3 RESULTS

A summary of the measured parameters is given in table 6.1. As may be seen there, the mean renal blood flows in all experimental groups were between 7.8 and 9.8 ml/min for the left and between 9.9 and 10.7 ml/min for the right kidney. Due to the first pass effect of renal extraction, local infusion reduced the systemic availability of the compounds. The ratio of the systemic Cr EDTA concentrations (C_{Sia}/C_{Siv}) was 0.74 for the right kidney and 0.62 for the left kidney. For IHA, this ratio was 0.15 during its administration via the right kidney (infusion rate 10 nCi/min). For the left kidney, this amounted to 0.26, infusing 40 nCi/min. The urinary excretions of the compounds were comparable to the actual infusion rates, confirming a steady-state situation.

There were no differences between the Cr EDTA steady-state concentrations perfusing the kidney (i.e. C_T) whether the compound was infused systemically or locally. For IHA, a significant ($p = 0.03$) difference was observed between the arterial concentrations entering the left kidney for the systemic and local administrations at the highest infusion rate (table 6.1).

Pharmacokinetic constants estimated from the experiments are presented in table 6.2. For the right kidney, the route of administration (i.a. or i.v.) did not influence the systemic clearance of Cr EDTA (3.2 ± 0.1 vs. 3.5 ± 0.2 ml/min for i.a. and i.v. infusion respectively) nor of IHA (8.3 ± 0.3 and 7.9 ± 0.3 ml/min following the infusion of 10 nCi/min i.a. and i.v., respectively; 8.7 ± 0.3 ml/min for the i.a. infusion of 40 nCi/min). Also for the left kidney, systemic clearance of Cr EDTA was independent of the route of administration: 3.4 ± 0.3 and 3.5 ± 0.2 ml/min for i.v. and i.a. infusion, respectively. The administration of IHA i.a. at a rate of 40 nCi/min showed a lower blood clearance in comparison to the clearance found for systemic infusion; 6.0 ± 1.0 vs. 9.0 ± 0.3 ml/min ($p < 0.01$). The clearance observed for IHA

Table 6.1: Summary of all measured parameters in the various experimental groups (mean $\pm$ SEM; n=7-8).

Left kidney										Right kidney									
51Cr EDTA					125IHA					51Cr EDTA					125IHA				
Infusion rate (cpm/min)		0.5x10 ⁴		2x10 ⁴		8x10 ⁴				0.5x10 ⁴		2x10 ⁴		8x10 ⁴					
Route of administration		ia		iv		ia		iv		ia		iv		ia		iv			
Q _T	7.8±1.0	9.8±1.1	8.9±0.7	-	7.8±1.0	9.8±1.1	9.9±0.3	10.7±0.4	9.9±0.3	10.7±0.4	10.3±0.2	-							
ml/min																			
C _g x10 ²	8.9±0.9	14.2±0.6	4.2±0.7	-	23.4±4.8 ^b	89 ±5.7	10.9±0.8	14.8±0.4	4.0±0.7	26 ±2	17.7±3	-							
cpm/ml																			
C _T x10 ²	15.3±0.7	14.2±0.6	27.4±2.7	-	148±22 ^a	89 ±5.7	16.2±1	14.8±0.6	23.8±0.6	26 ±2	96 ±4.2	-							
cpm/ml																			
U x10 ⁴	0.5±0.02	0.5±0.03	2.3±0.2	-	7.2±0.4	8 ±0.4	0.5±0.03	0.5±0.02	2.1±0.1	2.0±0.03	8.6±0.3	-							
cpm/mln																			
C _T ia/C _T iv	1.1				1.7		1.1		0.9										
C _S ia/C _S iv	0.62				0.26		0.74		0.15										

^ap<0.05; ^bp<0.01 vs. intravenous delivery.

ia = intrarenal administration; iv = intravenous administration; Q_T = renal blood flow; C_S = systemic arterial concentration; C_T = concentration in the renal artery; U = urinary excretion rate; C_Tia/C_Tiv = regional advantage; C_Sia/C_Siv = systemic advantage; Cl = clearance; E = extraction ratio.

infused i.a. at a rate of 10 nCi/min was not different from the clearance found for the systemic administration.

The clearances calculated from the urinary excretion rate ($Cl = U/C_T$) showed the same pattern as described above for the clearances calculated by $Cl = inf/C_T$ (table 6.2).

The renal extraction ratios of both substances ($E_T = U/C_T \cdot Q_T$) are also depicted in table 6.2. For Cr EDTA, this parameter was almost equal, i.e. 0.3 (0.28-0.35) in all experimental groups. For the right kidney, the extraction ratio of IHA (10 nCi/min) was slightly higher during local infusion than during i.v. infusion: 0.9 vs. 0.75. However, upon infusion of 40 nCi/min of IHA directly into the left kidney, the extraction ratio fell to 0.64 ± 0.03 which differed significantly from the extraction ratio during the systemic infusion of the same dose (0.89 ± 0.04 ; $p < 0.01$) and from that during intrarenal infusion of the lower dose (0.92 ± 0.04 ; $p < 0.01$).

6.4 DISCUSSION

We compared the pharmacokinetics of intrarenal administration of a high (IHA) and a low (Cr EDTA) renal clearance substance with those of their systemic administration. In accordance to what should be expected, intrarenal administration, in the right as well as in the left kidney, reduced the systemic availability of the compounds due to the first pass effect of elimination of the kidney. The extent of the reduction was in close agreement to $1 - E_T$, where the extraction ratio E_T was obtained from the urinary excretion rate (table 6.2). Differences were observed between the left and right kidney for the extraction of IHA. The right kidney showed an equal extraction for IHA independent of the route of infusion and the infusion rate. The left kidney showed a reduced extraction (-30%) following intrarenal administration of the highest IHA amount compared to its systemic administration or to its intrarenal administration at a 4-fold lower IHA infusion rate. Concomitant with the reduced IHA extraction, the IHA clearance decreased from 9 to 6 ml/min. The reduced clearance can partly be explained by the lower renal blood flow (-20%) observed in

Table 6.2: Summary of the calculated parameters in the various experimental groups (mean $\pm$ SEM; n=7-8).

	Left kidney						Right kidney					
	^{51}Cr EDTA			^{125}I HA			^{51}Cr EDTA			^{125}I HA		
	Infusion rate (cpm/min)			2×10^4			0.5×10^4			2×10^4		
	Route of administration											
Cl = Inf/ C_T ml/min	1a	iv		1a	iv	1a	iv	1a	iv	1a	iv	
	3.4 $\pm$ 0.4	3.5 $\pm$ 0.2		8.0 $\pm$ 0.6	-	6.0 $\pm$ 1.0 ^b	9.0 $\pm$ 0.3			3.2 $\pm$ 0.1	3.5 $\pm$ 0.2	
Cl = U/ C_T ml/min	2.8 $\pm$ 0.2	3.3 $\pm$ 0.2		8.5 $\pm$ 0.9	-	5.0 $\pm$ 0.8 ^b	8.7 $\pm$ 0.5			2.9 $\pm$ 0.1	3.2 $\pm$ 0.1	
E = U/ C_T · Q_T	0.35 $\pm$ 0.02	0.33 $\pm$ 0.01		0.92 $\pm$ 0.04	-	0.64 $\pm$ 0.03 ^b	0.89 $\pm$ 0.04			0.29 $\pm$ 0.01	0.28 $\pm$ 0.02	
										0.91 $\pm$ 0.06	0.75 $\pm$ 0.05	
												-

^b p 0.01.

For abbreviations, see table 6.1.

that experimental group, i.e. 7.8 ml/min (the other right kidney groups showed a renal blood flow of 8.9 and 9.8 ml/min; table 6.1). Saturation of elimination processes could be a suitable explanation for this reduced extraction. This, however, would suggest that there was a difference in the capacity of extraction between the left and right kidney. The extraction ratios of IHA in the left kidney during systemic infusion of 40 nCi/min and local infusion of 10 nCi/min were comparable with the extraction ratios observed in the right kidney during systemic and local infusion of IHA. This indicates that the overall extraction capacity in both kidneys was almost similar.

Fig. 6.2 Slices of the left kidney after injection of Evans blue via the suprarenal artery.

Apparently, a local (dose-dependent) phenomenon present only in the left kidney attributes to the observed saturation during i.a. infusion of IHA at the highest dose. We made a remarkable observation that might explain our findings; infusion of Evans blue via the suprarenal artery (i.e. the technique used throughout our experiments) into the left kidney showed that only parts of the kidney were colored (fig. 6.2). In contrast, right kidney preparations showed a homogenous distribution of the dye. The combination of laminar flow patterns and the short distance between the suprarenal entrance and the first branch of the renal artery, preventing a thorough mixing (fig. 6.3), explains this inhomogenous distribution. As only a fraction of the renal blood flow receives the compound, the part of the kidney perfused by it receives high concentrations and the tubular excretion process might become saturated locally. A lower extraction will be the result.

Fig. 6.3 The length (mm) of the renal artery between the aorta and the suprarenal artery at the right (a) and the left side (c) and the length of the renal artery between the suprarenal artery and its first branch to the kidney at the left (b) and the right side (d) measured in 10 WKY rats (mean $\pm$ SEM).

Non-linear pharmacokinetics have been shown to limit the advantages of 5-fluorouracil administration via the hepatic artery (Boublil et al, 1985). Other implications of saturation kinetics during local drug administration have been discussed recently (Smits and Thijssen, 1986).

As predicted by theory, chronic intrarenal drug infusion has no advantage if it is intended to reach higher local drug concentrations: steady-state concentrations in the renal artery were independent of the route of administration (table 6.1) with the exception of the high IHA infusion in the left kidney.

In summary, our experiments affirm the theoretical considerations about the pharmacokinetic advantages of local drug administration, i.e. advantages are obtained if either the target organ has a high extraction ratio for the drug or the systemic clearance of the drug is high with respect to the blood flow through the organ. Deviations from theoretical predictions can be expected because of saturation kinetics. This study shows that an inhomogeneous flow distribution can cause localized saturation kinetics in the target organ. Since renal size and vascularisation could be a function of genetic lines (McCurry et al, 1981; Mulvany and Korsgaard, 1983) care has to be taken to extrapolate our findings to other rat strains or other species.

7. LOCAL DRUG ADMINISTRATION: KINETIC EVALUATION OF LONG-TERM INTRARENAL AND SYSTEMIC INFUSION OF PROPRANOLOL IN CONSCIOUS WISTAR RATS

7.1 INTRODUCTION

In the previous chapter, it was shown that intrarenal administration of a drug cleared by the target organ reduces the systemic drug availability following the first pass effect of the drug by the target organ. Increases in regional drug concentration shall not be achieved.

In this study the suitability of intrarenal drug administration to increase the drug concentration in the kidney as compared to systemic drug administration will be investigated. In experimental pharmacology, intrarenal drug administration is an often applied instrument to study the direct effects of drugs on the kidney (Smits et al, 1986; Kleinjans et al, 1983). In experimental surgery it is used to treat the immunoresponse after kidney transplantation (Ruers et al, 1986).

As may be obvious from the theoretical considerations on the advantages of local over systemic drug administration (chapter 1), the ratio of the systemic clearance and the blood flow through the target organ indicates the increase of the concentration in the target organ during local drug administration. Applying this in the model of intrarenal drug administration a minimal regional advantage is to be expected, because of the relatively high blood flow through the kidney. Propranolol was used as a model substance since this drug has a high hepatic clearance (14-28 ml/min) in the rat.

7.2 EXPERIMENTAL SECTION

dl-Propranolol-HCl (Sigma, St. Louis, MO, USA) solved in isotonic saline was used for intrarenal and subcutaneous infusion.

7.2.1 Animal experiments

Male inbred Wistar-Kyoto rats, 250-300 g were used. The rats were on standard food and tap water. For intrarenal infusion, an elongated PE-10 catheter was introduced in a retrograde fashion in the right suprarenal artery as described in chapter 2. The other end was guided subcutaneously to the neck and connected to an osmotic minipump, (Model 2001, Alzet, Palo Alto, Ca, USA). Subcutaneous delivery of propranolol was performed by subcutaneous placing of a PE-10 catheter, attached to an osmotic minipump. The contralateral kidney was removed. Propranolol concentration was determined in plasma and kidney homogenates following the procedure described in chapter 2.3.

7.2.2 Experimental protocol

Propranolol was infused either intrarenally (i.r.) or subcutaneously (s.c.) at a constant rate of 1 mg/kg/d during 5 days. At day 5 the animals were euthanized and blood and kidney removed.

7.2.3 Statistical analysis

Data are presented as mean $\pm$ SEM. Significances were calculated using Student's t-tests. Differences were regarded to be statistically significant if $p < 0.05$.

7.3 RESULTS

The systemic plasma concentrations after intrarenal and subcutaneous administration of propranolol are shown in table 7.1. At steady state the plasma concentrations were comparable: 28.2 ± 3.4 ng/ml at i.r. and 20.0 ± 3.0 ng/ml at s.c. infusion. The concentration of propranolol in the kidney was 104 ± 6.0 ug/g at i.r. and 53 ± 5.4 ug/g at systemic administration. There was no systemic advantage of intrarenal over systemic propranolol administration. The regional advantage i.e. the ratio of the concentration of propranolol in the kidney during i.r. and s.c. infusion was 2.0.

The systemic plasma clearance, calculated by the quotient of the

infusion rate (ng/min) and the systemic concentration (ng/ml) was 6.5 ± 1.0 and 9.3 ± 1.5 ml/min at i.r. and s.c. infusion, respectively.

Table 7.1: Mean $\pm$ SEM (n=4) of the measured and calculated parameters during long-term constant rate infusion of propranolol (1 mg/kg/d) either intrarenally or subcutaneously in conscious male Wistar-Kyoto rats.

	Intrarenal	Subcutaneous
Systemic propranolol concentration (ng/ml)	28.2 ± 3.4	20.0 ± 3.0
Propranolol concentration in the kidney (ng/g)	104 ± 6.0^a	53.0 ± 5.4
Plasma clearance (ml/min)	6.5 ± 1.0	9.3 ± 1.5

a $p < 0.05$ as compared to subcutaneous infusion.

7.4 DISCUSSION

In this study, the model substance propranolol was infused intrarenally and subcutaneously at a constant rate of infusion during 5 days in conscious rats. It was found that intrarenal infusion of propranolol did not lower systemic concentrations as compared to systemic administration of propranolol. The drug concentration in the infused kidney, however, was two fold higher during intrarenal infusion as compared to systemic administration.

Long-term direct administration of propranolol into the kidney seems at first sight an obvious approach to investigate hemodynamics after specific long-term suppression of renal beta-adrenoceptors. The choice for target organ directed drug administration is then based upon the assumption that intrarenal delivery of the drug reduces the systemic drug concentrations and thus systemic effects and/or enhances the renal drug concentrations as compared to systemic administration (chapter 1). Kinetic analysis of this experiment, as presented in this study, indicates 1) that no systemic advantage is to be expected since propranolol is not cleared by the kidney (Iwamoto et al, 1985) and 2)

that the regional advantage of intrarenal over systemic propranolol administration is limited because of the relative high blood flow of the kidney (10 ml/min). The regional advantage assuming a propranolol clearance of about 14 ml/min after a single dose of 1 mg/kg s.c. (Smits et al, 1979), would be about 2.4. However, data presented here, show that the propranolol clearance after long term administration is decreased to 7-9 ml/min. This low clearance value decreases the regional advantage even more.

In summary, this study clearly demonstrates that long-term infusion of propranolol into the renal artery increases the propranolol concentration in the kidney only slightly which is in conformity with the theoretical predictions. It seriously questions the kinetic advantage of long-term intrarenal over systemic administration of propranolol.

8. LOCAL DRUG ADMINISTRATION: KINETIC EVALUATION OF LOCAL AND SYSTEMIC INFUSION OF THE HIGH HEPATIC CLEARANCE SUBSTANCE PROPRANOLOL IN RAT CARDIAC GRAFTS

8.1 INTRODUCTION

The local treatment of the immuneresponse after a tissue transplantation is a new and promising application of local drug administration. Recently, Ruers et al (1986) reported a beneficial effect of intrarenal over intravenous administration of prednisolone in the treatment of rat renal grafts. Intrarenally administered prednisolone (4 mg/kg/day) significantly prolonged the graft survival time, whereas intravenous administration of the same dose was ineffective.

The cardiac graft is used also in experimental surgery, to study the immuneresponse after tissue transplantation. In this model the direction of the blood flow in the transplanted aorta is artificially changed and the venous outflow of the transplanted heart drains entirely into the portal vein of the acceptor animal (fig. 8.1), putting the liver immediately downstreams from the transplanted heart. This experiment thus creates optimal kinetic conditions for high-advantage local administration of immunotherapeutic drugs that have a high hepatic clearance. The systemic concentration of such a drug when administered locally to the transplanted heart, should be very small. This because of the high first pass effect of elimination of the liver (Pond and Tozer, 1984). Low systemic concentrations should reduce the systemic side-effects, which often are limiting factors in immunosuppressive therapy.

In the present study, the actual kinetic advantages of local over systemic drug administration in the cardiac graft are determined. Propranolol was chosen as the representative of the kinetic class of substances with a high hepatic clearance (14-28 ml/min, chapter 4). The blood flow to the target organ was measured, because it is inversely related to the regional advantage of local over systemic drug administration. Flow was measured by means of an electromagnetic flowmeter placed on the artery supplying the cardiac graft.

8.2 EXPERIMENTAL SECTION

8.2.1 Materials

dl-Propranolol-HCl (Sigma, St. Louis, MO, USA) in isotonic saline (0.16 mg/ml) was used for direct infusion into the coronary arteries (i.c.) of the transplanted heart. Tritium-labeled l-propranolol (specific activity 18.5 Ci/mmol, NEN, Boston, MA, USA) was used for intravenous (i.v.) infusion.

8.2.2 Animal experiments

Male inbred Brown Norway and Lewis rats were obtained from the Central Animal Facilities of our University. Rats of 250-300 gr were used in all experiments. The rats were maintained on standard lab food and tap water available ad libitum. For these experiments rats with cardiac grafts were kindly provided by the Department of Surgery. The heart of a Brown-Norway rat was heterotopically transplanted into a Lewis rat, whereas the heart of the recipient animal remained functionally in situ (Ruers et al, 1987). Briefly, the aorta of the donor heart was anastomosed on the abdominal aorta of the acceptor and the donor heart's pulmonary artery was connected to the portal vein of the acceptor. The transplanted heart was placed in the right upper quadrant of the abdomen of the acceptor. All surgery was done under pentobarbital anesthesia.

A flow scheme of the status quo during the infusion of propranolol is shown in fig. 8.1: a fraction of the blood in the abdominal aorta reaches the aorta of the donor heart. It flows in a retrograde fashion through the aortic arch of the donor. Blood entering the heart flows into the coronary vessels in an orthograde fashion. The cavity of the left ventricle does not receive blood as the aortic valves are closed due to retrograde blood flow. The venous blood of the donor heart flows via the coronary sinus, right atrium, right ventricle and pulmonary artery into the portal vein and enters the liver.

The carotid artery of the implanted aorta was cannulated with a PE-10 catheter (see also chapter 2) for local infusion of propranolol. Two days after the heart transplantation, the rats were used for the propranolol experiments. At this time, animals had recovered from

Fig. 8.1: Flow diagram of the rat cardiac graft.

CL=clearance; E_t =extraction ratio; i.v.=intravenous administration; i.c.=intracoronary administration; C_s =systemic arterial concentration at steady state; C_t =concentration in the artery supplying the target organ; Q_s =cardiac output; Q_t =blood flow in the artery supplying the target organ.

operation and graft survival was optimal. In the experiments, a bolus injection of pentobarbital (30 mg/kg in isotonic saline) was given intraperitoneally for anesthesia. Anesthesia was maintained by repeated i.p. injections of 6 mg/kg of the pentobarbital solution every 60 min. The trachea was cannulated to provide a patent airway. PE-10 catheters were placed in the left femoral artery and vein for systemic arterial blood sampling and systemic intravenous infusion respectively. For measurements of the blood flow to the cardiac graft, an electromagnetic flow probe (internal diameter 1.8 mm, Skalar, Delft, The Netherlands) was placed around the transplanted aorta. The flow signals were measured with a sine-wave flowmeter (type MDL 400, Skalar, Delft, The Netherlands) and recorded on a Grass polygraph.

The body temperature was measured rectally and maintained at 37.5° C by a thermostatically controlled heat lamp.

8.2.3 Drug administration and sampling

dl-Propranolol was administered locally via the carotid catheter by a constant rate infusion of 4 ug/min. At the same time ^3H -l-propranolol was infused systemically by constant rate infusion of 115,000 dpm/min (i.e. 0.73 ng/min). These infusion rates were chosen to minimize the use of labeled drug as well as to be able to detect any locally delivered drug systemically, that had to be performed by an analytical procedure. To prevent dehydration the infusion volumes were 25 ul/min at both infusion sites (Maddox et al, 1977). Blood samples (500 ul) were taken at $t=120, 150, 180$ min via the femoral arterial catheter and collected in heparinized vessels. The lost blood volume was replaced by blood from litter mates. After centrifugation the plasma was stored at -20°C until assayed. At the end of the experiment ($t=180$ min), the animal was euthanized with an overdose pentobarbital i.v. and the donor heart removed. After washing and weighing the hearts were also stored at -20°C .

8.2.4 Pharmacokinetic analysis

Comparisons between propranolol concentrations from local and intravenous delivery are made after correction for the difference in infusion rate. Preliminary experiments showed that steady state was reached after 120 min of infusion. The plasma and flow data of each individual animal were obtained as the mean of the 3 values at 120, 150, and 180 min. The systemic plasma clearance (Cl_S) of intravenously administered propranolol was calculated by $\text{Cl}_S \text{ (ml/min)} = \text{inf}/C_S$, where inf equals the infusion rate (ng/min) and C_S the systemic arterial steady-state plasma concentration (ng/ml). The steady-state plasma concentration of propranolol in the artery supplying the target organ (C_T ; ng/ml) was calculated by $C_T = C_S + \text{inf}/Q_T$, where Q_T equals the plasma flow through the target organ (ml/min). Since the plasma concentration of propranolol equals its blood concentration (Bianchetti et al, 1980) calculations were made using the values of the blood concentration and blood flow. The systemic plasma (blood) clearance was calculated by $\text{Cl}_S = (1-E_T) \cdot \text{inf}/C_S$, where E_T equals the extraction ratio of propranolol by the liver. $(1-E_T)$ represents the quotient of C_S derived from local and intravenous infusion, i.e. the systemic

advantage of local over systemic drug delivery (chapter 1). The regional advantage of local over systemic propranolol delivery is expressed by the quotient of the concentration in the target artery from locally (C_{Tic}) and intravenously (C_{Tiv}) administered propranolol. This quotient equals $(1-E_T) + Cl_S/Q_T$ (Appendix 8.5).

8.2.5 Statistical analysis

All data are presented as mean $\pm$ SEM. Differences between the routes of administration were compared using Student's t-test. The 0.05 level of probability was used as the level of significance.

8.3 RESULTS

Eight rats were prepared and all were technically suited and used for the experiments. The blood flow through the transplant was 7.2 ± 0.5 ml/min. All data are corrected for the difference in infusion rates between intracoronary and systemic administration. The systemic plasma concentrations after i.v. and i.c. administration of propranolol are shown in table 8.1. At steady state, the plasma concentrations of propranolol at intracoronary infusion were much lower than those at simultaneous i.v. administration: 20 ± 3.3 and 357 ± 16 ng/ml plasma respectively. This finding is concomitant with the first pass effect of elimination of propranolol by the liver. The plasma clearance of propranolol was about 11 ml/min.

Higher propranolol concentrations in the transplanted hearts were observed after systemic administration : 791 ± 46 and 637 ± 36 ng/g heart at i.v and i.c. administration, respectively. The differences however, were not statistically significant. The blood concentration of propranolol in the artery supplying the transplant ($C_T = C_S + inf/Q_T$) was 581 ± 38 ng/ml at i.c. infusion. C_T of the i.v. infused propranolol equaled C_S hence it was 357 ± 16 ng/ml blood.

The quotient of the systemic concentrations at i.c. and i.v. administration was 0.05 ± 0.01 . This quotient represents the reduction in systemic concentration during local as compared to systemic administration, the so called systemic advantage of local over systemic drug

administration and indicates the bio-availability ($F=1-E_T$) of propranolol. The increase in drug concentration in the artery supplying the cardiac graft during local as compared to systemic drug administration, the so-called regional advantage of local over systemic drug administration, expressed by the quotient of C_{Tic} and C_{Tiv} , was 1.60 ± 0.4 . The regional advantage calculated by the formula $(1-E_T) + Cl_S/Q_T$ was 1.65 ± 0.4 .

Table 8.1: Mean values $\pm$ SEM, n=8 of the measured and calculated parameters during local and systemic infusion of propranolol.
a: measured; b: calculated; *: corrected for the difference in infusion rate.

Route of administration	Intracoronary	Intravenous
Infusion rate ^a (ng/min)	4000	0.73 ($=1.8 \cdot 10^{-4} \times IC$)
Blood flow ^a (ml/min)		7.2 ± 0.5
Systemic plasma (blood) concentration ^a (ng/ml)	20.0 ± 3.3	0.07 ± 0.005 (357 ± 16)*
Concentration in the transplanted heart ^a (ng/g)	581 ± 38	791 ± 46 *
Blood concentration in the artery supplying the target organ ^b (ng/ml)	581 ± 38	357 ± 16 *
Systemic advantage ^a (C_{Sic}/C_{Siv})		0.054 ± 0.01
Regional advantage ^b (C_{Tiv}/C_{Tiv})		1.60 ± 0.4
Regional advantage ^b ($[1-E_T] + Cl_S/Q_T$)		1.65 ± 0.4
Plasma (blood) clearance ^b (ml/min)	11.6 ± 0.7	10.9 ± 0.7

8.4 DISCUSSION

In this study, the kinetics of a high hepatic clearance substance, propranolol, were investigated in rats in which a donor heart was transplanted, under controlled experimental conditions. The advantages of local over systemic administration were determined, to provide a pharmacokinetic base for local immunotherapy in this model.

The cardiac graft, used here, has a unique flow pattern which offers, in theory, ideal conditions for advantageous local drug delivery. In these rats the venous outflow of the transplanted hearts drains into the portal vein of the acceptor. Hence, before reaching the systemic circulation, all of the blood leaving the transplanted heart flows through the liver of the acceptor animal. This implies that drugs with a high cardiac extraction and/or high hepatic extraction will be cleared very efficiently before entering the systemic circulation.

The observed propranolol clearances of about 11 ml/min are in agreement with literature data on rat liver flow (see also chapter 4). These values therefore suggest that in spite of the changed blood supply to the liver, the clearance capacity of this organ is not affected significantly.

Propranolol was infused locally at a high dose (i.e. 4 ug/min). Tracer amounts were given systemically (0.73 ng/min). The ratio of the systemic concentrations of propranolol from local and systemic infusion was 0.05 indicating the liver extraction to be 95% of the propranolol influx. This value is comparable with the value obtained in normal rats (Iwamoto, 1985). Saturation of the extraction process in the liver because of the local administration of high doses of propranolol apparently did not occur (Suzuki et al, 1981).

The ratio of the concentration of propranolol in the artery supplying the transplanted heart during local and systemic administration shows that in this model only minor regional advantages can be achieved by administering the drug locally ($C_{Tic}/C_{Tiv}=1.62\pm0.4$). This is in agreement with a theoretical analysis of this experiment, which predicts a regional advantage of about 1.6 (Appendix 8.5). The concentrations of propranolol derived in the transplanted hearts, whether

the drug was given i.c. or i.v., were not different. Thus intracoronary administration of propranolol did not enhance the local tissue concentration. The relatively high flow to the target organ ($Q_b = 7.2 \pm 0.5$ ml/min) limits any possible regional advantage. In general, a limited regional advantage of local over systemic drug administration can be expected to hold true for target organs with relatively high flows, such as the kidney and the liver. In low flow organs, however, as for example the testis, it can be shown that a great regional advantage can be achieved by local administration of the kinetically proper drug (chapter 10).

In this study, the target organ was artificially placed in series with the liver. In nature, an organ like the lung may act as a clearance organ situated in series with a target organ as was demonstrated by the advantages of intrarenal over systemic administration of bradykinin (Wedlund et al, 1983; Smits and Brody, 1984). In other words, not only the extraction of the locally infused drug by the target organ but also the extraction of any organ in series with the target organ will influence the systemic advantage.

To summarize, the clearance capacity of the liver in our cardiac grafts was not reduced. Hence, the first pass effect of elimination of the liver yielded a brisk reduction of the systemic drug concentration during local as compared to systemic delivery. Thus, the systemic advantage was very high. However, the regional advantage was limited. These results provide an experimental pharmacokinetic basis for local immunotherapy: by local delivery of an immunosuppressive agent with a high hepatic clearance it should be possible to reach therapeutic concentrations in the transplanted hearts but to reduce the systemic side-effects drastically, as compared to systemic delivery of the same drug at the same dose. Currently, experiments are being undertaken to extend these observations to the class of immunotherapeutic drugs.

The concentration in a systemic artery at steady state during systemic drug administration (C_S) is calculated by

$$C_S = \text{inf}/Cl \quad (1)$$

where inf equals the infusion rate and Cl the clearance of the drug. Here, C_S equals the concentration in the artery supplying the target organ (C_T).

During local (i.c.) infusion, C_S is defined by

$$C_S = \frac{(1-E_T) \cdot \text{inf}}{Cl} \quad (2)$$

where E_T represents the extraction ratio of the drug by (the target organ and) the liver. Here, the concentration in the artery supplying the target organ (C_T) is defined by

$$C_T = \frac{1-E_T \cdot \text{inf}}{Cl} + \frac{\text{inf}}{Q_T} \quad (3)$$

where Q_T equals the blood flow through the target organ.

The regional advantage of local over systemic drug delivery is expressed by the ratio of the concentration in the artery supplying the target organ at local (C_{Tic}) and systemic (C_{Tiv}) administration of the drug:

$$\frac{C_{Tiv}}{C_{Tic}} = \frac{\frac{(1-E_T) \cdot \text{inf}}{Cl} + \frac{\text{inf}}{Cl}}{\frac{\text{inf}}{Cl}} = (1-E_T) + \frac{Cl}{Q_T} \quad (4)$$

The systemic advantage of local over systemic drug delivery is expressed by the ratio of the systemic concentration at local (C_{Sic}) and systemic (C_{Siv}) administration of the drug:

$$\frac{C_{Sic}}{C_{Siv}} = \frac{\frac{(1-E_T) \cdot \text{inf}}{Cl}}{\frac{\text{inf}}{Cl}} = 1-E_T \quad (5)$$

9. LOCAL DRUG ADMINISTRATION: KINETIC EVALUATION OF INTRAHEPATIC AND INTRAVENOUS INFUSION OF S-ACENOCOUMAROL

9.1 INTRODUCTION

In a previous study (chapter 2 and Daemen et al, 1986a), the hepatic clearance of S-acenocoumarol (AC) was found to be dose-dependent. The hepatic clearance of AC was high at infusion rates below 0.6 ug/min and decreased at higher infusion rates. A saturable hepatic binding site of low capacity but high affinity was observed, which could account for this dose dependency.

According to theoretical considerations a regional advantage of direct intrahepatic administration of AC over its systemic administration is not to be expected, since the substance is eliminated by the target organ only. This latter characteristic also determines the systemic advantage. The extraction ratio of AC is small. Therefore, the systemic advantage will be limited. In a simulation study (Smits and Thijssen, 1986) on the advantages of local over systemic drug administration of a drug that is cleared by the target organ, it was shown that higher target organ concentrations can be achieved by the local administration of a drug as compared to its systemic administration, especially in the interval before steady-state conditions are achieved. Using the liver as a target organ this would imply that hepatic binding sites of low capacity but high affinity can become saturated by the local but not by the systemic infusion of AC at an infusion rate even below 0.6 ug/min. Although no regional advantage will be obtained at ultimate steady state, in pre-steady-state conditions higher local concentrations will be achieved. A saturated binding site would decrease the clearance of AC (see also chapter 2).

To examine these latter hypotheses, AC was administered via the systemic route and via the hepatic arterial route at an infusion rate of 0.5 ug/min in anesthetized rats. Liver AC concentrations were determined at different times in one animal with the biopsy technique (chapter 5).

9.2 EXPERIMENTAL SECTION

Male inbred Wistar Kyoto rats, weighing 250-300 g, were used. Before the experiment, the animals were on standard lab food and tap water. All experiments were performed in anesthetized animals. For anesthesia, 60 mg/kg pentobarbital was given intraperitoneally. The body temperature was controlled rectally and maintained at 37.5°C by a thermostatically controlled heat lamp. A PE-10 catheter was introduced in a retrograde fashion into the gastroduodenal artery (chapter 2) for hepatic arterial drug administration. Another PE-10 catheter was introduced into the femoral vein for intravenous drug administration as well as into the femoral artery for blood sampling. Liver biopsies were taken as described earlier (chapter 5).

9.2.1 Experimental protocol

In the first series of animals, the distribution of AC in the liver during its administration via the two routes was investigated. AC, dissolved in phosphate buffer, 0.1 M, pH 8.0, was given at a constant rate of infusion (0.5 ug/min) either via the femoral vein or the hepatic artery. Liver biopsies were taken at 30 min of infusion from the edge or the central part of each liver lobe.

To another series of animals (3 groups of 6 animals), AC was delivered also at an infusion rate of 0.5 ug/min by either one of the two routes. Here, liver biopsies from the edge and an arterial blood sample (100 ul) were taken at $t=15, 30, 60, 90, 120, 150$ and 180 min.

Samples were prepared for AC determination as described in chapter 2.

9.2.2 Statistical analysis

All data are presented as mean $\pm$ SEM (except for data in table 9.1). Data were evaluated by analysis of variance. Significant differences between the groups were determined by a modified t-test. Significance was defined at the $p<0.05$ level.

Table 9.1: Comparison between the concentration of AC (ug/g) in a biopsy taken from the centre (C) or from the edge of a liver (P) lobe, after 30 minutes of infusion, either systemically or intrahepatically.

Route of administration	Systemic				Intrahepatic			
Rat number	1		2		3		4	
Place of the biopsy	p	c	p	c	p	c	p	c
Left lateral lobe	0.70	0.76	0.78	0.87	0.35	0.33	0.34	0.39
Right lateral lobe	0.60	0.70	0.74	0.78	0.39	0.41	0.30	0.38
Median lobe left central part	0.60	0.69	0.78	0.73	0.38	0.44	0.43	0.40
Caudate lobe	0.65	0.71	0.75	0.80	0.38	0.39	0.37	0.43

9.3 RESULTS

9.3.1 Distribution of AC in the liver

In table 9.1, the hepatic concentrations of AC at different sites in the liver are depicted. Liver biopsies were taken from the periphery or the centre of the liver after 30 min of infusion (systemic or local). It can be seen that AC was evenly distributed in the liver. It can also be seen that at this time point, the hepatic AC concentration was dependent on the route of administration. At local administration the liver concentrations were about half of those at systemic administration.

9.3.2 Influence of the route of administration

The concentration time relations for AC in plasma and in liver tissue are depicted in fig. 9.1 and 9.2, respectively. As can be seen systemic plasma concentrations of AC were dependent on the route of administration. Plasma concentrations of AC administered intrahepatically were 25% lower than plasma concentrations during systemic administration ($p < 0.05$). The ultimate plateau concentration of AC in the

Fig. 9.1: Influence of the route of administration (systemic or intrahepatic) on the plasma concentration of AC ($\mu\text{g/ml}$) after a constant rate of infusion ($0.5 \mu\text{g/min}$) (mean $\pm$ SEM, $n=6$).

* $p < 0.05$ as compared to systemic infusion (0-180 min).

Fig. 9.2: Influence of the route of administration (systemic or intrahepatic) on the liver concentration of AC ($\mu\text{g/g}$) after a constant rate of infusion ($0.5 \mu\text{g/min}$).

* $p < 0.05$ as compared to systemic infusion (0-120 min).

liver (approximately 1 ug/g) was also dependent on the route of entry. Liver concentrations, however, were lower during local than during systemic AC administration in the time period before steady state was achieved.

9.4 DISCUSSION

As may be expected, systemic plasma concentrations of acenocoumarol were found to be dependent of the route of administration (fig. 9.1). Following the first pass effect of extraction by the liver AC plasma concentrations in the locally infused group were lower than in the systemically infused group. Concentrations of AC in liver biopsies taken at the same time from different sites in the liver indicate that AC is distributed homogeneously over the different liver lobes and that this distribution is independent of the route of administration (table 9.1). The outcome of preliminary experiments in which AC was administered via the portal vein using the same infusion protocol as above indicates that the systemic and hepatic concentrations at portal venous administration are comparable with those achieved by hepatic arterial infusion. These findings are in accordance with literature reports. The route of hepatic administration is known to influence the availability of high but not of low hepatic clearance substances (Shand et al, 1975; Øie and Huang, 1981; Ahmad et al, 1984; Didolkar et al, 1984). AC can be classified as a low hepatic clearance substance.

According to theoretical considerations on the advantages of local drug administration (chapter 1), no regional advantage of intra-hepatic over systemic AC administration may be expected at steady-state conditions. Indeed, as can be seen in fig. 9.2, no advantage was observed during hepatic arterial infusion of AC as compared to its systemic infusion. On the other hand, before steady-state conditions were achieved the drug concentration in the target organ should be higher during local than during systemic administration of the same drug at the same dose (Smits and Thijssen, 1986). This, however, was not observed in our experiments. We found that although the ultimate

steady-state levels were comparable, the liver did bind AC delivered via the systemic route faster than AC administered via the local route. This result suggests that the locally delivered AC is not as available for hepatic binding nor for hepatic elimination as systemically delivered AC. The preference of the hepatic binding site for systemically delivered AC might be due to a difference in the distribution of AC in the several intrahepatic spaces or to shunting of locally administered AC. Shunting is unlikely, since plasma concentrations of local administration were lower than those of AC during systemic administration. A third explanation might be that locally administered AC is not available for binding but only for elimination.

To summarize intrahepatic infusion of AC lowers the systemic drug concentrations as compared to systemic infusion, a finding concomitant with theoretical predictions. A regional advantage of intrahepatic over systemic AC infusion can not be achieved. Initially intrahepatic infusion even lowers the hepatic concentration of AC.

10. LOCAL DRUG ADMINISTRATION: KINETIC EVALUATION OF LONG-TERM INTRARENAL AND INTRATESTICULAR INFUSION OF S-ACENOCOUMAROL IN CONSCIOUS RATS

10.1 INTRODUCTION

This study was performed to substantiate the theoretical considerations on the advantages of local drug administration, by measuring the actual regional advantage during intratesticular infusion of S-acenocoumarol (AC) as compared to systemic administration and to provide a kinetic basis for the effects of intratesticularly administered AC on the vitamin K dependent systems in the testis (chapter 11).

By direct infusion of AC, at the same dose, into one kidney the effect of an increase of the blood flow of the target organ on the regional advantage was studied. In a physiological pharmacokinetic flow scheme the kidney and the testis differ in amount of flow by a factor 50 (about 10 and 0.2 ml/min respectively; Nishiyama et al, 1976).

The effect of an increase of the systemic clearance on the regional advantage was investigated by inducing the hepatic AC clearance with phenobarbital co-treatment.

10.2 EXPERIMENTAL SECTION

Wistar rats were purchased from Winkelman (Borchen, West Germany). Before and throughout the whole experiment the rats were on standard food and tap water.

10.2.1 Intratesticular administration

Male rats, aging 14-16 weeks, were used. Under light ether anesthesia, a femoral artery and a testicular catheter were placed, for blood sampling and drug administration respectively. The testicular catheter was placed as described in chapter 2.

Experimental protocol

AC, solved in 0.1 M phosphate buffer, pH 8.0, was infused at a constant rate of 1 ug/hr via an osmotic minipump (Model 2001, Alzet, Palo Alto, Ca, USA) during five days. Preliminary experiments showed that AC administered at a rate of 1 ug/hr subcutaneously for several days caused no bleeding complications. Arterial blood samples (0.5 ml) were taken on the third, fourth and fifth day of infusion. On the fifth day the animals were euthanized. Blood, liver, both testes and kidneys were removed.

Another group of animals received phenobarbital (1 mg/ml) in their drinking water, starting 3 days before the experiment, to increase the hepatic clearance of AC, and followed the protocol as above (intratesticular AC administration (1 ug/hr) during 5 days).

10.2.2 Intrarenal administration

AC (n=8) was infused directly into the right kidney of male Wistar rats, aging 14-16 weeks. For this purpose, an elongated PE-10 catheter was introduced in a retrograde fashion in the right suprarenal artery under a light ether anesthesia following the procedure described in chapter 2. The other end of the catheter was tunneled to the neck and attached to an osmotic minipump (Model 2001, Alzet, Palo Alto, Ca, USA), filled with AC (1 ug/ μ l). At the fifth day of infusion the animals were sacrificed and kidneys, testes, liver and blood removed.

10.2.3 Analytical procedures

After adding 0.1 M trisodium citrate (citrate : blood volume = 1:9) the blood samples were centrifugated and the plasma stored at -20°C until assayed. The removed organs were rinsed and homogenized in three volumes (w/v) of ice-cold buffer, 0.02 M tris HCl, 0.15 M KCl, pH 7.4, using the Potter technique.

AC concentrations were determined in plasma, liver, testis and kidney homogenate on HPLC according to the procedure described by Thijssen et al (1983b) and in chapter 2. Plasma coagulation activity was measured using the prothrombin time.

10.2.4 Statistical analysis

Data are presented as mean $\pm$ SEM. Groups were composed of 7-9 animals. Significances were calculated using Student's t-tests. Differences were regarded to be statistically significant if $p < 0.05$.

Fig. 10.1: Plasma concentrations of AC during its intratesticular infusion for 5 days with (●) or without (○) phenobarbital co-treatment (mean $\pm$ SEM, $n=9$).

10.3 RESULTS

10.3.1 Intratesticular administration

The plasma concentrations of AC on the third, fourth and fifth day of infusion are depicted in fig. 10.1. AC concentrations were between 5 and 6 ng/ml in normal rats, while co-treatment with phenobarbital reduced the plasma concentration to 3-4 ng/ml. As the half-life time of elimination of AC in the rat is short (approximately 24 min) steady state conditions during constant rate infusions are achieved within the first day of infusion.

On the fifth day, the liver contained 360 ± 30 ng/g, the kidneys 31 ± 2 ng/g AC (table 10.1). In the left testis 771 ± 90 ng/g AC was found, while the right testis contained only 26 ± 3 ng/g. Plasma clear-

Table 10.1: Mean $\pm$ SEM of the values of all measured and calculated parameters during intratesticular (with or without phenobarbital co-treatment) and intrarenal infusion of AC for 5 days (n=7-9). a: $p < 0.05$; b: $p < 0.01$ (as compared to intratesticular infusion); c: Ratio of the concentration in the target organ and its contralateral organ; d: Q_T (testis) = 0.2 ml/min, (Nishiyama et al, 1976); N: normal; n.d.: not determined.

	Intratesti- cular	Intratesti- cular + pheno- barbital	Intrarenal
Infusion rate (ug/hr)	1	1	1
AC concentration in			
plasma (ng/ml)	5.0 $\pm$ 1	3.6 $\pm$ 0.5 ^a	5.5 $\pm$ 1
liver (ng/g)	360 $\pm$ 30	340 $\pm$ 25	289 $\pm$ 17
left testis (ng/g)	771 $\pm$ 90	860 $\pm$ 30	25 $\pm$ 4
right testis (ng/g)	26 $\pm$ 3	12 $\pm$ 0.6 ^a	25 $\pm$ 4
right kidney (ng/g)	31 $\pm$ 2	n.d.	30 $\pm$ 2
left kidney (ng/g)	31 $\pm$ 2	n.d.	34 $\pm$ 3
Prothrombin time	N	N	N
Plasma clearance (ml/min)	3.4 $\pm$ 0.4	4.6 $\pm$ 0.3 ^b	3.0 $\pm$ 0.5
Liver to plasma concentration ratio	72 $\pm$ 8.0	98 $\pm$ 5.0 ^a	51 $\pm$ 7.0
Regional advantage ^c (measured)	22 $\pm$ 2.0	29 $\pm$ 2.0 ^a	0.9 $\pm$ 0.05
Regional advantage (calculated by $1 + Cl/Q_T$) ^d	18.0	23	1.3

ance was 3.4 $\pm$ 0.4 ml/min. Plasma coagulation activity was not affected.

Phenobarbital increased the hepatic clearance of AC to 4.6 $\pm$ 0.3 ml/min. It had no effect on the concentration in the left testis: 860 $\pm$ 30 ng/g. In the right testis AC concentration was reduced to 12 $\pm$ 0.6 ng/g. The hepatic AC content was comparable to the control experiment. Plasma coagulation activity was normal.

10.3.2 Intrarenal administration

As can be seen in table 10.1, the plasma and liver concentrations of AC on the fifth day of infusion were 5.5 ± 1 ng/ml and 289 ± 1.7 ng/g respectively. In the right kidney i.e. the target organ, 29.6 ± 2 ng/g of AC was found and in the left kidney 33.6 ± 3 ng/g. The concentrations in the testes were 25 ± 4 ng/g. Plasma clearance was 3.0 ± 0.5 ml/min. Again plasma coagulation activity was not affected.

10.4 DISCUSSION

Direct infusion into the testis of S-acenocoumarol, a drug which is cleared only by hepatic biotransformation, increased the drug concentration in the infused testis about 20-fold as compared to the contralateral, systemically infused testis. The infusion protocol used in this study was the same as that in the study on the effects of direct intratesticular infusions of AC on vitamin K dependent systems in the testis of the rat (chapter 11). Therefore, the results of this study provide a kinetic base for the effects observed in the latter study. The calculated values for the regional advantage during intratesticular and intrarenal infusion were comparable with the observed values (table 10.1). In this study, the regional advantage is defined by the ratio of the concentration in the directly infused organ and its contralateral, systemically infused organ. We studied the effects of an enhanced drug clearance on the regional advantage during intratesticular infusion of AC by adding phenobarbital (1 mg/ml) to the drinking water. Phenobarbital co-treatment increased the hepatic clearance about 35% while the observed regional advantage increased about 32%, as evidenced by a decreased concentration in the systemically infused testis.

A comparison of the regional advantages observed during intratesticular and intrarenal infusion of AC clarifies the importance of a low blood flow of the target organ for achieving high regional advantages: a high regional advantage achieved with intratesticular infusion vs non with intrarenal infusion.

The high regional advantage observed during intratesticular in-

fusion enabled us to reduce the infused dose in such a way that, on the one hand relatively high drug concentrations were achieved at the target site, while, on the other hand, the systemic concentrations were very low. In other words, the regional advantage was used to lower the dose and thus the systemic bleeding complications.

In summary, the results of these experiments give experimental support to the theories on local drug administration. Only the combination of a drug which is not cleared in the target organ and a target organ with a relatively low blood flow results in high regional advantages. The confirmation of the kinetic theory provides the possibility to reduce the systemic side-effects observed during equally effective systemic infusion, by reducing the infused dose during local drug administration.

11. SUPPRESSION OF VITAMIN K DEPENDENT ENZYME SYSTEMS IN THE TESTIS OF THE RAT DOES NOT AFFECT FERTILITY

11.1 INTRODUCTION

Oral anticoagulants of the coumarin type, as for instance acenocoumarol, warfarin and phenprocoumon, interrupt the vitamin K cycle in the liver by blocking the enzyme vitamin K epoxide reductase. This reduces the degree of carboxylation of several coagulation factors in the liver. Vitamin K dependent enzyme systems are also shown to be present in several non-hepatic tissues (Vermeer et al, 1982; Thijssen et al, 1986), as for instance the testis. To date any function of the vitamin K dependent systems in the testis is unknown. It is suggested that vitamin K dependent systems may play a role in fertility (Vermeer et al, 1984b).

The purpose of this study was to elucidate more of the function of these systems in the testis. A frequently used method for investigating the function of enzymes is to study the function of a system that lacks the enzyme, or to study the function of a system in which the activity of the enzyme is depressed. Since 4-hydroxycoumarins are known to depress the activity of vitamin K dependent systems in the testis of the rat (Thijssen et al, 1986), the second option is preferred here. Subsequently the route for the administration of the 4-hydroxycoumarin had to be chosen. On systemic administration coumarin derivatives are known to distribute poorly to the testis of the rat and to accumulate in the liver (Thijssen et al, 1985; Daemen et al, 1986a and chapter 2). Assuming a relationship between the concentration of a drug at a target site and its effect, relatively high concentrations have to be administered to the testis to depress its vitamin K dependent enzyme systems. Systemic administration of these high doses, however, would certainly depress the synthesis of coagulation factors in the liver and cause serious bleeding disorders. Direct administration of a coumarin derivative into the testis would on the one hand result in high concentrations in the testis and would on the other hand decline the drug concentration in the liver. Regarding the theoretical considerations of the advantages of local over systemic

drug administration the direct administration of a hepatically cleared drug into a target organ with a low blood flow is an ideal combination for achieving high local concentrations (chapter 1). For this purpose, S-acenocoumarol is the drug of first choice from the group of the 4-hydroxycoumarins, because of its relatively high liver clearance in the rat. Hence in theory, the local infusion of a drug, which is cleared in the liver, into a target organ with a low blood flow provides ideal conditions to study the effects of depression of vitamin K dependent enzyme systems on the function of the testis (i.e. on fertility). As was demonstrated in chapter 10, high testicular concentrations can indeed be achieved. In this study the effects of long-term administration of S-acenocoumarol on the vitamin K dependent systems in the testis are described.

11.2 EXPERIMENTAL SECTION

Wistar rats were purchased from Winkelmann (Borchen, West Germany). Before and throughout the whole experiment the rats were on standard lab food and tap water.

11.2.1 Intratesticular administration

Male rats, aging 14-16 weeks, were used. Under light ether anesthesia a testicular catheter was placed, for drug administration (see chapter 2). AC solved in 0.1 M phosphate buffer, pH 8.0 was infused at a constant rate of 1 ug/hr via an osmotic minipump (Model 2001, Alzet, Palo Alto, Ca, USA), connected to the other end of the catheter, during 5 days. Preliminary experiments showed that infusion of 5 ug/hr resulted in bleeding complications. An infusion rate of 1 ug/hr for several weeks revealed no bleedings. On the fifth day the animals were euthanized and the liver and both testes removed.

11.2.2 Fertility experiments

Male Wistar rats aging 7 weeks at the start of the experiment were used. According to the above described procedure, one end of a small silastic catheter was placed into the left testis, while the

other end was connected to an osmotic minipump placed subcutaneously between the shoulder blades. The right testis was removed. A group of 30 female Wistar rats (aging 9 weeks) was sterilized one week before the experiment by ligation of both uterine horns. A group consisting of 50 female animals underwent no surgery.

11.2.3 Experimental protocol

AC or saline (N=5/5) was infused at a constant rate (1 ug/hr of AC, 1 ul/hr of saline) for 8 weeks. New osmotic minipumps were placed every week. Each rat received either AC or saline throughout the whole experimental period. The first 50 days of the infusion a male rat was housed with three sterilized female rats to stimulate spermatogenesis. On the 55th day of the experiment, the three sterilized female rats were replaced by five nonsterilized female rats. On the 56th day of the experiment, all male animals were euthanized and plasma, liver and testis removed. From that day on, the female rats were housed separately and their offspring was counted.

11.2.4 Analytical procedures

The removed testes were rinsed extensively in ice-cold saline to wash out drug in the extracellular fluid and homogenized in three volumes (w/v) of icecold buffer, 0.02 M Tris HCl, 0.15 M KCl, pH 7.4, using the Potter technique.

Liver microsomes were made following the procedure of Thijssen et al (1986). The activity of the enzyme vitamin K epoxide (KO) reductase was measured separately in liver and testis homogenates on HPLC, following the procedure of Thijssen et al (1986). In short, 20 ul samples were incubated at 37° C for 3 min in 75 ul 0.02 M Tris-HCL, pH 7.4. DTT (5 ul, 0.1 M) was added and allowed to preincubate with the enzyme for 3 min at 37° C. The vitamin K epoxide reductase was started by adding 4 ug vitamin K epoxide in 2 ul isopropanol. The incubation lasted for 30 min. The reaction was stopped by adding 0.9 ml isopropanol containing 5 ug tocopherol acetate (Sigma, St. Louis, MO, USA) for internal standardization. Analysis of vitamin K and vitamin K epoxide was performed as follows. Substrate and product were extracted with 0.6 ml hexane containing 0.5 ml AgNO₃ (0.01 M) for removing DTT from

the organic phase. The hexane phase was washed with 0.5 ml of 1% NaCl in H_2O , where after it was evaporated at $37^{\circ}C$ under a nitrogen gas stream. The residue was dissolved in 50 μ l isopropanol and analyzed by HPLC.

Vitamin K and KH_2 dependent carboxylase activity was determined in testis microsomes, following the incorporation of $^{14}CO_2$ in a pentapeptide (FLEEL), following the procedure of Vermeer, 1984a. Plasma coagulation activity was determined using the prothrombin time. Protein content was determined following the method of Lowry et al (1951).

In the fertility experiment, the sperm cells in the cauda epididymis were counted in a counting chamber, immediately after euthanizing the animals. The mobile fraction of the total amount of counted cells was determined. After removing and weighing, the testis was frozen quickly (5 min at $-70^{\circ}C$) and a representative piece was cut with a knife, fixed in formalin (4% in phosphate buffer, 0.1 M, pH 7.4) and stained with haemato-oxyline eosine for morphological analysis. The remaining part was prepared for reductase and carboxylase assay, as described above.

11.2.5 Statistical analysis

Data are presented as mean $\pm$ SEM. Differences were compared using Student's t-test. The 0.05 level of probability was used as the level of significance.

11.3 RESULTS

11.3.1 Five days experiments

Following the administration of AC (1 μ g/hr) for 5 days into the left testis, vitamin K epoxide reductase activity in liver microsomes was reduced by about 50%. If the reductase activity in total liver homogenates was estimated a similar suppression was observed (fig. 11.1). We therefore decided to measure vitamin K epoxide reductase activity in testis in homogenates and not in microsomes, which enabled us to measure the activity in each individual animal (for measurements of vitamin K epoxide reductase and vitamin K dependent

carboxylase in testis microsomes, at least 3 testes have to be pooled). N.B.: Vitamin K epoxide reductase activity in tissue homogenates like microsomal reductase needs DTT to function and is equally susceptible to warfarin inhibition as microsomal activity. In the left testis, $26.4 \pm 5.0\%$ and in the right testis, $64 \pm 8\%$ of control testicular reductase activity was observed. Direct AC administration did not change the KH_2 dependent carboxylase activity in the infused testis as compared to the non-infused testis. K dependent carboxylase activity, however, was reduced to 22% (table 11.1). Plasma coagulation activity was not affected.

Table 11.1: The effect of 1 ug/hr AC delivered directly to the left testis during 5 days on vitamin K and KH_2 dependent carboxylase activity

Carboxylase activity^a

	KH_2 dependent	K dependent
Infused testis	1055	291
Contralateral testis	1432	1298

^a cpm/mg protein microsomes in AC treated animals.

11.3.2 Fertility experiments

Because of technical limitations, which included infection and disconnection of pump and catheter, only three of five AC treated animals finished the infusion period of eight weeks. Four of five animals in the control group completed the experiment. The values of the determined parameters are presented in fig. 11.1, right panel. The activity of vitamin K epoxide reductase in homogenates of the infused testis was $29.3 \pm 3.3\%$ of control, which is comparable with the value of the infused testis in the 5 day infusion group. Hepatic vitamin K epoxide reductase activity in AC treated animals was about half of the activity of the enzyme in the control group. Plasma coagulation acti-

vity was unaffected again.

Mating behavior in the two groups was similar. The number and mobility of sperm cells of animals treated with AC during a period of eight weeks were not different from control. The mating behavior in the two groups of animals was similar. Morphological analysis of fixed sperm cells and of testis tissue revealed no differences either. The litter size was 9.9 ± 1.0 in the AC treated group and 11.0 ± 0.6 in the control group (statistically no significant difference) (table 11.2).

Table 11.2: Effects of 1 ug/hr AC, delivered directly to the left testis during 8 weeks on fertility parameters.

	Control	AC
Mobility (% of 200 cells)	>75	>75 ^a
Litter size	11.0 ± 0.6	9.9 ± 1.0^a

a Statistically not significant from control.

11.4 DISCUSSION

Although the existence of specific vitamin K dependent proteins has been shown in various non-hepatic tissues, their precise function is not known yet. The vitamin K dependent bone protein osteocalcin probably prevents early calcification of the growing bone and regulates bone resorption (Lian et al, 1984). Any function of the vitamin K dependent protein found in human sperm (Vermeer et al, 1984b) is not known.

The apparent selectivity of 4-hydroxycoumarins to distribute to the liver (Thijssen et al, 1985; Daemen et al, 1986a) does not facilitate the study of chronic suppression of non-hepatic vitamin K dependent systems in the rat, since bleeding complications will terminate the experiment within a short time after start. The tool of target

VITAMIN K₀-REDUCTASE ACTIVITY

Fig. 11.1: The activity of the enzyme vitamin K epoxide (K₀) reductase in the liver, infused left testis and in the contralateral, non-infused, right testis during constant rate infusion of S-acenocoumarol for 5 days (left panel) and 8 weeks (right panel). Animals receiving an isotonic saline infusion served as control.

organ directed drug delivery was successfully applied here: on the one hand bleeding was prevented, while on the other hand high concentrations could be achieved in the testis (see chapter 10).

During the five days of infusion of 1 ug/hr AC K₀ reductase activity in the infused testis was about 25% of control. Administration of the same drug at the same dose for eight weeks reduced K₀ reductase activity to an almost equal level. Adaptation of the enzyme systems apparently did not occur. Vitamin K dependent carboxylase activity in the infused testis was reduced also to the about 25% of control. Notwithstanding these pronounced biochemical effects of direct intratesticular administration of AC, no effects on fertility parameters could be detected: the quantity and quality of sperm cells derived from 8 weeks AC treated animals were comparable with those from control animals. Several arguments can be put forward to explain this discrepancy: (1) 80% reduction of vitamin K₀ reductase and vita-

min K dependent carboxylase in the testis is not sufficient to affect spermatogenesis or sperm function; (2) the vitamin K cycle in the testis of the rat is different from that in the rat liver. Suppression of KO and K reductase might have been ineffective to interrupt the vitamin K cycle since other reducing pathways preserve the cycle; (3) the infusion period of 8 weeks was too short to affect spermatogenesis, although it is known that spermatogenesis in the rat lasts about 40 days (Bennett and Vickery, 1970); (4) vitamin K dependent enzyme systems have no function in spermatogenesis nor in male fertility.

In summary, suppression of the testis reductase by local administration of a coumarin derivative was successful but did not provoke detectable deficiencies in testis function. Since high testicular concentrations of S-acenocoumarol, which could only be reached by local administration of the drug, did not affect fertility, these data indicate that orally administered S-acenocoumarol will not influence fertility parameters. Therefore, it is not surprising that during oral anticoagulant therapy in clinical practice deficiencies in testicular function have not been reported.

12. CONCLUDING REMARKS AND OUTLOOK

In the previous chapters, experimental data have been presented to examine the hypotheses on the kinetic advantages of local over systemic drug administration at steady-state conditions during constant rate infusion. In all experiments, with the exception of the infusion of acenocoumarol in the testis, local drug administration was achieved by the administration of drugs into the artery supplying the target organ. Technically, this administration was possible by cannulation of a side-branch of the artery supplying the given target organ. Several substances, e.g. Cr EDTA, iodohippurate, propranolol and acenocoumarol, with distinct pharmacokinetic properties were infused into the artery of several target organs: the kidney, the liver, the transplanted heart and the testis. At steady-state conditions, the actual systemic and regional advantages of local over systemic drug administration were determined (table 12.1). As described in chapter 1, systemic advantages of local over systemic drug administration can be expected for drugs that are efficiently extracted by the target organ. Local infusion of iodohippurate, a high renal clearance substance, into the kidney reduces the systemic concentration by more than 80% as compared to systemic delivery. Local infusion of propranolol, a substance efficiently cleared by the liver, into the transplanted heart reduces the systemic drug concentration by more than 90%. In this latter model the clearance organ is situated in series with the target organ. Any drug infused directly into the transplanted heart reaches the liver and is subsequently extracted, before it reaches the systemic circulation. Local infusion of Cr EDTA, a low renal clearance substance, reduces the systemic drug concentration by approximately 30% only.

Saturation of elimination processes and/or inhomogeneous drug distribution in the target organ excepted, local infusion of drugs that are extracted by the target organ does not enhance the regional drug concentration as compared to systemic infusion.

If the infused drug is not cleared by the target, the ratio of the non-target organ clearance and the blood flow to the target organ

Table 12.1: A summary of the outcome of the experiments on the advantages of local over systemic drug delivery described in chapter 6-10 in this thesis

Drug cleared by the target organ	Remarks	Advantage	
		systemic	regional
low dose		0.15	0.9
high dose		n.d.	n.d.
low dose		n.d.	n.d.
high dose		0.26	1.7
propranolol in the transplanted heart		0.05	1.3
^{51}Cr EDTA in the right kidney		0.74	1.1
^{51}Cr EDTA in the left kidney		0.62	1.1
acenocoumarol in the liver		0.75	1
<u>Drug not cleared by the target organ</u>			
propranolol in the kidney		1	2.0
acenocoumarol in the kidney		1	0.9
acenocoumarol in the testis		1	22
acenocoumarol in the testis (phenobarbital co-treatment)		1	29
n.d. : not determined			
a systemic, i.e. non-target organ clearance		systemic advantage = $\frac{C_S \text{ at local administration}}{C_S \text{ at systemic administration}}$	
b blood flow to the target organ		regional advantage = $\frac{C_T \text{ at local administration}}{C_T \text{ at systemic administration}}$	

saturation because of inhomogeneous drug distribution

clearance organ in series with the target organ

(i.e. the systemic clearance) determines the advantage of local over systemic drug administration. This so-called regional advantage can be applied to lower the local infused dose, as compared to systemic drug infusion, and thus to reduce the systemic side-effects. This dose reduction is based on the assumption of a defined effective steady-state concentration in the arterial bed supplying the target organ that can be achieved by local as well as systemic administration.

Indeed, our experiments indicate that infusion of a drug with a low systemic clearance into a target organ with a high blood flow does not increase the regional drug concentration (acenocoumarol in the kidney, table 12.1). Administration of acenocoumarol into an organ with a low blood flow like the testis yielded a high regional advantage, which could even be increased after induction of the hepatic clearance of acenocoumarol by phenobarbital co-treatment.

In general, the experimental data confirmed the theoretical considerations. However, some pitfalls were observed.

12.1 PITFALLS OF LOCAL DRUG ADMINISTRATION

12.1.1 Saturation

The efficiency of the elimination processes in the target organ determines the reduction of the systemic concentration during local as compared to systemic drug administration. From this relation, a first pitfall of local drug administration can be derived. During intra-arterial administration, deviations from linear pharmacokinetics may occur. Smits and Thijssen (1986) demonstrated the effects of deviation from non-linear kinetics in a simulation study: in a physiologically based kinetic model comparable with fig. 1.3, the effects of saturation of carrier-mediated transport and of intracellular elimination mechanisms on the regional and systemic advantages of local vs. systemic drug administration were investigated. It was demonstrated that saturation of target organ transport mechanisms during local but not during systemic drug administration decreased the regional advantage of local drug administration. Saturation of elimination mechanisms in the target organ decreased the extraction ratio of the drug to almost

zero, resulting in a decreased systemic advantage. On the other hand, the increased concentration in the target organ enhanced the regional advantage. An example of the effects of saturation is found in the report of Boublil et al (1985) who described the effects of saturation of 5-FU elimination processes during its chronic intrahepatic delivery.

Non-linearity in plasma and/or tissue protein binding may also alter the expected advantages (Meijer et al, 1984; Mackichan, 1984; Rowland, 1984b; Harashima et al, 1985).

12.1.2 Inhomogeneous drug distribution

An inhomogeneous drug distribution over the target organ during local drug administration can be another pitfall. Laminar flow patterns in the artery supplying the target organ and/or a dual blood supply can cause this inhomogeneity. Inhomogeneous drug distribution may cause localized saturation of transport and/or elimination mechanisms which have the above-described effects on the regional and systemic advantage. An example of this pitfall is described in this thesis (chapter 6). Iodohippurate infused locally into the kidney was distributed inhomogeneously in the left, but not in the right kidney. This resulted in saturation of elimination processes in the infused part of the left kidney during the infusion of the highest dose. Subsequently the regional advantage increased but the systemic advantage decreased.

A remarkable phenomenon was observed in the experimental evaluation of intrahepatic delivery of acenocoumarol in the liver, a drug with a saturable hepatic binding site. Local drug administration did not increase but rather decreased the hepatic drug concentration.

A nice demonstration of the distinction between the study of drug effects (pharmacodynamics) and the study of drug concentrations (pharmacokinetics) (Powis, 1985) was shown in the experimental evaluation of local delivery of acenocoumarol in the testis. Notwithstanding the profound kinetic benefits of local drug delivery in this model, the effects of this intervention were poor.

12.2 TARGETING OF DRUGS - A PROBLEM OF SELECTIVITY

The experiments presented in this thesis indicate that pharmacokinetic research can provide a quantitative perspective with regard to the potential benefits of local drug administration. Certain drugs can be attractive candidates, while others are unattractive. Similarly some target sites are more favorable than others (Collins, 1984). These experiments also indicate that the probability of successful local drug administration can be substantially enhanced if the choices of drug and target are guided by pharmacokinetic principles (Schabel et al, 1983; Collins et al, 1986).

Recent pharmacochemical technologies give new possibilities for drug design. Specifically, drugs which are efficiently cleared by the target organ or which have a high systemic clearance can in some situations be favorable over drugs with a low clearance which are preferred in oral therapy.

Although the intra-arterial administration of drugs can lead to a more or less selective drug delivery, also some disadvantages are present. One important disadvantage is its invasiveness, that necessitates a surgical procedure. Surgery limits widespread use in human medicine since qualified persons and materials are not available at any time at any place.

Almost all attention has been paid to the administration of drugs into the blood vessel supplying a target organ since this is in the scope of the experimental section of this thesis. The many other approaches of selective drug delivery such as liposomal drug delivery systems, antibody-drug carrier systems, cellular drug carriers or microspheres and macromolecules as drug carriers and pro-drug delivery systems are described briefly in the first chapter of this thesis.

All systems of selective drug delivery have to deal with and are actually designed to cope with the so-called targeting problem (Poznansky and Juliano, 1984): a drug has to be delivered to that particular cell, where desirable effects can be achieved, while a cell where toxic effects can be achieved has to be passed by. The administration of a drug into the blood vessel supplying the target offers a kinetic approach to the solution of this problem, but is a relatively

non-selective cell targeting approach. The solution of the targeting problem presumes the existence of a molecular characteristic that differs between a target and a non-target cell. Subsequently reagents, i.e. drug carrier conjugates, have to be developed that bring the drug in or to the selected target cell (Chabner et al, 1984). Here, an additional problem may be that the target cell may not be a static but a dynamic entity, which can loose its specific receptors, can have a down regulation of receptors or can become otherwise insensitive to the targeted drug.

The key to selectivity will be found in the design of agents intervening in the pathogenesis of diseases at a very fundamental level. In cancer research oncogenes or the products of oncogenes may be seen as selective agents that are not subject to any escape mechanism. Also the discrimination of unique receptor molecules offers the possibility to manipulate processes in specific cell populations by antireceptor antibodies. The design of drugs selective in this respect is a plan for the future. Until that time, drug targeting can be achieved by the local administration of less selective drugs.

Appendix I: A PHYSIOLOGICALLY BASED COMPUTERIZED MODEL TO SIMULATE
PHARMACOKINETICS - SIMULATIONS ON THE PHARMACOKINETICS OF
S-ACENOCOUMAROL IN THE RAT -

I.1 INTRODUCTION

In chapter 2, studies are described on the apparent dose-dependent hepatic clearance of S-acenocoumarol (AC) in the rat. A saturable hepatic binding site ($K_D = 2 \text{ ng/ml}$; $B_{\text{max}} = 1.4 \text{ ug/g}$ of liver) was found that could account for this dose-dependency. The results did not allow differentiation between a mere binding protein (for instance ligandin or protein Y) or a transport system (Daemen et al, 1986a). A binder was suggested by the slower elimination from the liver than from the plasma (Thijssen et al, 1983a; Daemen et al, 1986a). A transporter, however, should be more compatible with the dependency of the drug's blood clearance on its infusion rate. The simulations described below were performed to give more insight in the effects of a hepatic binding protein on the pharmacokinetics of S-acenocoumarol.

Fig. I.1: Scheme of a physiological model for the pharmacokinetics of S-acenocoumarol in the rat.

i.s.=interstitial space; i.c.= intracellular space.

A physiologically based pharmacokinetic computerized model was created according to the scheme described in fig. I.1. A flow diagram of the model is given in fig. I.2. It includes a systemic compartment (fig. I.2.A) to which a drug can be delivered and from which a drug can be cleared. The vascular/interstitial space of the liver is represented by block B. Via this compartment the drug enters or leaves the liver. From the interstitial space drug is rapidly exchanged with the intracellular space, C. In the intracellular space the drug can be bound or cleared. The free concentration of the drug in the intracellular space is calculated by the formula

$$C_f = 1/2 [C_{tot} - (K_D + B_{max}) + \sqrt{[(K_D + B_{max}) - C_{tot}]^2 + 4K_D \cdot C_{tot}}]$$

where C_f represents the free concentration of the drug in the intracellular space, K_D the dissociation constant and B_{max} the total number of binding sites. This calculation is performed in block D.

In the simulations S-acenocoumarol was used as the example drug. Values of the different parameters were based on physiological and pharmacological data on the pharmacokinetics of AC in the rat. Drug could be delivered via the systemic route or via the local route (i.e. intrahepatically). The blood flow to the liver was 20 ml/min, which is comparable with rat liver flow. The dissociation constant was 2 ng/ml, while the value of the total binding sites was 1.4 ug/g (Daemen et al, 1986a). The apparent intrinsic clearance was 3 ml/min, which is comparable with the steady-state clearance of AC during the infusion of high doses, assuming all binding sites to be saturated.

Processes were simulated using THTSIM (Technische Hogeschool Twente, Enschede, The Netherlands) as simulation language.

Fig. 1.2: Flow diagram of the model used in the first set of simulations.

Table I.1: Listing of the simulation model described in fig. I.2

1 pls	23 gai 15
2 int 1	24 int 23
3 sum 2, 22, -6, -8	25 sum 24, -39, -41
4 att 3	26 att 25
5 gai 4	27 lim 26
6 int 5	28 sum 35, -27
7 gai 4	29 abs 28
8 int 7	30 pwr 29
9 pls	31 sum 33, 30
10 att 9	32 sqt 31
11 sum 10, 4	33 gai 27
12 gai 11	34 sum -35, 27
13 int 12	35 con
14 sum 13, 41, -17, -24	36 sum 32, 34
15 att 14	37 att 36
16 gai 15	38 gai 42
17 int 16	39 int 38
18 gai 15	40 gai 42
19 sum 18, 20	41 int 40
20 gai 4	42 lim 37
21 gai 19	43 sum 27, -42
22 int 21	

Abbreviations

pls = pulse input
 int = integrator
 sum = summation
 gai = gain
 att = attenuator
 lim = limiter
 abs = absolute value
 pwr = power
 sqt = square root
 con = constant

Table I.2: Value of important parameters used in the simulation models I and II.

Block no.	description	value
4	blood volume	10 ml
5	systemic clearance	0 ml/min
10, 12	liver flow	20 ml/min
15	interstitial volume	25 ml
21	cardiac output	100 ml/min
23, 43	diffusion constant	100
26	intracellular volume	10 ml
33	$4 \times K_D$	8 ng/ml
35	$K_D + B_{\max}$	1.402 ug/g liver
38	elimination rate	3 ml/min

I.2. SIMULATIONS

I.2.1 "Systemic and intrahepatic administration of AC"

A constant rate of infusion of 0.1, 0.5 or 1.0 ug/min via the systemic route was simulated. The infusion lasted from time 0 until time 180 min. The values of C_S (the systemic arterial concentration (ug/ml)) and C_b (the concentration at the binding site in the liver (ug/g)) in time are described in fig. I.3 (upper part). The clearance, calculated by inf/C_S , at steady state, was about 8 ml/min during the infusion of 0.1 ug/min. The clearance decreased to 2.6 ml/min during the constant rate infusion of 0.5 and 1.0 ug/min. Infusion of 0.5 and 1.0 ug/min saturated the binding site completely. The ratio of the concentration of drug bound in the liver and the systemic plasma concentration was high (about 80) at the lowest infusion rate and decreased to about 3 at the highest infusion rate (1 ug/min). Note that the liver concentration almost equalled the plasma concentration during the infusion of 12.5 ug/min (fig. I.4). Both these phenomena, the dose-dependent clearance and the inverse relation between the

Fig. I.3: Outcome of simulations of systemic (C_s ; ug/ml) and hepatically bound (C_b ; ug/g) concentrations of acenocoumarol after constant rate infusion of 0.1, 0.5 and 1 ug/min, via the systemic route (upper part) or via the local route (lower part; C_t represents the concentration in the artery supplying the target organ).

liver/plasma ratio and the infusion rate, state the results of experimental data on the kinetics of AC in the rat (Daemen et al, 1986a and chapter 2).

In fig. I.4, short-term constant rate infusion of 12.5 ug/min via the systemic route was simulated. C_b , C_{li} and C_s were followed during 450 min. At $t=100$ (the end of the infusion), the total drug concentration in the liver was approximately 5.5 ug/g liver, the concentration in plasma 4.8 ug/ml and the concentration bound in the liver 1.4 ug/g. Plasma clearance was 3 ml/min. Initially, the decrease of the AC concentration in the plasma was rapid ($t_{1/2}$ approximately

Fig. I.4: Outcome of simulations of C_s and C_b levels of acenocoumarol after a constant rate infusion of 0.5 ug/min via the systemic route for 100 min.

Fig. I.5: Outcome of simulations of C_s and C_b levels of acenocoumarol after a bolus injection of 1, 10, and 100 ug via the systemic route.

15 min). From about $t=180$, $t_{1/2}$ increased. The disappearance of AC from the liver was slower than from the circulation.

Local infusion of the drug was simulated also (fig. I.3, lower part). At the lowest infusion rate, steady-state conditions were not achieved after 180 min of infusion nor was the binding site saturated. The systemic concentrations during the local infusion of 0.5 and 1.0 ug/min were somewhat lower than during their systemic infusion (0.17 and 0.34 at local and 0.19 and 0.38 ug/ml at systemic infusion of 0.5 and 1.0 ug/min, respectively). The concentration in the artery supplying the target organ almost equalled the systemic concentration. Hence, the regional advantage was minimal. The small systemic and regional advantage at steady state are in accordance with the predictions. The saturation of the binding site decreases the clearance, decreasing the systemic advantage. On the other hand, the high blood flow to the target organ ($Q_T = 20$ ml/min) in combination with no systemic clearance of the drug limits any regional advantage. Note that before saturation is achieved the concentration in the artery supplying the target organ was higher than the systemic drug concentration.

In fig. I.5, the kinetic processes after systemic bolus injections of 1, 10 and 100 ug AC are described. Here, one sees a biphasic dose dependent disappearance of the drug from the circulation. There is an initial fast disappearance of the drug from the circulation, especially to the binding site. Subsequently, this binding site with a high affinity is a limiting factor for elimination of the drug (so called deep compartment).

The initially fast disappearance of the drug from the circulation, followed by a very slow rate of disappearance after small bolus injections, is also observed after injection of tracer amounts of warfarin in rats (Thijssen, personal communication).

Fig. I.6: Flow diagram of the extension of the model described in fig. I.2, used to simulate the effects of changes in target flow (Q_T) and systemic clearance (Cl_S) on the advantages of local over systemic drug delivery.

1.2.2 "Intrarenal and intratesticular administration of AC"

The flow diagram (fig. I.2) is extended with two organs linked in parallel as in fig. I.6. This extension is necessary to describe the processes during the infusion of a drug in other target organs than the liver, for instance the kidney and the testis (chapter 10 and 11). The new target organ (blocks 50-59) has a volume of 5 ml and a blood flow of 4 ml/min. It does not clear the infused substance. Local infusion into the new target organ is possible. A parallel-linked identical organ is described by the blocks 60-69, representing the non-infused kidney or testis. The value of the parameters of the parallel-linked organs are identical. S-acenocoumarol is used as the example substance. The hepatic clearance is 3 ml/min. All other parameters are identical to those described in the first set of simula-

tions. The infusion rate was set at 0.5 ug/min during 180 min.

The concentration in the artery supplying the target organ (C_T), the concentration in a systemic artery (C_S) as well as the concentration in the artery supplying the parallel-linked (C_{NT}) were followed in time. Their respective values are depicted in fig. I.7A. The concentration in the artery supplying the non-infused organ equals the systemic concentration. The concentration in the artery supplying the infused target organ is slightly higher than the concentration in the artery supplying the non-infused organ. Hence, there is only a small regional advantage (approximately 1.6).

The effects of an increased hepatic clearance (cf. phenobarbital co-treatment; chapter 10) was simulated (fig. I.7B). Both C_T and C_{NT} decreased. The regional advantage ($= C_T/C_{NT}$), however, increased: $C_T/C_{NT} = 2.5$.

In fig. I.7C, the outcome of simulations of local infusion into a target organ with a low blood flow are described. The flow to the

Table I.3: Listing of the simulation model described in fig.I.6

48 pls	19 sum 59, 18, 20, 69
49 att 48	60 pls
50 sum 49, 4	61 att 60
51 int 51	62 sum 4, 61
52 int 51	63 gai 62
53 sum 52, -58, -56	64 int 63
54 att 53	65 sum -68, 64
55 gai 54	66 att 65
56 int 55	67 gai 66
57 gai 54	68 int 67
58 int 57	69 gai 66
59 gai 5	

Fig. I.7: Outcome of simulations on the systemic concentrations (C_S ; ug/ml), the concentrations in the target organ (kidney or testis; C_T ; ug/g) and the identical, parallel-linked, non-target organ (non-infused kidney or testis; C_{NT} ; ug/g). The infusion rate was set at 0.5 ug/min.

- A. $Q_T = 4$ ml/min; $Cl_S = 3$ ml/min
 B. $Q_T = 4$ ml/min; $Cl_S = 10$ ml/min
 C. $Q_T = 0.2$ ml/min; $Cl_S = 3$ ml/min.

target organ is 0.2 ml/min. The hepatic clearance is 3 ml/min again. All other parameters are as above. One can see that C_T is increased: 2.7 ug/ml at steady state during a blood flow of 0.2 ml/min and 0.31 ug/ml during a blood flow of 4 ml/min. The ultimate level of C_S and C_{NT} are comparable with the values described in fig. I.6A. The time course, however, is different. The ratio of C_T and C_{NT} is very large before steady state is achieved (at $t=50$ min, this ratio equals almost 100). At steady-state conditions, the ratio is still large: 13.5.

I.3 CONCLUSIONS

1. In the first set of simulations (fig. I.3, I.4 and I.5), it was described that a hepatic binding site with a low capacity but a high affinity could indeed account for a dose-dependent clearance of a systemically infused drug. A binding site for acenocoumarol is consistent with the outcome of these simulations.
2. Local infusion of the model substance yielded minimal regional and systemic advantages. At high infusions rates, steady state was achieved sooner than at lower infusion rates. These latter findings are in comparison with the experimental data described in chapter 9. The decreased concentration in the liver during local as compared to systemic infusion, however, cannot be explained by this model.
3. In the second set of simulations (fig. I.7), the effects of changes in the systemic clearance and the blood flow through the target organ were described. A target organ with a relatively high flow as compared to the systemic clearance of the locally infused drug is unsuited for achieving high regional advantages (chapter 10; fig. I.7A). By increasing the systemic clearance, the regional advantage is increased. Lowering of the blood flow to the target organ increased the regional advantage, especially in the time period before steady state is achieved (chapter 10; fig. I.7B,C).

SUMMARY

This thesis reports on an experimental pharmacokinetic study in the rat on the possibilities and limitations of local drug administration. Local drug administration means the administration of a substance directly in the artery supplying a target organ. This special way of drug delivery is widely applied, often based upon the assumption that local administration of a drug increases the local and decreases the systemic drug availability. The theoretical pharmacokinetic principles, however, the basis of this way of drug administration, are not as known as the widespread use of local drug administration suggests.

Pharmacokinetic research on the possibilities and limitations of local drug administration is rare. It might, however, be a guide for advantageous local, i.e. intra-arterial, drug administration.

In chapter 1, an overview is given of the many applications of local drug administration in clinical practice, like cancer chemotherapy and thrombolytic therapy, and in experimental research. The theoretical principles of local drug administration are described in the second part of the chapter. An advantage of local over systemic drug administration can either mean an increase of the local drug concentration and/or a decrease of the systemic drug concentration. The increase of the local drug concentration is proportional to the systemic clearance of the drug and inversely related to the blood flow of the target organ. The decline of the systemic concentration is related to the extraction ratio of the drug by the target organ.

Chapter 2 describes the methods that are used in our experiments for the administration of substances into the artery supplying a target. Access to the artery supplying the target organ is gained by cannulating a side-branch of this artery. The different infused substances, which were chosen because of their distinct pharmacokinetic properties, are described.

In literature the clearance of indocyanine green is seen as an indicator of hepatic blood flow. Experiments, described in chapter 3, indicate that pentobarbital anesthesia and hypothermia during the

anesthesia reduce the clearance of indocyanine green. Experiments in anesthetized rats, in which hepatic blood flow is measured simultaneously by electromagnetic flowmeters and the indocyanine green clearance method (chapter 4), seriously question the suitability of indocyanine green to indicate hepatic blood flow.

In chapter 5, a method of following the hepatic drug concentration in time in one experimental animal by taking small liver biopsies is evaluated.

In chapter 6 to 10, local infusions of several substances are compared with their respective systemic infusions at the same dose. The results of experiments described in chapter 6 indicate that local, i.e. intrarenal, administration of hippuric acid, a substance with a high renal extraction ratio, reduces its systemic concentration (as compared to systemic infusion). During intrarenal infusion of Chromium EDTA, a substance with a low renal extraction ratio, the reduction of the systemic concentration is much smaller. Inhomogeneous drug distribution during local but not during systemic infusion may decrease the extraction of the drug by the target organ, resulting in higher systemic concentrations. Intrarenal infusion of propranolol, a substance with a high hepatic clearance, hardly reduces its systemic concentration (chapter 7). Intracardial or in fact intrahepatic administration of the same substance (chapter 8), minimizes its systemic concentration, following the effect of extraction of propranolol by the liver. The experiments described in chapter 8 provide a pharmacokinetic base for the local administration of immunosuppressive drugs with a high hepatic clearance in the described graft. As is demonstrated in chapter 9, local, i.e. intrahepatic, administration of acenocoumarol, a hepatically cleared oral anticoagulant, decreases its systemic concentration. The local, hepatic, concentration does not increase but even decreases. In accordance with theoretical predictions, intrarenal administration of acenocoumarol does not decrease its systemic concentration nor increases its local concentration (chapter 10). Local, intratesticular, administration yields relatively high testicular concentrations, which, because of serious systemic side-effects, cannot be achieved by systemic administration of acenocoumarol.

In chapter 11, experiments are described in which the local, in-

tratesticular infusion of acenocoumarol was applied to investigate the function of vitamin K dependent systems in the testis. The relatively high testicular concentrations of acenocoumarol declined the activity of several biochemical parameters of the function of vitamin K dependent systems, but did not affect fertility.

In chapter 12, the possibilities and limitations of local drug administration, now based on experimental evidence, are described. A discrete outlook in future indicates that as long as a really selective drug is not yet available, local drug administration of more or less selective substances can provide a useful alternative.

SAMENVATTING

In dit proefschrift word verslag gedaan van een experimenteel farmakokinetisch onderzoek in de rat naar de mogelijkheden en beperkingen van lokale toediening van geneesmiddelen. Lokale toediening wil zeggen: toediening van een middel direkt in het aanvoerend bloedvat van een bepaald doelorgaan. Deze speciale toedieningsvorm wordt op vrij uitgebreide schaal toegepast. De achterliggende aanneming is dan dat als een geneesmiddel lokaal wordt toegediend, dit ter plekke leidt tot hoge concentraties van dit middel, terwijl er in de rest van het lichaam maar zeer weinig van het middel terecht komt. Ondanks de wijdverbreide toepassingen van lokale toediening zijn de farmakokinetische principes, die de theoretische basis vormen van deze toedieningsvorm, aan velen onbekend. Er is slechts weinig farmakokinetisch onderzoek gedaan naar de mogelijkheden en beperkingen van lokale toediening. Dit onderzoek echter zou een leidraad kunnen zijn bij het lokaal toedienen van geneesmiddelen.

In hoofdstuk 1 wordt een overzicht gegeven van de vele toepassingen van lokale toediening in de klinische praktijk, zoals kankerchemotherapie, thrombolytische therapie en in experimenteel onderzoek. In het tweede deel van dit hoofdstuk worden de theoretische principes van lokale toediening beschreven. Voordeel van lokale toediening ten opzichte van systemische toediening kan of een verhoging van de lokale concentratie en/of een verlaging van de systemische concentratie betekenen. De verhoging van de lokale concentratie is evenredig met de systemische klaring van de stof en omgekeerd evenredig met de bloeddoorstroming in het doelorgaan. De verlaging van de systemische concentratie is evenredig met de extraktieratio van de stof in het doelorgaan.

Hoofdstuk 2 beschrijft de methoden die gebruikt worden om stoffen toe te dienen in het aanvoerend bloedvat van een doelorgaan. Toegang tot dit aanvoerend bloedvat wordt verkregen door het kanuleren van een zijtak van het bloedvat. De verschillende stoffen, gebruikt in onze studies en gekozen om hun verschillende farmakokinetische eigenschappen, worden eveneens beschreven.

In de literatuur wordt de klaring van indocyanine groen wel gezien als een goede indikator van de leverdoorbloeding. Uit de resultaten van experimenten beschreven in hoofdstuk 3 blijkt dat pentobarbitalverdooving en een lagere lichaamstemperatuur tijdens die narkose de klaring van het indocyanine groen verlaagt. De resultaten van experimenten in verdoofde ratten, waarin met behulp van elektromagnetische flowmeters en met behulp van de indocyanine groen klaringsmethode de leverdoorbloeding wordt gemeten (hoofdstuk 4), doen ernstige twijfel ontstaan aan de waarde van de klaringsmethode als indikator voor de leverdoorbloeding.

In hoofdstuk 5 wordt aangetoond dat het met behulp van kleine leverbipten mogelijk is om bij één proefdier de leverkoncentratie van een stof in de tijd te volgen.

In hoofdstuk 6 tot en met 10 wordt de lokale infusie van een aantal stoffen in een aantal doelorganen vergeleken met systemische infusie in dezelfde dosering van dezelfde stof. Uit de uitkomsten van de experimenten in hoofdstuk 6 blijkt dat lokale, dat wil zeggen intrarenale, toediening van para-aminohippuurzuur, een stof met een hoge extraktie in de nier, de systemische concentratie vermindert vergeleken met systemische toediening. Bij intrarenale toediening van chroom EDTA, een stof met een lage extraktie in de nier, daalt de systemische concentratie veel minder. Inhomogene verdeling van de lokaal toegediende stof over het doelorgaan kan de extraktie van de stof verminderen en leiden tot hogere systemische concentraties. Intrarenale toediening van propranolol, een stof met een hoge leverextraktie, vermindert de systemische concentratie niet of nauwelijks (hoofdstuk 7). Wordt dezelfde stof intracardiaal of in feite direct aan de lever aangeboden, dan daalt de systemische concentratie evenredig aan de extraktieratio van propranolol in de lever (hoofdstuk 8). De experimenten in dit hoofdstuk bieden een farmakokinetische basis voor lokale toediening van immuunsuppressiva met een hoge leverklaring in het beschreven harttransplantaat. Zoals blijkt in hoofdstuk 9 vermindert intrahepatische toediening van acenocoumarol, een oraal antistollingsmiddel, dat door de lever geklaard wordt, de systemische concentratie. De concentratie van de stof in de lever neemt echter niet toe, zij neemt zelfs af. Geheel in overeenstemming met theoretische voorspel-

lingen leidt intrarenale toediening van acenocoumarol noch tot lagere systemische noch tot hogere lokale concentraties (hoofdstuk 10). Direkte toediening van acenocoumarol in de testis leidt tot relatief hoge lokale concentraties die niet bereikt kunnen worden door de stof systemisch toe te dienen.

In hoofdstuk 11 wordt beschreven hoe met behulp van direkte chronische toediening van acenocoumarol in de testis onderzoek gedaan kan worden naar de functie van vitamine K afhankelijke systemen in de testis. De relatief hoge concentraties van acenocoumarol in de testis verminderen wel de aktiviteit van een aantal biochemische parameters van vitamine K afhankelijke systemen, maar leiden niet tot een vermindering van de fertiliteit.

In hoofdstuk 12 worden de, nu experimenteel onderbouwde, mogelijkheden en grenzen van lokale toediening beschreven. Een voorzichtige blik in de toekomst geeft aan dat zo lang een echt selektief werkend geneesmiddel nog niet beschikbaar is, de lokale toediening van min of meer selektieve middelen een bruikbaar alternatief kan zijn.

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CURRICULUM VITAE

The author of this thesis was born in Heerlen on April 20, 1959. From 1971 until 1977 he spent his school years at the Coriovallum College (gymnasium). Afterwards, he studied medicine at the University of Limburg in Maastricht. In the 4th year of the medical study, he was a student research-assistent at the Department of Biochemistry (dr. K. Hamulyák and dr. C. Vermeer). He graduated in June 1983.

In the second half of 1983, he was a research-fellow at the Department of Pathology. The studies described in this thesis were performed at the Department of Pharmacology (head: prof. dr. H.A.J. Struyker Boudier) where the author started working in December 1983.

From April 1987 on, he will be a member of the Department of Pathology (head: prof. dr. F.T. Bosman) at the University of Limburg in Maastricht.

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