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Experimental myocardial infarction : a proteomics point of view

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Chapter 7

Summary and conclusions

Summary

The first goal of this thesis was to investigate the potential cardioprotective effects of the antioxidant 7-monohydroxyethylrutoside (monoHER) in an *in vivo* mouse model of cardiac ischaemia-reperfusion (IR). The second goal was to determine the early alterations in cardiac protein expression in this IR model in order to delineate the mechanisms of action of monoHER in this setting.

As the *in vivo* mouse model of cardiac IR was the study object of this thesis we compared in **chapter 2** the long-term structural and functional consequences of this myocardial infarction model to the ones induced by permanent ischaemia (PI).
The majority of studies on cardiac IR injury have focused on the short-term consequences of reperfusion. Using myocardial infarct size or neutrophil influx as parameters, the progression of cardiac tissue damage has been described in the time frame of a few hours up to one or a few days after the initiation of reperfusion. The long-term effects of IR injury, in terms of weeks or months, are less well examined in this *in vivo* mouse model. This is, however, a relevant aspect for extrapolation to the clinical setting as it may be that the outcome of interventions that are beneficial shortly after initiation of reperfusion is different when the evaluation takes place at later stages.
In chapter 2 we showed that the long-term structural and functional consequences following cardiac IR are different from those observed after PI. Contractility of the heart was depressed for at least 2 weeks after IR, but was nearly restored after 8 weeks of IR. After cardiac IR, the gross architecture of the ventricular wall was preserved and the wound healing process was characterized by extensive calcification, minor hypertrophy and minor dilatation. In the PI model, the ischaemic area was not calcified, but completely replaced by connective tissue. In addition, the ventricular wall was severely dilated, the remaining myocardial muscle was strongly hypertrophied and loss of cardiac function was permanent.

The potential protective effects of exogenously administered antioxidants on cardiac reperfusion injury have been studied extensively. However, as described in detail in **chapter 1**, the beneficial effects of antioxidant treatment in these models remain controversial and need further clarification. Moreover, most of these studies only investigated potential short-term protective effects after initiation of reperfusion and did not examine sustained protective effects.
In **chapter 3**, we evaluated the cardioprotective effects of the antioxidant flavonoid monoHER in the *in vivo* mouse model of cardiac IR up till two weeks after IR, a time point when wound healing processes are completed. There were three indications that monoHER might act protective against injury in this model. These were: 1/ strong radical scavenging and iron chelating properties of monoHER; 2/ proven protective effects of monoHER against doxorubicin-induced cardiotoxicity *in vivo* in mice; 3/ *in vitro* anti-inflammatory effects of monoHER by reducing neutrophil adhesion.
We showed that monoHER exerted a sustained cardioprotective effect in this *in vivo* mouse model of cardiac IR. This was demonstrated at two time points (24 hours and

2 weeks after IR) by the fact that monoHER significantly reduced myocardial neutrophil influx, infarct size and maintenance of cardiac contractile responses when the heart was stressed with dobutamine and volume loading.
To our knowledge, this was the first time that sustained protective effects (in terms of weeks) of a flavonoid are demonstrated in this cardiac IR model.
The timing, route and dose of monoHER administration in this study (chapter 3) were based on earlier *in vivo* mouse studies in which monoHER protected against doxorubicin-induced cardiotoxicity [1, 2]. In these studies monoHER was administered intraperitoneally one hour before doxorubicin injection.

Obviously, to minimize myocardial damage evoked by chemotherapeutic agents such as doxorubicin it is logical to pre-treat patients with radical scavenging agents such as monoHER. However, when preventing "reperfusion injury", administering an antioxidant as a protective drug before the onset of ischaemia, has less clinical relevance. In addition, recent pharmacokinetic data in mice revealed that following intravenous injection of monoHER maximal myocardial tissue concentrations of the drug were reached almost immediately [3].
Therefore, in **chapter 4** we set out a study to test if monoHER would also be effective when administered intravenously five minutes before reperfusion. Administration of monoHER at this time point neither affected the influx of neutrophils in the injured tissue nor improved stimulated cardiac contractility, 24 hours after reperfusion.
Thus monoHER protects in an *in vivo* mouse model of cardiac IR when administered intraperitoneally one hour before ischaemia but lacks these properties when applied intravenously five minutes before reperfusion.
This discrepancy in results (between chapter 3 and 4) indicates that the time point of monoHER administration is very crucial to obtain cardioprotective effects.
Among the explanations for the lack of a clear pharmacodynamic effect of monoHER in the latter study (chapter 4) are: 1) the potential restriction in myocardial uptake of monoHER in conditions of cardiac IR; 2) the necessity of formation of active metabolites of monoHER; and 3) the possibility that cardioprotective effects are, directly or otherwise indirectly via antioxidant effects, due to interference of monoHER with specific signal transduction pathways during the ischaemic period. Such signal transduction pathways might be associated to the protective mechanism of action of monoHER. As signalling pathways consist of proteins, it is essential to study changes in protein expression or post-translational modifications of proteins to verify this hypothesis.

To search for those, two sets of proteomic studies were performed in which we used a technique of 2-DE combined with mass spectrometry (MS). We studied early (210 min) changes in protein expression because we were primarily interested in finding the reason for the protective effects of monoHER. In addition we were interested in identifying potential new targets for cardioprotection that are beneficial in the first few hours of myocardial infarction. Previous studies showed that by using this technique

of 2-DE and MS it is possible to detect early alterations in the proteome induced by cardiac IR [4-6].
In **chapter 5** we compared protein density changes in the area at risk of the mouse heart following *in vivo* PI and IR. We analyzed cytosolic as well as membrane fractions and found a total of thirty-two protein spots (out of about 600 matched spots) that were differentially expressed. With this technique we were able to find twenty protein spots that were changed in expression level after PI alone, four protein spots that were changed in expression level after IR alone, and eight spots that changed commonly in both models. The results of this study are therefore a source for further studies in which the specific functional relevance of these proteins in models of myocardial infarction can be investigated.
Identified proteins (by MS) could be classified in seven functional groups being the 1/ anticoagulant proteins (Annexin A3 and Annexin A5), 2/ structural proteins (cardiac troponin T, α-tropomyosin and α-myosin heavy chain), 3/ inflammatory-related proteins (serum amyloid P-component precursor), 4/ transcription- and translation-related proteins (Prohibitin and Histidine Triad Nucleotide Binding Protein 1, Heterogeneous Nuclear Ribonucleoprotein K), 5/ heat shock proteins (Heat Shock Protein 20&27), 6/ metabolism-related proteins (Adenylate Kinase 1, Pyruvate Dehydrogenase E1 Component Beta Subunit, Catechol-O-Methyltransferase) and 7/ miscellaneous (dual specificity phosphatase, similar to Zn-alcohol dehydrogenase).
A remarkable finding was the IR specific translocation of annexins (A3 and A5) from the cytosolic to the membrane compartment, a phenomenon that was verified by Western blotting. The fact that this phenomenon occurred after reperfusion only and not after permanent ischaemia suggests a specific related mechanism that triggers translocation of annexins to the membrane fraction during reperfusion. These findings may give new impetus for further studies in relation to annexins and early diagnosis of reperfusion injury in connection with earlier studies from our laboratory [7].
Four proteins were changed in expression level at multiple spot locations, characterized by a difference in isoelectric point. In the case of cardiac troponin T and HSP-20, these changes were also dependent on the model. In addition, one spot for the proteins adenylate kinase 1, cardiac troponin T and HSP-20 was uniquely present in the IR and/or PI and not in the respective sham groups. Further research is necessary to determine the character of possible pathology related post-translational modifications.

A total of twelve protein spots changed significantly in expression level after cardiac IR (**chapter 6**). Ten of these protein spots could be characterized by MS. These were: Heat Shock Protein-20 and Heat Shock Protein 27, Heterogeneous Nuclear Ribonucleoprotein K, Pyruvate Dehydrogenase E1-β, Annexin A3 (at 2 spot locations), Dual Specificity Phosphatase-3, Catechol-O-Methyltransferase, and Adenylate Kinase 1 (at 2 spot locations).
Intraperitoneal administration of monoHER, one hour before ischaemia, did not alter this protein expression profile related to cardiac IR. This means that the proven cardioprotective effects of monoHER as evidenced at a later time point on

histological and functional level were not detected on proteome level at an early time point by using the current techniques.
The discrepancy between these two studies in chapters 3 and 6 may be explained by the possibility: 1/ that monoHER did not affect changes in protein expression after cardiac IR; 2/ this time point was not relevant to detect alterations caused by monoHER; 3/ the technique we used in this study may not have been able to detect alterations in the proteome specifically triggered by monoHER.

Conclusions and implications

The discrepancy in outcome with antioxidant treatment related to cardiac IR
As summarized in the general introduction to this thesis, numerous *in vitro* and *in vivo* studies have examined the role of antioxidant enzymes and less specific antioxidant interventions in reducing cardiac IR injury. The results of these studies are very conflicting since the number of studies in which protective effects were proven was comparable to the number that could not. Furthermore, it is reasonable to assume that numerous studies have been performed, that did not reach the stage of publication, because protective effects of the antioxidant under investigation was disputable or even could not be proven under certain conditions.
Although research on this topic started already in the early eighties, a clear explanation for this discrepancy is, up to now, not known. Among the experimental variables that have been postulated to explain these conflicting results are: 1/ animal species; 2/ general features of the experimental model (e.g. anaesthetized or awake model, open- or closed- chest model); 3/ the presence or absence of critical stenosis; 4/ duration of ischaemia; 5/ duration of reperfusion; 6/ pharmacokinetic considerations; 7/ methodology to quantify infarct size; and 8/ inclusion or omission of measurements to control for baseline predictors of infarct size (e.g. collateral blood flow). However, up to now, there is no study available in literature that really proved that one of these factors or a combination of them is the cause for this discrepancy.
Studies in transgenic mice with altered levels of antioxidant enzymes consistently yield results in support of a detrimental role for reactive oxygen species (ROS) in experimental myocardial IR injury (chapter 1). This means that the lack of effect of these exogenous antioxidants may be (in part) due to a restricted access to the sites of ROS generation. As the capacity of an antioxidant to gain entry to the target tissue and cells and the relevant ROS-producing intracellular compartments or sites is an important condition, this ability needs to be verified. It will not be surprising if therapies fail if they do not successfully enter these compartments.

Pharmacokinetic aspects (time point of delivery)
In addition to this explanation, our study with monoHER revealed that the time point of administration of the antioxidant, to act protective, might be very crucial. MonoHER exerted protective effects when it was administrated one hour before cardiac ischaemia. In contrast, no (protective) effect was found when it was applied intravenously right before reperfusion. This phenomenon has been observed also in the doxorubicin mouse model. MonoHER protected against the cardiotoxicity of

doxorubicin when it was administered one hour before doxorubicin treatment but not when it was applied five minutes before doxorubicin treatment (unpublished observation, Prof. Aalt Bast). The data suggest that this time-dependency may be a specific characteristic of monoHER. Studies with superoxide dismutase [8] and a glutathione analogue [9] in dogs, as well as an α-tocopherol analogue in rats [10] and melatonin in mice [11] demonstrated that the time point of antioxidant administration did not influence the protective capacity of these antioxidants in *in vivo* models of experimental cardiac IR. In addition, studies in which the time point of administration of the antioxidant was shown to be crucial for its protective effect are not available in literature. This means that the observation that the efficacy of antioxidant therapy is dependent on the time point of administration is not a general concept. Further research is therefore necessary to determine this specific mechanism of action of monoHER.

The dominant concept of antioxidant therapy during cardiac IR is that the antioxidant agent reduces the detrimental effects of oxidative stress specifically during the initial phase of reperfusion by scavenging ROS [9, 12]. Recent pharmacokinetic studies with monoHER showed that the peak concentration of monoHER in heart tissue of mice was reached almost immediately after intravenous administration [3]. This implies that also in our current cardiac IR model the highest cardiac tissue levels of monoHER, when administered intravenously, right before reperfusion, are reached very early (within one minute) after the initiation of reperfusion.
Since this corresponds with the phase at which the production of ROS is the highest [9, 13], it was very unexpected that monoHER treatment lacked any beneficial effects when administered right before reperfusion. At the moment we do not have a clear explanation for this. Analyzing the pharmacokinetics of monoHER specifically in our cardiac IR model and determining the correlation between cardiac tissue levels of monoHER and ROS during reperfusion gives the opportunity to test if the dominant concept (see above) applies for monoHER too. If this is not the case, a logic explanation may be that the antioxidant monoHER protects against detrimental effects during the period of ischaemia and not during the initial phase of reperfusion. In this case, it is also evident that there are doubts about the truth of this dominant concept that ROS act detrimental initially after reperfusion (see above).

Despite the dominant concept (ROS are produced primarily with the reintroduction of oxygen following reperfusion), several investigators have observed ROS generation during ischaemia [14]. In addition, the fact that our studies showed that monoHER acts protective when applied before ischaemia but not when applied right before reperfusion also indirectly indicate possible detrimental effects of ROS during the experimental ischaemic phase. In our view it is therefore essential to further determine to which extent and at which stage ROS act detrimental during cardiac IR injury in order to deliver the agents in a time-sensitive fashion.

Clinical implications

Several clinical studies have evaluated the influence of antioxidants alone or in various combinations on IR injury in patients undergoing reperfusion therapy after myocardial infarction. Despite many research efforts and a wide availability of antioxidant agents,

there is at the moment no clinical evidence for the routine use of antioxidants in the clinical setting of cardiac IR. With respect to the clinical relevance it is evident that antioxidants are administered right before reperfusion therapy. In relation to our findings with monoHER this pleads in favour of a more thorough and critical analysis of a specific antioxidant at several time points of administration in an experimental setting before specific clinical trials should be done. However, the fact that some antioxidants act protective in animal models when applied right before or during reperfusion and not in a clinical setting of cardiac IR makes an explanation even more complex. For example the free radical scavenger recombinant h-SOD when administered before [12] or at the moment of reperfusion [15] acted protective in an *in vivo* dog model of cardiac IR but not in patients who underwent PTCA [16].
Myocardial infarction related to ischaemia-reperfusion is not a homogenous insult as shown by the patchy distribution of granulation and scar tissue. It is possible that some antioxidant compounds have failed because they protect the most affected regions of the area at risk but also contributed to additional injury in the adjoining tissue, as the border zones. This nearby tissue may loose preconditioning effects by the (non-specific) addition of antioxidants that scavenge ROS and these antioxidants are therefore interfering in adaptive natural cardioprotection.

Pharmacodynamic aspects
In our opinion it is necessary to define in more detail the patho-mechanism specifically related to cardiac repefusion injury and to determine the influence of ROS and antioxidants on this mechanism. This underlines the need for studies in which the analysis of patho-mechanisms of permanent ischaemia and IR is compared next to each other in one study in order to determine targets specifically related to reperfusion injury.
The comparison of the effects of cardiac IR and permanent ischaemia (PI) on the proteome can provide a first view at this mechanism. Proteomic studies of human heart tissue are, however, complicated by factors such as small size of sample, availability, disease state, tissue heterogeneity, genetic variability, medical history, and therapeutic interventions [17].
Our proteomic study was set-up in order to resolve part of this mechanism of action of monoHER in an *in vivo* mouse model of cardiac IR.
Our study demonstrates that *in vivo* mouse myocardial infarction models are suitable to study *in vivo* changes in the proteome early after induction of PI or IR.
We were able to identify proteins that were changed in expression level specifically after IR or PI and proteins that were commonly changed in level in both models.
Three proteins, Annexin A3&A5 and dual specificity phosphatase were significantly changed in expression level after cardiac IR but not after PI. This means that the changes in expression level of these proteins are specifically triggered by the process of cardiac IR. Their functional significance needs to be investigated in order to determine their impact on IR injury or the contribution to its mechanism.
Twenty protein spots were significantly changed in expression level after PI alone and are therefore potential targets for further study specifically on ischaemic injury.

However, we showed that the infarct size is almost 4 times greater after cardiac PI compared to IR (chapter 2). This means that the affected area is possibly bigger after PI and alterations in proteome are consequently easier to intercept. To overcome this problem, more specific techniques, as for instance laser capture micro-dissection, will be necessary to select more specifically the affected area for analysis. This is mainly true for the IR model as the infarct is patchy distributed in the area at risk.
In addition it is difficult to distinguish between detrimental effects triggered by the ischaemic period, the reperfusion period or the combination ischaemia and reperfusion. Studying the changes in protein expression specifically triggered by IR with different time-combinations of ischaemia and reperfusion can reduce part of this problem.
The protective effects of monoHER visible on histological and functional level were not detected on proteome level by using a technique of 2-DE combined with mass spectrometry.
This implies that more specific tools as for example subcellular proteomics (chapter 1), the detection of oxidation of proteins [18] or of intermolecular protein disulfide formation [19] are necessary to determine changes in the proteome due to oxidative stress in order to determine parts of the mechanism of IR injury.
We conclude that the specific alterations in protein expression that take place after cardiac ischaemia-reperfusion and permanent ischaemia may stimulate the search for new tools to resolve specific mechanisms and to find specific targets for therapy for these pathologies.

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Chapter 7

Samenvatting

Het eerste doel van dit proefschrift was om in een *in vivo* muizenmodel van cardiale ischemie-reperfusie (IR) na te gaan of de antioxidante stof 7-monohydroxyethylrutoside (monoHER) een beschermende werking heeft op het muizenhart. Om het werkingsmechanisme van monoHER in dit IR model te analyseren, was het tweede doel de vroege veranderingen in proteïneëxpressie in het muizenhart te onderzoeken bij dit model.

Het *in vivo* muizenmodel van cardiale IR is het studieobject van deze thesis. Daarom vergeleken wij in **hoofdstuk 2** de structurele en functionele effecten van cardiale IR op het muizenhart, met deze geïnduceerd door permanente ischemie (PI).
Het merendeel van de studies met betrekking tot dit model hebben het accent gelegd op de kortetermijneffecten van reperfusie. Door gebruik te maken van parameters als oppervlakte van het infarct en neutrofielinfiltratie werd de vooruitgang van weefselschade beschreven binnen een tijdsbestek van een paar uren tot een paar dagen na de inductie van reperfusie. De langetermijneffecten van IR-schade, in termen van weken of maanden, werden slechts in beperkt mate onderzocht in dit *in vivo* muizenmodel. Echter, voor extrapolatie naar een klinische situatie is het nagaan van de effecten op lange termijn zeer relevant, aangezien deze kunnen verschillen van de effecten op korte termijn.
In hoofdstuk 2 toonden we aan dat de structurele en functionele effecten van cardiale IR op lange termijn, verschillen van deze bij cardiale PI. De contractiliteit van het hart was aanzienlijk verminderd gedurende minstens 2 weken na IR, maar was min of meer hersteld 8 weken na IR. Na cardiale IR was de algemene architectuur van de wand van het linker ventrikel intact gebleven en was het proces van wondheling gekarakteriseerd door uitgebreide calcificatie, beperkte hypertrofie en beperkte dilatatie.
In het PI model was de ischemische zone niet gecalcificeerd, maar volledig vervangen door bindweefsel. Bijkomend was het linker ventrikel, ten gevolge van PI, sterk gedilateerd, het resterende spierweefsel sterk gehypertrofieerd en het verlies van de hartfunctie permanent.

Het potentieel beschermend effect van exogeen toegediende antioxidanten op cardiale reperfusieschade zijn al uitgebreid bestudeerd. Echter, zoals in detail beschreven in hoofdstuk 1, blijken de effecten van behandeling met antioxidanten in deze modellen controversieel en moeten deze verder uitgeklaard worden. Bovendien, de meeste van deze studies hebben enkel kortetermijneffecten en geen langetermijneffecten van IR bestudeerd.
In **hoofdstuk 3** hebben we de beschermende werking van de antioxidante stof monoHER op het hart, in het *in vivo* muizenmodel van cardiale IR, geëvalueerd. Dit tot 2 weken na IR, het tijdstip waarop het wondhelingsproces vervolledigd is.
Er zijn drie redenen waarom we voorop stelden dat monoHER mogelijk beschermend werkt in dit cardiaal IR model. Deze zijn: 1/ sterke radicaal opvangende en ijzer-bindende eigenschappen van monoHER; 2/ de aangetoonde beschermende werking van monoHER tegen de cardiotoxiciteit geïnduceerd door doxorubicine; 3/ *in vitro* anti-inflammatoire effecten van monoHER door een vermindering van de neutrofieladhesie.

We toonden aan dat monoHER blijvende beschermende effecten heeft in dit *in vivo* muizenmodel van cardiale IR. We hebben op 2 tijdstippen (24 uur en 2 weken na IR) aangetoond dat een behandeling met monoHER de infiltratie van neutrofielen vermindert, de oppervlakte van het infarct reduceert en bijkomend zorgt voor een behoud van de contractiele eigenschappen van het hart bij zowel stimulatie door dobutamine als door volumebelasting.
Voor zover we weten is deze studie de eerste die blijvende beschermende effecten aantoont van een flavonoïd in dit muizenmodel van cardiale IR.
Het tijdstip, de route van toediening en de dosis van monoHER waar in deze studie (hoofdstuk 3) voor gekozen werd, zijn gebaseerd op voorgaande studies in dewelke aangetoond werd dat monoHER een beschermend effect heeft in het model van doxorubicine geïnduceerde cardiotoxiciteit [1, 2]. In deze studies werd monoHER intra-peritoneaal, één uur voor doxorubicine injectie, toegediend.

Om de oxidatieve schade op het hart, veroorzaakt door een chemotherapeutisch middel zoals doxorubicine, te verminderen, is het logisch om patiënten vooraf te behandelen met een antioxidante stof als monoHER. Echter, om "reperfusieschade" te verminderen is het toedienen van een antioxidante stof, vóór de ischemische fase, vanuit klinisch oogpunt, niet relevant. Bovendien tonen recente farmacokinetische studies in muizen aan dat de maximale weefselconcentratie van monoHER bijna onmiddellijk na intraveneuze toediening bereikt wordt [3].
In **hoofdstuk 4** onderzochten we daarom of monoHER tevens effectief was wanneer het intraveneus, vijf minuten vóór reperfusie, toegediend werd. Toediening van monoHER beïnvloedde de infiltratie van neutrofielen in het hartweefsel, noch had het een beschermend effect op de cardiale contractiliteit, 24 uur na reperfusie.
MonoHER heeft dus een beschermende werking in het *in vivo* muizenmodel van cardiale IR wanneer het intraperitoneaal, één uur vóór ischemie toegediend wordt, maar niet wanneer het intraveneus, vijf minuten vóór reperfusie toegediend wordt.
Deze discrepantie in resultaten (tussen hoofdstuk 3 en 4) toont aan dat het tijdstip van monoHER toediening zeer cruciaal is om een zijn beschermend effect te realiseren.
Mogelijke verklaringen voor het ontbreken van een farmacodynamisch effect van monoHER in deze laatste studie (hoofdstuk 4) zijn: 1/ de mogelijke beperking in de opname van monoHER door het hartweefsel in het model van cardiale IR; 2/ de noodzaak voor de vorming van actieve metabolieten van monoHER; en 3/ de kans dat beschermende effecten, enerzijds direct of anderzijds indirect via antioxidante effecten, te wijten zijn aan een wisselwerking van monoHER met specifieke signaaltransductiecascades gedurende de periode van ischemie. Deze signaaltransductiecascades zouden kunnen geassocieerd zijn aan de beschermende werking van monoHER.
Aangezien signaaltransductiecascades opgebouwd zijn uit proteïnen is het essentieel om, ten einde deze hypothese te verifiëren, veranderingen in proteïneëxpressie of post-translationele modificaties van proteïnen te bestuderen.

Daarom werden 2 reeksen 'proteomics' studies uitgevoerd d.m.v. de techniek van 2-D gelelectroforese gecombineerd met massaspectrometrie (MS) voor de karakterisatie van de proteïnen.
Aangezien we in eerste instantie geïnteresseerd waren in de oorzaak van de beschermende werking van monoHER, bestudeerden we vroege (na 210 minuten) veranderingen in proteïneëxpressie. Bijkomend waren we geïnteresseerd in het identificeren van potentieel onbekende 'protein targets' die een invloed hebben in de vroege fase na het ontstaan van een hartinfarct. Voorgaande studies van andere laboratoria toonden aan dat deze techniek in staat is om vroege veranderingen in het proteoom, geïnduceerd door cardiale IR, te detecteren [4-6].
In **hoofdstuk 5** vergeleken we de veranderingen in proteïnedensiteit in de 'area at risk' van het muizenhart bij *in vivo* PI en IR. We analyseerden zowel cytosolische als membraanfracties en vonden een totaal van 32 proteïnespots (uit ongeveer 600 'matched' spots) die differentieel tot expressie kwamen.
Met deze techniek waren we in staat om 20 proteïnespots te bepalen die enkel na PI veranderden in densiteit, 4 proteïnespots die enkel na IR veranderden in densiteit en tenslotte 8 proteïnespots die gemeenschappelijk in beide modellen veranderden in densiteit. De resultaten van deze studie zijn daarom een basis voor verdere studies in dewelke de specifieke functionele relevantie van deze proteïnen in modellen van hartinfarct onderzocht kunnen worden.
De geïdentificeerde proteïnen (door MS) kunnen worden geclassificeerd in zeven functionele groepen, met name: 1/ anticoagulante proteïnen (Annexin A3&A5), 2/ structurele proteïnen (cardiac troponin T, α-tropomyosin and α-myosin heavy chain), 3/ inflammatoir-gerelateerde proteïnen (serum amyloid P-component precursor), 4/ transcriptie- en translatie- gerelateerde proteïnen (Prohibitin and Histidine Triad Nucleotide Binding Protein 1, Heterogeneous Nuclear Ribonucleoprotein K), 5/ heat shock proteïnen (Heat Shock Protein 20 en Heat Shock Protein 27), 6/ metabolisme-gerelateerde proteïnen (Adenylate Kinase 1, Pyruvate Dehydrogenase E1 Component Beta Subunit, Catechol-O-Methyltransferase) en 7/ overige (dual specificity phosphatase, similar to Zn-alcohol dehydrogenase).
Een opmerkelijke bevinding was de translocatie van annexines (A3 en A5) van het cytosolische naar het membranaire celcompartiment, tijdens cardiale IR. Een bevinding die tevens bevestigd werd d.m.v. 'Western blotting'. Het feit dat dit fenomeen enkel optreedt na reperfusie en niet na permanente ischemie suggereert een specifiek mechanisme dat de translocatie van annexines induceert gedurende reperfusie. Deze bevindingen zijn een bijkomende stimulans voor verdere studies in relatie tot annexines en de vroege diagnose van reperfusieschade met betrekking tot voorgaande studies in ons laboratorium [7].
Vier proteïnen waren op meerdere spotlocaties veranderd in expressieniveau, gekarakteriseerd door een verschil in isoelectrisch punt. In het geval van troponine T en HSP-20 waren deze veranderingen ook afhankelijk van het model. Bijkomend, één spot voor de proteïnen adenylate kinase 1, troponine T en HSP-20 was uniek aanwezig in de IR en/of de PI groep en niet in de respectievelijke sham groepen. Voortgezet onderzoek is nodig voor het bepalen van pathologie gerelateerde post-translationele modificaties.

Een totaal van 12 proteïnespots veranderde in expressieniveau na cardiale IR (**hoofdstuk 6**). Tien van deze proteïnespots konden gekarakteriseerd worden met MS. Deze waren: Heat Shock Proteïne 20 en Heat Shock Proteïne 27, Heterogeneous Nuclear Ribonucleoprotein K, Pyruvate Dehydrogenase E1-β, Annexine A3 (op 2 spotlocaties), Dual Specificity Phosphatase-3, Catechol-O-Methyltransferase, and Adenylate Kinase 1 (op 2 spotlocaties).
De intraperitoneale toediening van monoHER, één uur vóór ischemie, had geen invloed op het profiel van proteïneëxpressie gerelateerd aan cardiale IR. Dit betekent dat de beschermende effecten van monoHER op lange-termijn, die aangetoond werden op histologisch en functioneel niveau, niet geassocieerd waren met detecteerbare vroege veranderingen in het proteoom, nagegaan met 2-D gel electroforese. Mogelijke verklaringen voor de discrepantie in resultaten tussen beide studies in hoofdstuk 3 en 6 zijn: 1/ monoHER heeft geen invloed op de veranderingen in proteïneëxpressie na cardiale IR; 2/ het gekozen tijdstip is niet relevant voor het detecteren van veranderingen in het proteoom veroorzaakt door monoHER; 3/ de techniek die we gebruikt hebben was niet in staat om veranderingen in het proteoom, specifiek veroorzaakt door monoHER, aan te tonen.
We concluderen dat de specifieke veranderingen in proteineëxpresssie die plaatsvinden na cardiale ischemie-reperfusie en permanente ischemie de zoektocht stimuleren naar middelen voor het ontrafelen van specifieke mechanismen en het vinden van specifieke 'targets' die als therapie kunnen gebruikt worden.

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