Protection, adaptation or cell death as response to toxins

Valorization addendum

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1.1 Relevance

In this thesis, the response of a cell to a toxic compound (toxin), namely protection, adaptation or cell death, was investigated. In particular, the cellular response to the compounds acrolein, silver nanoparticles (AgNPs) and the flavonoids rutin and quercetin was examined. In the cellular response to these substances, redox (regulated) mechanisms via the induction of oxidative stress play a pivotal role.

Oxidative stress does not only play a role in the cellular toxicity of these compounds, it also plays a pivotal role in various age-related diseases including chronic obstructive pulmonary disease (COPD), cardiovascular disease (CVD) and cancer.

1.1.1 Chronic Obstructive Pulmonary Disease

Worldwide 65 million people have COPD. And every year, 3 out of 1000 are diagnosed with COPD (1, 2). It is a severe disease and current treatment is mostly aimed at preventing disease progression and reducing the symptoms, since a cure has not been found. Approximately 5 % of all deaths are due to COPD. This makes COPD the fifth leading cause of death worldwide and it has been predicted that in 2030, it will even be the third leading cause of death. Especially, smoking is involved in causing COPD. The prevalence of COPD in smokers is 3 times higher than in non-smokers (3). Next to smoking also other factors like age and gender are risk factors for developing COPD.

1.1.2 Cardiovascular disease

CVD results in almost 20 million deaths globally every year (4-6). This makes it the primary cause of death worldwide. The mortality risk is expected to increase by 36 % from 2017 to 2030. CVD is not one disease, but an umbrella term for a collection of diseases including cardiomyopathy, heart failure, coronary artery disease and cerebrovascular disease including stroke. Once developed stroke, the risk of reoccurrence is approximately 30 %. Many risk factors are involved in the development of CVD including age, gender, high blood pressure or physical inactivity. Remarkably, in 50 % of the strokes, hypertension plays a central role (7). The resulting hospitalization is the main contributing factor to the enormous health care costs of approximately 313 billion euro worldwide annually. The prevalence of hypertension is about 1000 million people. The prevalence of hypertension increases with age. Additionally, almost the whole population is at risk of developing CVD, since according to the Framingham Heart Study, 90 % of all the people will develop hypertension (8). Additionally, current diagnostic and treatment methods are lacking. Diagnosis is mainly focused on late stage risk factors and treatment is symptomatic rather than curative or preventive.

1.1.3 Cancer

Worldwide 25 million people have cancer (9, 10). And every year it is estimated that 10 million people are newly diagnosed with cancer. And this number is even increasing to 12 million new cases each year by 2030. The associated mortality is also very high, namely around 8 million deaths globally each year. This makes cancer the second leading cause of death worldwide. Cancer is a very heterogenous disease and can affect nearly any organ, most deaths are attributed to lung, liver, colorectal, stomach and breast cancer. The treatment is not very specific and is most successful when applied as early as possible. Unfortunately, most cancer types are diagnosed at late stage and screening methods still need to be improved. It is estimated that only 30-50 % of the cancers currently are preventable and the health care costs amount almost 1 trillion euro annually.

This extensive burden of the oxidative stress-related diseases including COPD, CVD and cancer underlines the need to develop strategies to treat patients suffering from the disease or prevent development or progression of the pathologic process (11-14). These diseases are complex and different factors are involved, but the common characteristic is the pivotal role of oxidative stress in their etiology. Acrolein induces oxidative stress and is mainly associated with an increase in the prevalence of COPD (15-17). The toxic effects of AgNPs are still enigmatic, although induction of oxidative stress seems to be involved (18-22). Evidence is emerging that the flavonoids, rutin and quercetin can protect against oxidative stress-related diseases, because of their antioxidant function (23). They can scavenge reactive oxygen species (ROS) and thereby protect against oxidative stress-related damaging effects to protein, lipid or DNA (24). Epidemiological studies regarding the expected beneficial effect of rutin or quercetin in the treatment or prevention of CVD or cancer are however controversial (25-31). In this thesis it was therefore further investigated via which mechanism these toxins induce their effects. Apparently, besides inducing protection (rutin, quercetin) or cell death (acrolein, AgNPs), all of these substances have also shown to induce adaptation (32-34). This implies that exposure to a low dose of the chemical for a relative short time is not toxic and can upregulate endogenous antioxidant systems that protect against a second challenge with a toxic, relative high dose of the specific toxin or exposure to the toxin for a relatively long time (35).

Risk assessment of toxins (36) starts with hazard identification and exposure assessment and in the European Union this is organized by REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) (37) and OECD (Organization for Economic Co-operation and Development) that also makes rules and regulations based on this risk characterization. Some chemicals have a threshold and others no threshold for their (toxic) effect. For some substances, even zero exposure is recommended. Current risk assessment procedures do not include the phenomenon of adaptation. Risk

assessment of especially acrolein and AgNPs with a non-observed adverse effect level (NOAEL) of 0.1 µg/m^3 and 100 µg/m^3 respectively should be reconsidered (38, 39).

Regarding rutin and quercetin, a better understanding of their molecular mechanism inducing adaptation or toxicity could result in a more targeted use in the treatment of CVD or cancer. The results in this thesis indicate that, after *in vivo* validation, rutin could may contribute to the therapy against CVD. Rutin is present in the daily human diet, mainly in fruit and vegetables like citrus fruits and buckwheat, it is a food component. If rutin is going to be used as a drug against hypertension and concomitant CVD, it will be a food component or nutrient that is used as a pharmaceutical drug, which can be defined as a nutraceutical. The most striking difference compared to a pharmaceutical, is that a nutraceutical mostly has multiple targets and effects (40).

Rutin in the presence of oxidative stress has been shown to upregulate Nrf2 and associated antioxidant genes, which could function as an early biomarker of CVD, which is likely better than the many current circulating biomarkers like C-reactive protein or triglycerides or a decreased high density lipoprotein. In the end it may lead to an aggressive decrease of atherosclerosis modifiers (ADAM) or development of new types of interventions and prevent early vascular aging (EVA) (41).

With regard to quercetin it is remarkable that in BSO-treated A375 tumor cells in regions with high oxidative stress Nrf2-mediated expression of endogenous antioxidant genes is decreased, while apoptosis is induced, whereas in (surrounding) non-tumor HEK293 (pTRAF$^{Nrf2/HIF/NF-\kappa B}$) reporter cells Nrf2-mediated protection by increasing endogenous antioxidant systems is enhanced. This indicates that the effect of the flavonoid is dependent on the presence of oxidative stress. To be able to use quercetin in current clinical practice as a treatment against cancer, more research is needed. The findings should be verified *in vivo*. The effects of quercetin will be dependent on the circumstances, level of oxidative stress and endogenous antioxidant presence, pointing at a very personalized potential regarding treatment or prevention of cancer by personalized nutrition.

1.2 Target groups

The findings from this thesis after verification *in vivo* could contribute to preventing or treating oxidative stress-related diseases like COPD, CVD or cancer. The knowledge obtained could also be expanded to the prevention or treatment of other oxidative stress-related diseases, for example diabetes. By this way it could be aimed to decrease the disease burden, to improve the life of the concerned patients and to diminish the related health care costs also resulting in some economical benefits. Furthermore, rules and regulations especially regarding risk assessment of the investigated toxins should be reconsidered on a national level, by the 'Rijksinstituut voor

Volksgezondheid en Milieu' (RIVM) or international level for example by the REACH or OECD from the European Union or the World Health Organization (WHO).

1.3 Activities and products

In particular, with regard to the antioxidant flavonoids, the chemical structure can be modified to either improve the uptake and bioavailability or activity of the specific flavonoid (42, 43). By this way a better therapeutic agent can be achieved with higher reactivity or better metabolic profile. Pharmaceutical companies and food industries will be interested, since on this modified flavonoid a patent can be requested and this nutrient can be developed to a new pharmaceutical treatment, a nutraceutical, which can be launched. This treatment can be personalized, since each patient may respond differently and the flavonoid could be changed in structure accordingly.

1.4 Planning and realization

Before a modified flavonoid can be launched as a new pharmaceutical product on the market or Nrf2 can be used as a biomarker, further studies are required. First, the found effects need to be validated further in *in vitro* and *in vivo* studies. Additionally, before the modified flavonoid can be commercialized, it needs to be validated in Phase I-IV trials, which will take a couple of years. Besides this pharmaceutical approach it might also be considered to adjust the current human diet. This can be done in a personalized way, resulting in personalized nutrition. In some cases the use of supplements of flavonoids could be recommended. Finally, it is aimed to change the current treatment recommendations in the European medicine evaluation board (MEB) or the 'Nederlands huisartsen genootschap' (NHG) standard for the investigated oxidative stress-related diseases including COPD, CVD and cancer. Regarding, the risk assessment of the toxins acrolein and AgNPs, NOAEL can be changed relatively fast by the REACH, OECD or the RIVM based on the current study of adaptive effects of the concerned chemicals and future supportive additional findings.

1.5 Innovation

In this thesis it has been described that the original view of Paracelsus on protection and toxicity should be extended by incorporating the level of adaptation or hormesis. A subtoxic dose of a chemical selectively targets specific pathways that induce adaptation and contributes to an enhanced protection on a longer term. It is found that this principle applies for the toxins acrolein, AgNPs and the flavonoids rutin and quercetin in the presence of oxidative stress. Additionally, we introduce a new term, the phenomenon of transhormesis, indicating that a low dose of one chemical induces a protective system (adaptation) that protects against the toxic dose of another chemical

for which a similar protective mechanism is induced by the adaptive response. Finally, the flavonoids rutin and quercetin only induce adaptation in the regions with a high level of oxidative stress. This makes the process selective and explains that despite of their short half-life, these flavonoids can be beneficial on a longer term. Apart from inducing adaptation, we also found that under specific circumstances flavonoids also enhance apoptotic cell death. This could explain the controversial epidemiologic findings regarding flavonoid intake in patients suffering from oxidative stress-related diseases. Furthermore, this adds another dimension to the paradigm of Paracelsus. The induction of adaptation or toxicity could both be beneficial or detrimental depending on the circumstances. For a healthy cell protective adaptation is beneficial, whereas in a tumor cell apoptotic cell death could be advantageous. This finding opens new directions to develop a personalized treatment or nutrition with flavonoids. In addition, the increased level of Nrf2 could be developed into a new biomarker that inversely predicts the progression of CVD. It could be an earlier biomarker than for example hypertension and most current tissue biomarkers. But in these promising innovative findings time plays an important role and it will also take some time before they are implemented in current clinical practice.

2 References

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