Exploring Redox Biology in physiology and disease

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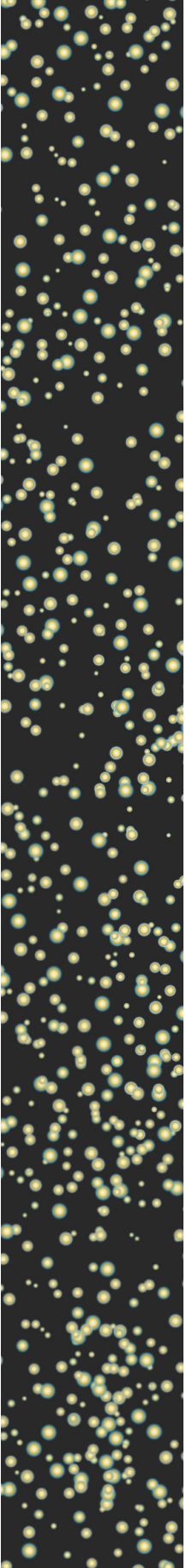

Chapter 12

Summary and Future Perspectives

Summary and discussion

Redox signalling is involved in a wide range of physiological processes and disturbance of the redox balance is a key factor in the development of disease.(1,2) Studying components of the redox network can provide valuable information to advance our understanding of disease conditions, to predict outcome and possibly to identify novel intervention targets. In **this thesis** we have studied elements of redox signalling, including serum free thiols and metabolites of gasotransmitters, hydrogen sulfide (H_2S) and nitric oxide (NO), in human populations and have related these to risk and outcome of cardiovascular and renal disease. Also, variants of the *cystathionine β-synthase (CBS)* gene - a gene involved in the regulation of H_2S production - were assessed for their relation to renal graft survival in humans. Finally, the therapeutic potential of H_2S was studied in rat models of renal disease.

As discussed in **Chapter 2** the reactive species interactome provides the rapid sensing and signalling that allows for adequate adaptation to internal as well as environmental changes. Central to this regulatory system are thiols. Because of their important role in transferring signals among distinct elements of the redox network, these functional groups, consisting of a sulfur and an hydrogen atom, are often called redox-switches. **Part 1** of this thesis focuses on the role of thiol biology in heart failure.

Chapter 3 describes the entanglement of various metabolic changes in heart failure and excessive production of reactive oxygen species (ROS), in particular by mitochondria. These reactive species can oxidize their targets by reacting with thiols. The remaining free thiol concentration may be seen as a direct reflection of the balance between oxidants and antioxidant capacity and thus the overall level of oxidative stress.(3,4) Accordingly, in **Chapter 4**, we show that in a small cohort of 101 stable chronic heart failure (CHF) patients *above*-average protein-adjusted serum free thiol concentrations are associated with a favorable disease outcome. Especially considering the fact that this study concerned patients with stable disease, serum free thiols appear to provide a robust reflection of redox status and confirmation of this finding in a larger cohort is currently underway. Compared to most conventional oxidative stress biomarkers, the advantage of free thiols is that they are active components of the antioxidant defense, which are known to be receptive to therapeutic modulation, for example by cysteine derivatives such as N-acetylcysteine.(5–7) The inconsistent and often disappointing results of therapies aimed at restoring redox status are likely related to hampering of the physiological functions of reactive species. However, in selected patients, with aberrant endogenous antioxidant capacity, these compounds may well prove to be effective. Furthermore, although beyond the scope of this thesis, there may be compositional variations in the thiol pool specific to CHF or other disease conditions worth pinpointing in the future, especially in the context of drug development. Alternatively, pharmacological stimulation of molecules that induce reversible protein modifications, including NO and H_2S, may offer opportunities to influence the amount of free thiols, as well as to favourably steer protein function.(4,8,9) **Part 2** of this thesis is dedicated to these gaseous signalling molecules and their metabolites.

In **Chapter 5** we provide an extensive review of the available literature on the roles of H_2S in renal physiology, disease and transplantation. We found evidence to support a positive

effect of H_2S on renal function by increasing blood flow, glomerular filtration rate (GFR) and electrolyte excretion. Data also suggest that H_2S holds promise as a treatment modality in renal disease as many of its biological functions, including vasodilatory, antioxidant, anti-apoptotic and anti-inflammatory properties, directly counteract the mechanisms involved in renal disease progression. This is supported by **Chapter 8** in which H_2S treatment, in the form of both sodium hydrosulfide (NaHS) and sodium thiosulfate (STS), is shown to protect against Angiotensin II-induced hypertension, proteinuria and renal damage. Interestingly, our group has also shown that in this model H_2S producing enzymes, CBS, cystathionine γ-lyase and 3-mercaptopyruvate sulfurtransferase, are down-regulated.(10) This finding implicates impaired endogenous H_2S production in the pathophysiology of hypertensive renal disease and thereby provides additional rationale for H_2S therapy in this context. In spite of numerous experimental studies demonstrating benefit of H_2S treatment in renal disease, among other pathological conditions, clinical translation of these findings has proven challenging. This is mainly due to the toxicity of H_2S at high concentrations, in combination with its volatile nature that complicates controlled administration. In this regard, the protective effects of H_2S therapy in the form of STS, shown in **Chapter 8**, are particularly promising as this compound can safely be administered to humans. In fact, it is clinically applied in doses of multiple grams, e.g. for the treatment of calciphylaxis in dialysis patients.(11) While thiosulfate is often dismissed as a breakdown product of H_2S, evidence suggests its reconversion into H_2S *in vivo*.(12–15) Studies reviewed in **Chapter 5** also indicate a beneficial role for both endogenous and administered H_2S in renal ischemia-reperfusion injury (IRI) and transplantation. In **Chapter 6** of this thesis a single nucleotide polymorphism (SNP) in the *CBS* gene of renal transplant recipients was found to be associated with increased graft survival. Although this association lost its statistical significance after adjustment for possible confounders, this was possibly due to the relatively low number of patients that carry this gene variant. As renal IRI has previously been shown to impair CBS activity, this finding may be of considerable importance and warrants confirmation in a larger cohort.(16,17) Increasing renal H_2S availability, through exogenous administration or stimulation of endogenous production, may be a promising strategy to minimize IRI during renal transplantation. Whereas the results from various experimental studies support this notion, in our unilateral renal IRI experiment in rats, described in **Chapter 7**, no protective effects of NaHS and STS treatment were found. This discrepancy may be explained by our particular treatment scheme of twice-daily intra-peritoneal injections. In fact, detailed review of available evidence revealed that application of treatment close to or even during the reperfusion phase may be essential to *in vivo* protection of the kidney against IRI. In turn, this may be related to the fact that renal blood flow is relatively unresponsive to hypoxia or hyperoxia and rather controlled by mechanisms that regulate renal function.(18) On reperfusion, arterial-to-venous oxygen shunting is likely to be inadequate, rendering the kidney susceptible to hyperoxia and consequent oxidation. Interestingly, *ex vivo* experiments in **Chapter 7** show that NaHS is able to induce metabolic suppression in isolated rat kidneys. While H_2S-induced whole body hypometabolism in larger mammals, including humans, is inapplicable, this finding implies a possible role for H_2S therapy in renal graft preservation. Unfortunately, STS appears to be unsuitable to serve this purpose, as previous research

indicates that the reconversion of TS into H_2S predominantly occurs in the liver and under hypoxic circumstances.(15) Accordingly, in Chapter 8 STS, in contrast to NaHS, was found to have no effect on intra-renal pressure in the isolated perfused kidney setup.

Apart from the potential toxicity and volatile nature of H_2S, another factor that complicates clinical translation is the absence of a reliable and convenient method for direct detection of H_2S in biological samples.(19,20) Nevertheless, H_2S metabolites may in part reflect endogenous H_2S levels. Interestingly, daily urinary excretion of TS and sulfate have been associated with a favourable cardiovascular risk profile in renal transplant recipients and preservation of renal function in patients with diabetes.(21–23) In Chapter 9 a strong inverse association was found between daily urinary sulfate, but not TS, excretion and all-cause mortality in a large sample of subjects from the general population. In this regard, it is important to note that the urinary excretion of sulfate - or any metabolite - is not determined by renal function. The mechanism that does underlie the association remains to be elucidated. Contrary to our expectation, we did not find a particularly strong association of TS or sulfate excretion with cardiovascular events or related mortality in this population. Meanwhile, Chapter 10 shows that in stable CHF patients daily urinary sulfate excretion is positively associated with favorable disease outcome, albeit not as strongly as renal sulfate clearance. Possibly, TS and sulfate are part of an adaptive response that is elicited in disease conditions - either directly or through their precursor, H_2S. This would explain the absence of a strong association between baseline values of TS and sulfate and cardiovascular outcome in predominantly healthy individuals from the general population. The apparent significance of the renal handling of sulfate in CHF patients calls for further investigation. Interestingly, certain factors that promote or interfere with H_2S production, similarly promote or interfere with tubular reabsorption of sulfate.(24–26) While vitamin D promotes H_2S production and tubular reabsorption of sulfate, glucocorticoids interfere with both.(24–26) Perhaps these processes serve a common purpose, which could be to increase sulfate levels. However, there is also the possibility that sulfate, contrary to the current notion, is somehow reconverted into H_2S *in vivo*.

In turn, the final oxidation product of NO, nitrate, is widely known to be recycled through the entero-salivary pathway, in which bacteria in the oral cavity reduce nitrate to nitrite.(27) However, the potential influence of the renal handling of nitrite and nitrate on their circulating levels has largely been disregarded. In fact, existing literature comprises almost no information about the handling of either anion by the kidneys. As described in Chapter 11 our study is the first to provide reference values for nitrite and nitrate clearances in humans. Moreover, we have shown that nitrite and nitrate are handled by the kidneys in separate ways. The association of nitrate, but not nitrite, clearance with favourable outcome of disease in CHF patients indicates the clinical relevance of this distinction. Finally, next to the entero-salivary pathway, renal tubular reabsorption represents another important route of nitrite and nitrate salvage that should be taken into account by future research.

Future perspectives

Even though data from human cohorts remain scarce and observational, the available evidence underscores the significance of redox signalling in physiology and disease, as implied by experimental research. Augmenting free thiol availability, either directly by cysteine derivatives or indirectly through pharmacological stimulation of reversible protein modifications, may prove to be beneficial in selected patients. Further research is needed to establish the way in which modifications by small molecules, including NO and H_2S, affect protein function to allow this concept to be exploited therapeutically.

The obstacles that challenge the clinical translation of H_2S research are not likely to be overcome in the near future. However, H_2S-related drugs that can safely be administered to humans, such as STS and possibly other compounds, do hold promise for clinical application and their investigation in human populations is warranted. In accordance, the start of a clinical trial of STS treatment in myocardial infarction is pending.

To expand our knowledge of redox signalling and to establish its actual significance in humans, interesting study subjects include healthy individuals, under physiological conditions or subjected to various forms of stress. In this respect, we are currently evaluating serum free thiol concentrations in a large population based cohort. Furthermore, dietary precursors and metabolites of primary elements of the redox network, such as cysteine and sulfate for sulfur and arginine and nitrate for nitrogen species, conceivably influence redox signalling and thereby may affect aging or development of disease. Likewise, studying redox signalling in various human diseases is of great interest.

While many research efforts have focussed on pinpointing a single targetable cause for a disease, a systems based approach would allow simultaneous analysis of various redox signalling elements and provide insight in their interactions. This type of information would greatly enhance our understanding of the network's architecture and its functioning in physiological and pathophysiological conditions. In turn, this will likely contribute to the successful therapeutic modulation of redox signalling.

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