Exploring Redox Biology in physiology and disease

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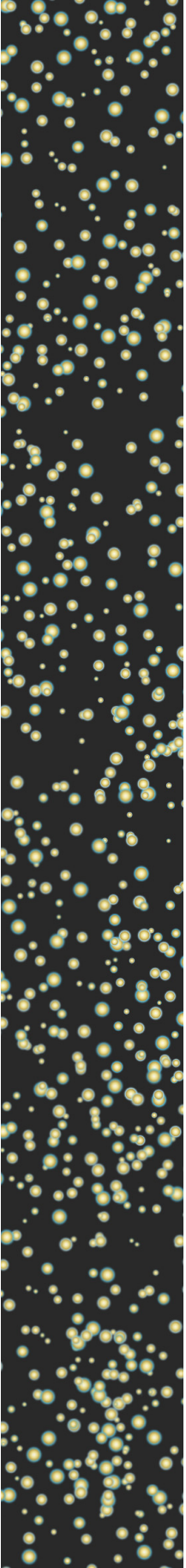

Chapter 1

Introduction

Redox biology

Redox reactions - characterized by the transfer of electrons from one chemical species to another - are responsible for the regulation of numerous bodily processes.(1) Under physiological conditions there is a fine-tuned balance between oxidants and reductive capacity. In these circumstances, reactive oxygen, nitrogen and sulfur species (ROS, RNS and RSS) are involved in various physiological cellular processes, including immunity, cell growth and apoptosis.(2–4) Common examples of these species are super oxide (O_2^-), hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$) and thiyl radical ($RS\cdot$). Unaccompanied by adequate upregulation of antioxidant defence mechanisms, excess production of reactive species leads to oxidative stress, which in turn has been implicated in the development of many disease conditions, including cardiovascular and renal disease.(2,5) The redox biology field has long focussed on the development of oxidative stress biomarkers. Even though these can be useful to determine the degree of oxidative stress and thereby the severity of a certain condition, most of these markers are oxidation products rather than active components of the redox signalling network and therefore provide little mechanistic insight, let alone intervention targets.(6)

Thiols

Thiols, on the other hand, are critical active components of the antioxidant machinery. These functional groups, consisting of a sulfur and an hydrogen atom, play an important role in transferring signals from distinct elements of the redox network and accordingly are often called redox-switches.(7–11) Since reduced (or free) thiols are readily oxidized by reactive species, their level may be interpreted as a direct reflection of the overall redox status.(11,12) So far, most studies looking into antioxidant machinery have focused on low molecular weight (LMW) thiols, in particular glutathione and cysteine. However, whereas LMW thiols are key actors in intracellular antioxidant defence, in the extracellular compartment these are strongly outnumbered by protein thiols.(7,13) In fact, the majority of thiols in serum (~0.6 mM) is accounted for by the single thiol group of albumin, the most abundant serum protein. Therefore, compared to the concentration of LWM thiols, the total free thiol level may be a more relevant circulatory marker of oxidative stress and, thereby, of disease severity.

Gasotransmitters

Reversible oxidative posttranslational modifications of protein thiols by several small molecules may protect proteins from irreversible oxidative damage and in certain cases alter protein function.(11,13,14) Among these redox-active small molecules are gasotransmitters nitric oxide (NO) and hydrogen sulfide (H_2S), which are able to induce S-nitrosylation and S-sulfhydration, respectively.(11) S-sulfhydration is indirectly brought about by H_2S, either through reactions of oxidized thiols with sulfide or through reactions of sulfide oxidation products with thiols, giving rise to persulfides.(15–17)

While gasotransmitters - further including carbon monoxide - were initially dismissed as toxic gases causing environmental hazard, over the past decades it became apparent that

these molecules are enzymatically produced and exert important regulatory functions in the mammalian body.(18) NO formation from L-arginine is regulated by endothelial, inducible and neuronal NO synthase (NOS), of which endothelial NOS, expressed by endothelial cells, is primarily responsible for NO production in the cardiovascular system. In turn, all H_2S producing enzymes, cystathionine β-synthase (CBS), cystathionine γ-lyase and 3-mercaptopyruvate, are abundantly present in the vasculature. Both NO and H_2S have been acknowledged for their versatile role in (patho)physiological processes and feature vasodilatory, antioxidant and anti-inflammatory properties, to name a few.(18)

In short, by constantly relaying signals the redox network is involved in the majority of bodily processes. Studying its components, such as thiols and gaseous signalling molecules, can provide valuable information to advance our understanding of pathophysiological conditions and may even reveal novel targets for therapy.

Scope of the thesis

The general aim of this thesis was to explore elements of redox signalling in physiology and disease. To this end, we have assessed concentrations of reduced thiols and gasotransmitter metabolites in blood and urine samples from a variety of human populations and have related these to risk or 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 investigated in relation to graft survival in human renal transplantation. Finally, the therapeutic potential of H_2S was studied in rat models of renal disease.

Chapter 2 comprises a more extensive introduction to redox biology and presents the concept of redox regulation by the reactive species interactome. The remainder of the thesis is divided into two parts.

Part 1 of this thesis focuses on the role of thiol biology in heart failure. In **Chapter 3** the link between metabolic changes in heart failure and excessive ROS production is reviewed and free thiols are introduced as a potential biomarker and target for therapy. In **Chapter 4** serum free thiol concentrations were determined in a cohort of stable chronic heart failure patients are associated with outcome of disease.

Part 2 of this thesis is dedicated to gaseous signalling molecules and their metabolites. **Chapter 5** provides an elaborate discussion of the available literature on the potential roles of H_2S in renal physiology, disease and transplantation. In **Chapter 6** single nucleotide polymorphisms in the *CBS* gene – a gene that regulates the expression of one of the H_2S producing enzymes – are studied in relation to renal graft survival in humans. H_2S treatment has previously been shown to protect against renal ischemia-reperfusion injury (IRI). While in mice protection is ascribed to H_2S-induced metabolic suppression, in larger animals metabolism is generally found to be unaffected by H_2S.(19–27) However, this gaseous signalling molecule is also considered to be protective independent of metabolic suppression, e.g. by means of its vasodilatory, anti-inflammatory and anti-oxidant properties.(18) In **Chapter 7** we investigated the therapeutic effects of sodium hydrosulfide (NaHS) and sodium thiosulfate (STS) in a model of unilateral renal IRI in rats and the potential of NaHS to induce hypometabolism in isolated rat kidneys. Although thiosulfate is generally

dismissed as a break down product of H_2S, there is evidence to support its reconversion into H_2S *in vivo*.(28–31) As a treatment modality, STS is of particular interest as it can safely be administered to humans. In **Chapter 8** the renoprotective properties of NaHS and STS were studied in a rat model of Angiotensin II-induced hypertensive renal disease.

A logical first step towards translation of H_2S research would be to study endogenous H_2S metabolism in various human populations, ranging from healthy subjects to patients with severe disease. However, unfortunately a reliable method for direct detection of H_2S in biological samples is lacking.(32,33) Alternatively, quantification of its metabolites, thiosulfate and sulfate, may be used to evaluate changes in H_2S metabolism, although other sources of these metabolites as well as their potential direct (patho)physiological roles should be taken into account when doing so. In **Chapter 9** urinary excretion of these sulfur metabolites is assessed in relation to risk of cardiovascular events and all-cause mortality in a large sample of subjects from the general population. **Chapter 10** explores changes in sulfate clearance in CHF patients compared to healthy individuals, as well as the relationship between sulfate clearance and outcome of disease.

Whereas H_2S has only recently been recognised as a cardiovascular signalling molecule, NO has been acknowledged for its role in cardiovascular physiology ever since its identification as endothelial derived relaxing factor back in 1987.(34–37) Circulating concentrations of its metabolites, nitrite and nitrate, are often used as markers of endogenous NO production. However, the potential influence of the renal handling of these anions on circulating levels has largely been disregarded. In fact, surprisingly little is known about the handling of either anion by the kidneys. In **Chapter 11** we therefore aimed to elucidate the renal handling of nitrite and nitrate and to explore its relevance to clinical outcome in CHF.

In **Chapter 12** the content of this thesis is summarized and discussed. To conclude, we provide our perspective on the future of redox biology research and the therapeutic potential of redox modulation in aging and disease.

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