Can aminothiols be distinguished from reactive oxygen species?

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Refers to: Patel R.S. *et al.* A Novel Biomarker of Oxidative Stress is Associated with Risk of Death in Patients with Coronary Artery Disease. Circulation (2015).

Glutathione (GSH) is considered the major natural antioxidant, protecting cells from oxidative stress (OS). In patients with coronary artery disease, OS was quantified by plasma levels of aminothiols: cysteine and GSH. The ratio between these species levels was associated with increased mortality. Is this a new approach for clinical risk stratification?

Redox thiol status is a dynamic system which is probably linked to the extracellular antioxidant defence system. The glutathione-dependent system is one of the key systems regulating cellular redox balance[1]. Cysteine is present in its oxidized form, cystine, in the extracellular space, and is the rate-limiting substrate for glutathione (GSH) synthesis. GSH is present in cells in millimolar concentrations and is considered the major natural antioxidant, protecting cells from oxidative stress (OS). The availability of cysteine/cystine is the rate-limiting step in GSH synthesis. Cysteine is transported into cells via neutral amino-acid transport systems, whereas cystine, the predominant form in plasma and extracellular body fluids, is carried by the anionic amino-acid transport system[2] (Figure 1).

Elevated plasma total homocysteine (tHCys), an intermediate metabolite of methionine, is a risk factor for early-onset cardiovascular disease[3]. Hyperhomocysteinemia increases OS and is closely related to the accumulation of asymmetric dimethylarginine (ADMA), an endogenous nitric oxide (NO) synthase (NOS) inhibitor[4]. Reduced, oxidized and protein-bound forms of HCys, cysteine and cysteinylglycine (Cys-Glyc) in plasma interact via redox and disulphide exchange reactions, and these aminothiol species comprise a dynamic system referred to as the redox thiol status[5].

In a recent study, Patel *et al.*[6] drew attention to the importance of alterations in aminothiol markers of non-free-radical-mediated OS in patients with coronary artery disease. OS was quantified by plasma levels of aminothiols, cysteine and GSH, and the ratio between these levels was associated with mortality in patients with coronary artery disease (CAD).

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In this study, participants aged 20-90 years were recruited as part of an ongoing prospective cohort of patients (1,411) enrolled prior to undergoing coronary angiography for the investigation or management of CAD. Blood was drawn with a syringe through the arterial sheath at the time of catheterization. Recruited patients were stable at the time of enrolment and were about to undergo an elective procedure, although stable patients with non-ST elevation myocardial infarction, defined using international criteria, were also included. Fifteen patients (1%) were lost to follow-up and were excluded from the analysis, leaving 1,411 patients with complete biomarker and follow-up data. Plasma cysteine, cystine, GSH, and its oxidized form GSH-disulphide (GSSG) were measured in all subjects using high-performance liquid chromatography (HPLC) coupled to mass spectrometry. Serum concentrations of hs-CRP, as an inflammation biomarker, were measured. The primary end-point was a composite of all-cause mortality and hospitalization for a cardiovascular event. The cause of death was identified in all cases. The cohort was prospectively followed for a mean of 4.7 +/- 2.1 years to determine the primary outcome of all-cause death and the secondary outcomes of cardiovascular death and the composite of death/or non-fatal MI (n=247). High levels of cystine (oxidized) and low levels of GSH (reduced) were associated with increased risk of death ($p<0.001$ both) before and after adjustment for covariates. A high cystine/GSH ratio was significantly associated with mortality and was independent to the hs-CRP level.

Important conclusions can be drawn from this study by Patel and colleagues. First, they showed that the cystine/GSH ratio was significantly associated with mortality and that this effect was independent of inflammation as assessed by hs-CRP. Second, the investigators indicated that a combination of biomarkers, reflecting aminothiol-mediated OS, and inflammation quantified by CRP, may offer a helpful approach for clinical risk stratification. Patel and colleagues argued that their results supported the use of these aminothiols as biomarkers of OS and suggested that they could potentially contribute to the development of new anti-oxidant therapies. Nevertheless, we agree with their comments that the use of these aminothiols in clinical practice requires further studies. The incidence of diet on the evolution of plasma aminothiol levels needs to be investigated as does the relationship between thiol-containing medications such as N-acetylcysteine and the metabolism of these emerging aminothiol markers.

It is tempting to think that in the plasma, the cystine/GSH ratio, which is significantly associated with mortality in patients with CAD, 1) does not reflect intracellular defences and 2) is the result of chain-breaking antioxidants acting by combining with chain-propagating radicals[7]. It should be pointed out that antioxidant inhibitors have more than one mechanism of action. It is now well-established that one major antioxidant action of blood plasma is the prevention of transition metal ions (Fe^{3+}/Fe^{2+}) from accelerating damaging free-radical reactions (figure 1). The reactive species are present at very low concentrations and are difficult to measure directly. An important point in the redox-signalling pathways is that compartmentation is an important aspect of cell signalling mechanisms [1]. Intracellular

GSH is compartmentalized as different redox pools within the cellular compartments of the cytosol, endoplasmic reticulum and nucleus. A distinct mitochondrial GSH (mtGSH) pool preserves the integrity of mitochondrial proteins and lipids and controls mitochondrial ROS generation.

Within cells and in the extracellular compartment, iron is in a dynamic equilibrium and the interplay between iron and reactive oxygen species and antioxidants, including GSH/GSSG, is complex[8]. It is also important to remember that cysteine transport is upregulated in conditions of OS and that electrophilic agents, such as diethylmaleate, are GSH-depleting agents[2]. NADPH/$NADP^+$ contribute to antioxidant defences by controlling cellular OS and the GSH/GSSG redox balance. NADPH is pivotal in GSSG reduction and NADPH/$NADP^+$ were found to influence cellular signalling and ROS production by the electron transport chain or by NADPH oxidases[4].

Finally, we would like to comment on two issues. Firstly, the concept developed by Patel *et al.* that: "free radicals may not constitute clinically important sources of oxidants and that non-free radical species may be of equal or greater importance" should be tempered since one major role of *in vivo* antioxidant defences is to attenuate free-radical reactions. However, the second issue concerns the concept that cystine/GSH ratio is associated with higher mortality: it is a major innovative result concerning the identification of pathophysiological mechanisms related to cardiovascular risk.

Competing interest

The authors declare that they have no competing interests.

Figure 1: Interplay between aminothiol species and reactive oxygen species

ASC: alanine, serine, cysteine neutral amino acid transport system

Cys: cysteine 

Cys-Gly: cysteinyl glycine

CGS: cystine-glycine synthase

G6PDH : Glucose-6 phosphate dehydrogenase

GCL: glutamyl-cysteine ligase

Glu: glutamic acid 

Glu-Cys: L-γ-glutamyl cysteine

Gly: glycine 

GSH: glutathione (L-γ-glutamyl cysteinyl glycine)

GSSG: glutathione disulphide

GS: glutathione synthase

GST: glutathione-S-transferase

HCys: homocysteine 

mtGSH: mitochondrial glutathione

MRPs: multi drug resistance-associated proteins

SOD: superoxide dismutase

xCT/4F2hc: cystine transporter

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