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## Oxidative stress and atherosclerosis in early chronic kidney disease

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### Oxidative stress and cardiovascular disease: fundamental aspects

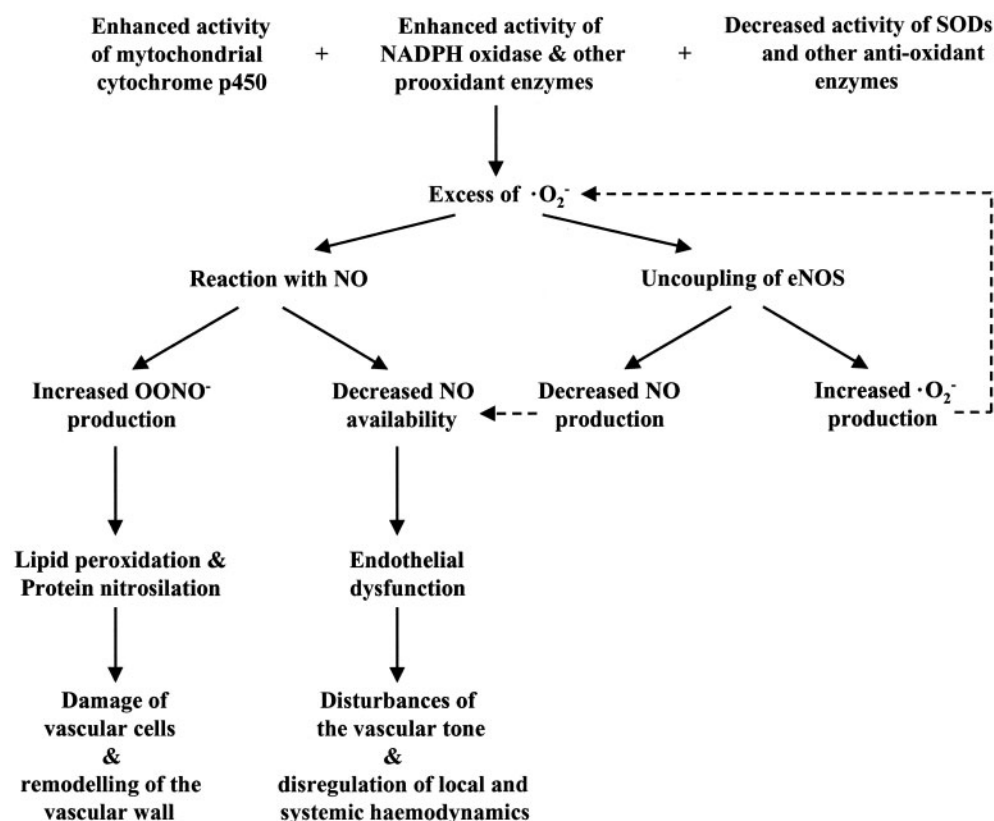
#### *The chemical basis of oxidative stress*

Reactive oxygen species (ROS) are intermediary metabolites that are normally produced in the course of oxygen metabolism. Under physiological conditions, ROS play a critical role as signal molecules, and ROS produced by

activated leucocytes and macrophages are essential for defence against the invading micro-organisms. In addition to a mitochondrial origin, ROS can be generated by a great number of enzymes including oxidases, cyclo-oxygenases and lipoxygenases. Normally, ROS are contained by a wide array of antioxidant enzymes and endogenous and dietary antioxidants. The excess production of ROS or impaired antioxidant defense capacity leads to oxidative stress, in which uncontained ROS cause oxidation of macro-molecules, tissue damage and dysfunction.

The primary ROS produced in the body is superoxide anion ( $\cdot\text{O}_2^-$ ) generated from a one-electron reduction of molecular oxygen. The nicotinamide adenine dinucleotide phosphate oxidase or NADPH oxidase is the main source of  $\cdot\text{O}_2^-$  in mammalian cells [1]. NADPH oxidase is a multicomponent enzyme that has a membrane portion collectively known as cytochrome *b*<sub>558</sub>, which is inactive until it is associated with the cytosolic components. Steady-state levels

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**Fig. 1.** This flow diagram illustrates the multifactorial origin, the intermediate pathways and final detrimental consequences of an excess of superoxide anion ( $\cdot\text{O}_2^-$ ) in the vascular system.

of  $\cdot\text{O}_2^-$  are dependent on both its rate of production as well as activity of various superoxide dismutases (SODs). In mammals, there are three isoforms of SOD (cytosolic or copper-zinc SOD, manganese SOD localized in mitochondria and an extracellular form of copper-zinc SOD); each are products of distinct genes but catalyse the same reaction: dismutation of  $\cdot\text{O}_2^-$  into hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) plus molecular oxygen [2].

#### $\cdot\text{O}_2^-$ -mediated oxidative stress and atherosclerosis

A number of findings support the notion that enhanced  $\cdot\text{O}_2^-$  levels play an important role in the pathophysiology of atherosclerosis (Figure 1). For instance,  $\cdot\text{O}_2^-$  may inactivate nitric oxide (NO) and diminish its bioavailability, thus inducing endothelial dysfunction [3]. Alternatively,  $\cdot\text{O}_2^-$  may promote oxidation of the endogenous NO synthase cofactor tetrahydrobiopterin, leading to NO synthase uncoupling with decreased NO production and increased  $\cdot\text{O}_2^-$  production from the enzyme [4]. In addition, the reaction product between  $\cdot\text{O}_2^-$  and NO, peroxynitrite, constitutes a strong oxidant molecule, which is able to oxidize proteins, lipids and nucleic acids, causing vascular cell damage [5]. Finally,  $\cdot\text{O}_2^-$  facilitates oxidative modification of low-density lipoproteins (LDL) that play a key role in the formation of atherosclerotic lesions [6].

Several studies have demonstrated the involvement of vascular NADPH oxidase in experimental atherosclerosis [7]. In addition, findings from different studies suggest a contributing role of NADPH oxidase present in phagocytic cells infiltrating the vascular wall in the development of the atherosclerotic lesion in humans [8–10]. On the other hand, the use of genetically altered animals provides evidence that a decreased expression and activity of SODs in the vessel wall may contribute to the development of the functional and morphological alterations present in atherosclerotic vessels [11,12]. In fact, reduced extracellular SOD activity in patients with coronary artery disease has been reported; this may be contributing to endothelial dysfunction in these patients [13].

#### Oxidative stress and cardiovascular disease in chronic kidney disease (CKD)—emerging aspects

##### *The connection of atherosclerosis with oxidative stress in CKD*

It is accepted that patients with advanced CKD (i.e. stages 3–5 according to glomerular filtration rate or GFR) are at greater risk for the development of atherosclerosis and associated morbidity and mortality [14]. In addition, recent evidence suggests that this process of cardiovascular damage starts very

early during progression in well-defined CKD, long before end-stage renal disease is developed (i.e. stages 1–2 according to GFR) [15,16].

The links between CKD and cardiovascular disease are probably numerous, since both share a number of common aetiological factors, and the circumstances derived from disease in one system can negatively influence the other organ system [17]. Thus, patients with CKD present an excess of traditional cardiovascular risk factors (i.e. age, gender, arterial hypertension, left ventricular growth and dysfunction, diabetes and dyslipidaemia); however, after adjusting for these factors, the association of CKD with prevalence of cardiovascular disease is seen to persist [16]. Therefore, it is likely that non-traditional risk factors (identified and as yet unidentified) are at play as well.

In this context, compelling evidence has emerged during the last few years pointing to a potential role of oxidative stress in the pathogenesis of atherosclerosis and other alterations in advanced CKD [18]. Oxidative stress in these patients has been attributed to the effects of processes specifically linked to the loss of renal function and/or the renal replacement therapy [19]. However, some recent findings indicate that oxidative stress is already present in early stages of CKD.

#### Exaggerated $\cdot\text{O}_2^-$ generation in early CKD

NADPH oxidase-dependent  $\cdot\text{O}_2^-$  generation has been reported to be abnormally increased in peripheral mononuclear cells (lymphocytes and monocytes) from patients with stages 1–2 CKD [20] (Figure 2). Since Galli *et al.* [21] reported abnormally enhanced NADPH oxidase-mediated  $\cdot\text{O}_2^-$  production in neutrophils from haemodialysis patients, it is likely that phagocytic NADPH oxidase overactivity may represent an early alteration that is maintained throughout the evolution of kidney disease.

The exaggerated activity of NADPH oxidase in phagocytic cells from patients with stages 1–2 CKD might be the result of a state of pre-activation of these cell types. In fact, it has been shown that pre-activated monocytes from patients with CKD exhibit enhanced ROS production and increased release of cytokines upon stimulation [22]. In addition, some extracellular stimulating factors of the NADPH oxidase enzymatic system deserve to be considered. On the one hand, insulin has been shown to stimulate NADPH oxidase activity in human peripheral mononuclear cells [23]. The pathophysiological meaning of these data is remarked by the finding that insulin levels were associated with phagocytic NADPH oxidase activity in patients with stages 1–2 CKD [20]. On the other hand, *in vitro* experiments show that advanced oxidation protein products (AOPP) activate NADPH oxidase in human mononuclear cells [24]. Interestingly, it has been reported that *in vivo* AOPP levels elevated early in the course of CKD (i.e. GFR > 80 ml/min/1.73 m<sup>2</sup>), increase with the progression of the

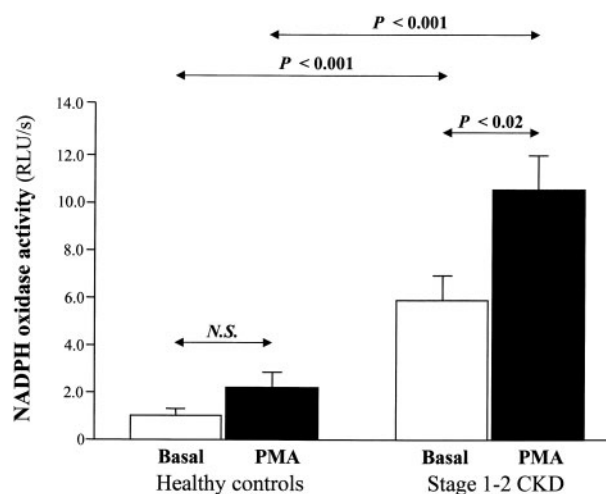


Fig. 2. Phagocytic nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity in control subjects and patients with stages 1–2 chronic kidney disease (CKD) measured in basal conditions (open bars) and after stimulation with phorbol myristate acetate (PMA) (closed bars). Bars represent the mean + SEM (Adapted from [20]).

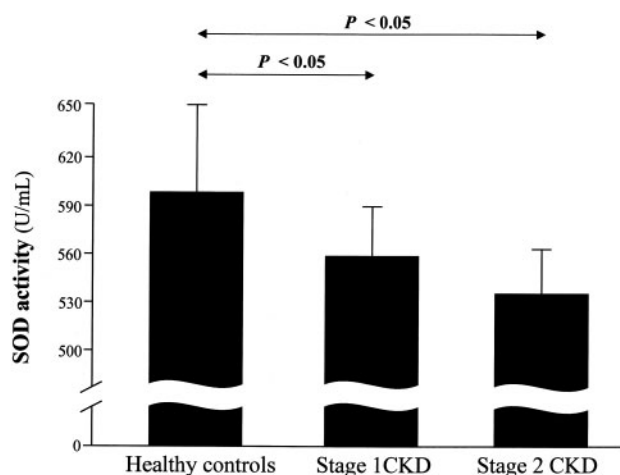
disease and are closely related to monocyte activation state [25].

The potential clinical relevance of enhanced phagocytic NADPH oxidase activity in patients with stages 1–2 CKD is further supported by the recent observation that this alteration is associated with enhanced carotid intima-media thickness (IMT) in asymptomatic subjects [26]. In fact, evidence substantiates the fact that carotid IMT correlates with the presence of coronary atherosclerosis and that enhanced carotid IMT represents an independent risk factor for coronary heart disease events, stroke and transient cerebral ischaemia [27].

#### Deficient $\cdot\text{O}_2^-$ scavenging capacity in early CKD

Recently, Yilmaz *et al.* [28] reported that erythrocytes from patients with stages 1–2 CKD exhibit lower SOD activity and lower levels of trace elements zinc and copper than cells from healthy controls (Figure 3). Erythrocytes from patients with stages 1–2 CKD also present reduction in another antioxidant enzyme, glutathione peroxidase activity, as compared with controls. Interestingly, these alterations worsen in parallel with the decline of GFR, thus patients with stage 5 CKD exhibit the most reduced values for the above parameters. Collectively, these findings suggest that the compromise of antioxidant mechanisms is also an early and progressive phenomenon in the evolution of CKD.

Interestingly, Yilmaz *et al.* [28] also found that the above antioxidant markers were negatively correlated with serum levels of asymmetric dimethylarginine (ADMA) but positively correlated with brachial artery endothelium-dependent vasodilatation. ADMA inhibits NO synthase by competing with L-arginine



**Fig. 3.** Erythrocyte superoxide dismutase (SOD) activity in control subjects and patients with stages 1 and 2 chronic kidney disease (CKD). Bars represent Mean + SD (Adapted from [28]).

and thus causes endothelial dysfunction. In addition, Zoccali *et al.* [29] reported that ADMA level is a strong and independent predictor of cardiovascular outcome and mortality in patients with advanced CKD. Although ADMA level is regulated by its renal clearance, it has been proposed that the activity of the enzyme dimethylarginine dimethylaminohydrolase that regulates the generation of ADMA is sensitive to oxidative stress [30]. Therefore, it is tempting to speculate that in early stages of CKD, decreased antioxidant defence mechanisms contribute to oxidative stress which, in turn, may induce ADMA-mediated mechanisms that will facilitate endothelial damage and cardiovascular damage.

### Towards a new paradigm with clinical impact?

The classical view is that oxidative stress represents an emerging threat to patient cardiovascular outcome in end-stage renal disease. From the evidence reviewed here, it is reasonable to consider that oxidative stress (probably due to the contribution of both stimulation of NADPH oxidase and inhibition of SOD) is already present at the earlier stages of CKD, and thus it is a potentially important mechanism of atherosclerosis from the beginning of the renal disease process. By assuming this paradigm, it is clear that measures aimed to detect and reduce oxidative stress cannot be restricted only to patients with advanced stages of CKD, but must also be expanded to patients with early stages. In this regard, the vast majority of studies based on antioxidants in the general population have produced negative results [31], thus oxidative stress should be better countered by decreasing  $\cdot\text{O}_2^-$  generation (i.e. inhibiting NADPH oxidase activation) [32]. Nevertheless, since there is still no solid evidence that oxidative stress is a modifiable condition, further clinical studies are required to test whether such an approach will add effectiveness to the prevention of

cardiovascular morbidity and mortality in CKD patients.

*Conflict of interest statement.* None declared.

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## The protean face of sarcoidosis revisited

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**Keywords:** glomerulopathy; granulomatosis; hypocomplementaemia; interstitial nephritis; immunotactoid; kidney; sarcoidosis

### Introduction

Sarcoidosis is a multisystem disorder characterized by non-caseating, epithelioid granulomas. Sarcoidosis predominantly affects the lungs; however, almost every organ can be involved including the kidneys. In fact, the absence of pulmonary findings by no means excludes sarcoidosis. The aetiology of the disease is still not fully elucidated. On the one hand, we have strong evidence from various sources suggesting that increased macrophage and CD4 helper T-cell activity

results in accelerated inflammation. This extensive local response ultimately causes the granuloma formation. On the other hand, sarcoidosis patients show suppressed immune responses to *in vivo* and *in vitro* antigen challenges, such as tuberculin. The latter state of affairs is in stark contrast to the accelerated inflammation hypothesis and suggests an anergic state. Anergy is believed to be responsible for the increased risk of sarcoidosis patients to acquire opportunistic infections and cancer.

Interesting new findings from affected patients show that expanded CD4<sup>+</sup>CD25<sup>bright</sup>FoxP3<sup>+</sup> accumulate in the periphery of sarcoid granulomas [1]. These cells represent regulatory T-lymphocytes with a strong anti-proliferative effect. However, this suppressive effect was not sufficient to completely inhibit tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) secretion in autologous lymphocytes. This finding would probably explain why granulomas still occur, for TNF- $\alpha$  is central to granuloma formation. The regulatory T-cells were nonetheless sufficient to prevent lymphocyte interleukin-2 (IL-2) secretion. The latter fact may induce a state of anergy by preventing antigen-specific memory responses.

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