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REVIEW ARTICLE

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Is uric acid a true antioxidant? Identification of uric acid oxidation products and their biological effects

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ABSTRACT

Uric acid (UA), the final product of purine metabolism in humans, exhibits a dual role as an anti or pro-oxidant, depending on the microenvironment. The two-electron oxidation of UA by biological oxidants can neutralize such harmful molecules. Additionally, UA chelates metals and can activate adaptive response against oxidation. However, some products of the reaction between UA and oxidants are not inert and, therefore, do not confer the anticipated antioxidant protection. A direct pro-oxidant effect is favoured in the one-electron oxidation of UA by heme-peroxidases yielding free radical intermediates that can initiate or propagate a radical-chain reaction. Additionally, an indirect pro-oxidant effect has been proposed by eliciting the expression or activation of enzymes that catalyse oxidant production, e.g. NADPH oxidase (NOX). This review brings together fundamental concepts and the molecular mechanisms of the redox reactions involving UA. The signature metabolites from these reactions are discussed to give valuable insights on whether these intermediates are being formed and what role they may play in disease pathogenesis. It proposes that, through identifying specific products, it may be possible to elucidate whether a harmful or protective action is linked to downstream bioactivities.

KEYWORDS

Uric acid; oxidation products; antioxidant; pro-oxidant

Introduction

Uric acid (UA) (7,9-Dihydro-1*H*-purine-2,6,8(3*H*)-trione) (Figure 1) is the final product from purine metabolism in humans and some primates. It is ubiquitously produced by cell metabolism but also by food digestion in the gastrointestinal tract [1]. The evolutionary gene silence for hepatic uricase in humans and certain primates dampened UA degradation, resulting in higher physiological levels in these upper-order mammals. This selective increase in UA may offer physiological benefits such as increasing blood pressure that contributes to postural evolution [2]. The balance between production and excretion of UA maintains physiological plasmatic levels in a relatively stable state, variating from 4.3 to 7.1 mg/dL [3]. However, it can be influenced by numerous factors such as age, sex, diet, body mass, physical activity, and medications [4].

At physiological pH, UA is predominantly as a mono-anion, termed urate. Urate is an efficient electron donor and can react with several biologically relevant reactive oxygen species (ROS). In 1981, a hypothesis was proposed that UA provides an additional low-molecular-weight antioxidant defence in humans [5]. It is worth mentioning that in the hydrophobic environment, UA loses its antioxidant ability [6] and so, there is some limitation in the ability of urate to interact with substrates that have high hydrophobicity.

Most notably, UA accounts for two-thirds of the total plasma antioxidant capacity [7]. Many studies demonstrated its protective role in different chemical and biological contexts. UA can act as a plausible antioxidant to prevent systemic oxidative damage in obese people [8]. The reduction in serum UA levels by rasburicase, a recombinant urate oxidase that decreases circulating UA ~90% [9], led to a decrease in serum and saliva antioxidant capacity, as assessed by the total radical-trapping antioxidant potential (TRAP) and ferric-reducing antioxidant potential (FRAP) assays. This diminished antioxidant capacity led to an increase in oxidative stress, as judged by the measurement of urinary isoprostanes and skeletal muscle protein carbonylation in obese subjects [8]. Irrespective of the mechanism, the protective action of UA is well-established especially in neurodegenerative diseases, protecting against the pathogenesis of Alzheimer's disease [10]. Peripheral blood total antioxidant capacity along with UA is decreased in Alzheimer's disease patients, whereas markers of lipid peroxidation are elevated and copper metabolism is dysregulated [11]. Similarly, studies have consistently described links between Parkinson's disease and low levels of UA [12,13]. Determining if low levels of UA are involved in the progression of neurodegenerative diseases is challenging. Accordingly, a strategy to increase UA levels in these patients has been proposed as an alternative treatment due to the notably UA plasma antioxidant effect.

On the other hand, accumulation of UA in circulation can lead to precipitation in the joints, manifesting as gout. Hyperuricemia is also linked with hypertension, atherosclerosis, insulin resistance and diabetes [14]. Commonly in humans, a decrease in UA urinary excretion is responsible for hyperuricemia [15,16]. Dietary factors such as the prevalence of a high-purine diet, fructose beverages, and alcohol consumption are linked to increased purine intake and consequently elevated UA levels, leading to increased incidence of hyperuricemia [17]. A recent study showed that serum UA

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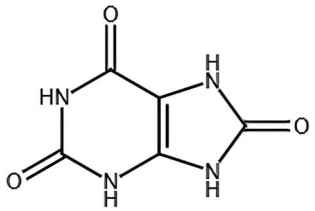

Figure 1. Uric acid chemical structure.

contemporary reference ranges were increased, now documented at 4.0–9.2 mg/dL for men and 2.8–6.9 mg/dL for women [18]. This study supports the hypothesis that lifestyle changes can impact UA levels, highlighting the importance of clinical management in controlling these levels to prevent the harmful effects of hyperuricemia.

The main described UA deleterious mechanisms are related to crystal deposition and gout arthritis. In this condition, monosodium urate crystals induce lysosome membrane disruption with activation of NLR family pyrin domain containing 3 (NALP3) inflammasome and production of active interleukin (IL)-1β and IL-18 [19]. UA in the soluble form also can activate the NLRP3 inflammasome and induce the production of IL-1β. This study suggests that the mechanism driving soluble UA-induced inflammation is dependent on redox state changes along with mitochondrial oxidant production [20]. In fact, Kimura et al. also demonstrated that physiological concentrations of soluble UA led to NLRP3-mediated secretion of IL-1β from human peripheral blood mononuclear cells. It was mediated by mitochondrial oxidants dependent on AMPK (AMP-activated protein kinase)-mTOR (mammalian target of rapamycin) [21]. Additionally to the activation of NALP3, monosodium urate crystals (MSU) are well-known activators of neutrophils [22] and by inducing the release of neutrophil extracellular traps (NETs) [23,24]. Therefore, UA can trigger/modulate inflammation by different and complementary ways and a full cover of this topic is beyond the focus of the present review. A better review of the pro-inflammatory mechanisms of UA was recently published by Li et al. [25].

Molecular effects of UA can be directly or indirectly linked to a pro-oxidant action. A direct pro-oxidant effect involves the transformation of UA into reactive intermediate species that can react with different biomolecules [26–29]. Stable metabolites resulting from UA oxidation can provide important insights into the identity of intermediates involved and their potential contribution to disease pathogenesis. They will be comprehensively analysed in this review article. UA can also exert an indirect pro-oxidant effect through induction/activation of ROS-producing enzymes like nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX) [7,30]. These pro-oxidant mechanisms are potential factors that can impact on diseases mentioned before. In this sense, this review article seeks to cover the mechanisms of action involved in the oxidant–antioxidant paradox of UA and propose reflections and new avenues in this aspect.

The indirect anti or pro-oxidant effects of UA

Antioxidant effect

One explanation for UA beneficial effects is the upregulation of signalling pathways that increase gene expression of endogenous antioxidant defence. In homeostasis, the transcript factor NF-E2-related factor-2 (Nrf2) is bound to Kelch-like ECH-associated protein 1 (Keap1) in cytosol and constantly ubiquitinated for degradation. In an oxidative environment, the cysteine sulfhydryl groups of Keap1 are oxidized, releasing Nrf2 that migrates to the nucleus and induces the expression of response genes [31,32]. An indirect antioxidant adaptive effect of UA can be attributed to the increase in Nrf2-responsive genes, including γ-glutamyl cysteine ligase (GCL) catalytic subunit (GCLC), heme oxygenase-1 (HO-1), and NAD(P)H quinone oxidoreductase 1 (NQO1) [33]. For example, chicken enterocytes were shown to synthesize and secrete UA in response to an insult with hydrogen peroxide, in a Nrf2-dependent mechanism. Additionally, the organic peroxide tert-butyl-hydroquinone (TBHQ) induced Nrf2 migration to the nucleus and mRNA expression of the UA transporters ATP-binding cassette sub-family G member 2 (ABCG2) and solute carrier family 2 member 9 (SLC2A9). In parallel there was an increase in UA and glutathione peroxidase activity [34], suggesting a link between Nrf2 and UA metabolic pathways. In human umbilical vein endothelial cells (HUVECs), UA decreased Nrf-2 ubiquitination, increasing the nuclear localization of this transcription factor and this was associated with a protection against the damage caused by oxidized LDL [35]. UA also induced resistance against oxidative stress and extended the life span of *Caenorhabditis elegans* (*C. elegans*) by activating the stress response factors SKN-1 (NRF2 ortholog) and DAF-16 (Forkhead box O transcription factor, FOXO homolog) [36]. In this sense, UA can activate key signalling pathways that are also responsive to redox signalling molecules as hydrogen peroxide. However, whether they share or not the same mechanism for this activation is still unknown.

UA at levels commonly encountered in humans effectively preserved and enhanced SOD activity partially inactivated in atherosclerotic vessels of ApoE−/− mice. SOD is an antioxidant enzyme that converts two superoxide radicals into oxygen and hydrogen peroxide and UA may regulate the vascular redox state by preserving SOD activity [37]. UA extended the lifespan of female mice with a decrease in protein nitration and lipid peroxidation in muscle and brain tissues of urate oxidase (UOX)+/− mice under metabolic and oxidative stress, emphasizing UA's role in mitigating the negative effects of aging [38]. In vascular endothelial cells, UA treatment induced an upregulation of antioxidant enzymes after 24 h. Thus, Peroxiredoxin 1, 4 and 6, Thioredoxin reductase 1 and Glutaredoxin-3 all increased as an adaptation to a primary oxidative stress since at earlier, within 1:30 h UA treatment, there was a down-regulation in these same families of antioxidant proteins. Additionally, this same UA treatment induced an overall pro-oxidant status within 2 h as measured by the oxidation of the intracellular probe Grx1-roGFP [39]. This duality of action for UA complicates the attribution of solely anti or pro-oxidant effects, as an adaptive antioxidant response likely involves UA acting via a primary pro-oxidant pathway.

Undoubtedly, the central nervous system is the body region where the most beneficial effects of UA have been documented, neuroprotective effects were reported in Alzheimer's and Parkinson's diseases and multiple sclerosis [33,40,41]. Enhanced oxidative stress and the dysregulation of metal levels are commonly and strongly associated with the pathogenesis of these diseases. The lipid structure of neuronal membranes with unsaturated fatty acids makes

neurons extremely sensitive to lipid peroxidation [42] and metals are known to catalyse lipid peroxidation [43]. UA is a metal chelator [44], which explains, at least in part, its protective role in the central nervous system. When the brain blood barrier is impaired, as typified in the case for Alzheimer's disease, levels of UA in the central nervous system can be concomitantly increased, thereby contributing to a substantial enhancement of local antioxidant capacity. UA levels in human cerebrospinal fluid (average = 17.7 μM) was positively correlated with levels in serum (average = 172.3 μM, $r = 0.669$, $p = 0.001$) and the impairment of the blood brain barrier led to higher UA levels in cerebrospinal fluid [45]. This can help to explain a high correlation between decreased UA levels and central nervous system diseases [46].

Supplemented UA (100 μM) protects cultured rat hippocampal neuronal cells from oxidative stress and administration of 370 μM UA 24 h prior to middle artery occlusion attenuated brain injury induced by acute ischaemia in rats [47]. However, under conditions favouring the one-electron oxidation of UA, as depicted below, the formation of urate free radical could be harmful rather than protective. Therefore, it is expected that UA is not acting solely as an antioxidant in this situation and may act in conjunction with ascorbate. Reduction potentials at pH 7.0 for urate ($HU^{\bullet-}$, $H^+/UH2^-$) and ascorbate ($Asc^{\bullet}$, H^+/Asc^- mono-anion) are, respectively, 0.59 and 0.28 V. Therefore, ascorbate can repair urate radical showing the potential synergy of these antioxidants (Figure 2) [48]. A similar synergism can be observed between ascorbate and α-tocopherol (vitamin E) [49,50]. The reduction potential of α-tocopherol (α-TO•, H^+/α-TOH) = 0.48 V is also higher than of ascorbate [51]. Therefore, ascorbate would be the common radical sink for urate and α-tocopherol-derived radicals, in aqueous milieu and membranes, respectively. Additionally, ascorbate concentration is around 10 mM in neurons [52] and the obvious question is whether urate and ascorbate act jointly to contribute to total cellular antioxidant capacity in neurodegenerative diseases. Waugh suggested that oral supplementation of ascorbate in combination with a metabolic precursor of UA like inosine or hypoxanthine, can prevent or slow Alzheimer's disease progression and amnestic mild cognitive impairment [53]. However, this study has certain limitations: (i) it was conducted exclusively through *in vitro* experiments and (ii) the potential synergistic interactions between UA and ascorbate were not directly investigated. To our knowledge, there are no currently *in vivo* studies that support this hypothesis. Therefore, conducting research to elucidate this relationship *in vivo* would hold considerable scientific significance with the potential to advance the treatment of neurodegenerative disease. Additionally, it was observed that UA at a concentration of 10 μM increased cysteine (Cys) uptake, enhancing glutathione synthesis in hippocampal slices [54]. Therefore, UA may contribute to the protection in nervous tissue through indirect or direct antioxidant activity.

Nonetheless, research on the potential neuroprotective role of UA in relation to neurodegenerative disorders has been controversial. Latourte et al. demonstrated that the risk of dementia increased with increasing serum UA levels in a cohort of 1,598 participants, and that the link was

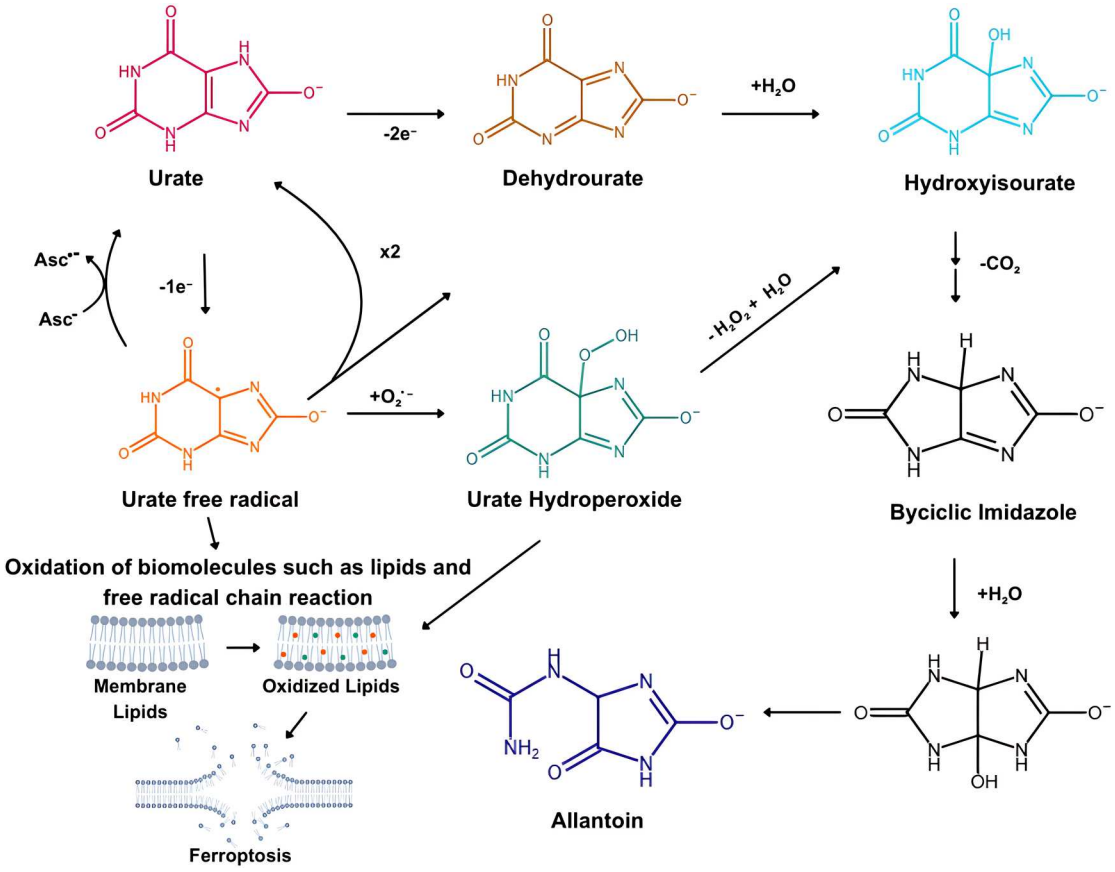

Figure 2. Uric acid undergoes two distinct oxidation pathways: the two-electron oxidation generates dehydrourate, which is hydrated to hydroxyisourate and eventually forms allantoin through four reactions; the one-electron oxidation produces urate free radical, which can dismutate into urate and dehydrourate. Alternatively, urate free radical can react with ascorbate (Asc^-) to regenerate urate while forming ascorbyl radical ($Asc^{\bullet-}$). Whether oxidizing other biomolecules, urate free radical can trigger a free radical-chain reaction or combine with superoxide $O_2^{\bullet-}$ to form urate hydroperoxide (HOOU).

stronger with vascular and mixed forms of dementia as compared to Alzheimer's disease [55]. Moreover, UA at 40 μM markedly decreased cell viability of differentiated SHSY5Y neuroblastoma cells and potentiated the pro-apoptotic effect of amyloid β. Under these conditions, the generation of 4-hydroxynonenal and the expression of PPAR β/δ promoted by amyloid β was intensified indicating a pro-oxidant effect in an *in vitro* model of Alzheimer's disease [56]. Together these results introduce uncertainty regarding the potential protective or detrimental role that UA may have in neurodegenerative disorders. In Alzheimer's and Parkinson's diseases, pathophysiological changes contribute to the low levels of UA including mitochondrial dysfunction, inherently low purine metabolism, dietary habits, changes in gastrointestinal motility or microbiome, or low physical activity [57]. Another point worth consideration is the fact that these patients tend to have other comorbidities like cardiovascular disease (CVD) burden, leading to a concomitant use of varying medications that may affect serum urate levels and complicate the evaluation of data from these patient cohorts. Hence, experimental design should be carefully planned to establish whether there is or not an association between serum urate and the condition of interest. UA levels measuring may not accurately reflect an individual's inherent level.

Pro-oxidant effect

Despite its well-documented antioxidant role, it is known that depending on the context, UA can act as pro-oxidant, including during its biosynthesis. UA is formed from the oxidation of hypoxanthine to xanthine and then to UA. The reaction is catalysed by xanthine oxidoreductase (XOR), an enzyme that can transfer the electrons to NAD^+ if acting as xanthine dehydrogenase (XDH) or to oxygen, if acting as xanthine oxidase (XO). The prevalence of one to another depends on the surrounding conditions and XO predominates under oxidative circumstances. Reduction of oxygen by XO generates the superoxide anion radical, contributing to the redox imbalance toward a more oxidative environment [14,58–60].

Supplementing UA decreased the expression of Sirtuin 1 (SIRT1) mRNA, and increased endoplasmic reticulum stress and oxidative stress in endothelial cells [61]. SIRT1 is a histone deacetylase involved in DNA repair, inflammation repression and autophagy and prevented ferroptosis cell death by regulating responsive genes to p53 and Nrf2 [62]. In this sense, the decrease in SIRT1 expression and its protective functions could be another possible mechanism involved in elevated oxidative burden mediated by UA.

Indirect pro-oxidant mechanisms of UA include an increase in the production of oxidants in mitochondria [20] and activation of NOX [63], with the generation of superoxide anion radical and hydrogen peroxide. In mature adipocytes, stimulation of NOX-dependent ROS by soluble UA triggered activation of p38 and ERK1/2 MAP kinases, decreased nitric oxide ($NO^{\bullet}$) bioavailability, increased protein nitration and lipid peroxidation, and could be an indirect promoter of insulin resistance [63]. NOX activation by UA also induced monocyte chemotactic protein-1 (MCP-1) mRNA synthesis and protein secretion, an adipokine that promotes a pro-inflammatory state in adipocytes in obesity. The increase in MCP-1 production was prevented by either superoxide scavenging or NOX inhibition. Additionally, decreasing UA levels in obese mice led to decreased macrophage infiltration in adipose tissue and improved insulin sensitivity [30]. Later, it was demonstrated that UA enhanced adipogenesis by upregulating NOX expression and by increasing the adipogenesis markers C/EBPα, PPARγ, and Mest, while reducing small lipid droplets and the anti-adipogenic factor Wnt10b.

Changes in adipocytes phenotype induced by UA were prevented by incubating cells with cobalt protoporphyrin (CoPP), a heme oxygenase (HO)-1 inducer, which promoted a decrease XO and NOX expression [64]. HO-1 is a powerful antioxidant response element; thereby suggesting that an indirect redox regulation is involved in the adipogenesis induced by UA. UA also induced a significant increase in NOX4 mRNA and protein expression, with elevations in oxidant production and changes in the mitochondrial membrane of the proximal tubule cells. In this study, authors found that apoptosis was dependent on NOX expression/activity and on UA internalization via URAT1 [65].

Soluble UA (100 μM) doubled superoxide production in human leukemic cells differentiated into neutrophils (dHL-60) that were activated with phorbol myristate acetate (PMA) within 30 min [26]. This increase was likely due to a direct modulation on NOX activity and not overexpression. NOX2, the most representative from the NOX family in phagocytes, comprises the cytosolic associated subunits, $p47^{phox}$, $p67^{phox}$ and $p40^{phox}$ that, upon activation, undergo phosphorylation, leading to translocation to the plasma membrane and association with flavocytochrome b_{558} heterodimer [66]. PMA activates protein kinase C that phosphorylates $p47^{phox}$ triggering superoxide production. More recently, it was demonstrated that $p47^{phox}$ glutathionylation was secondary to protein phosphorylation and sustained oxidant production in neutrophils. Cysteine at $p47^{phox}$ was glutathionylated either after PMA activation or hydrogen peroxide incubation with neutrophils, showing that oxidants can directly induce this post-translational modification independent of other mechanisms of activation [67]. Since UA is promptly oxidized in neutrophils to form urate hydroperoxide (HOOU) [26], it is reasonable to propose that it could directly upregulate NOX2 activity by inducing $p47^{phox}$ glutathionylation. However, further studies are necessary to confirm whether UA would exhibit such post-translational NOX2 modification. Despite the efforts to understand the role of UA in the pathogenesis of inflammatory diseases, it is presently unclear whether UA oxidation products could modulate such intracellular redox signalling pathways.

The paradoxical direct anti or pro-oxidant effect of UA

Currently, the precise biological effects of UA remain paradoxical and widely debated in the scientific community. As mentioned, UA can be an efficient electron donor (one-electron reduction potential = 0.59 V, pH 7.0, $HU^{\bullet-}$, $H^+/UH_2^{\bullet-}$) capable of reacting with oxidizing species. Nevertheless, breakdown products from UA oxidation can be different depending on the reagents present in the medium and these products can react with biomolecules and propagate oxidative damage. Also, UA appears to offer protective benefits only under certain conditions and is not effective in neutralizing all potentially damaging free radicals.

The two-electron oxidation of UA produces dehydrourate which is then hydrated to yield hydroxyisourate [68–70]. The

subsequent decomposition of hydroxyisourate follows four downstream reactions to yield allantoin as the final product. No free radical intermediates are generated in this two-electron oxidation process. Alternatively, UA can donate one-electron by reacting with free radicals or as a substrate for heme-peroxidases, yielding urate free radical. Dismutation of two urate free radicals recovers UA and produces dehydrourate, breaking a free radical-chain reaction. Alternatively, urate-free radicals can abstract one-electron from another biomolecule to propagate a free radical-chain reaction. The prevalent mechanism will depend on the local environment, since biomolecules with lower redox potential will be able to donate one-electron to urate free radical, as reported for ascorbate (Figure 2).

Urate free radical can react with superoxide radical anion at diffusion limiting rate (8×10^8 $M^{-1}s^{-1}$) [71]. It is expected to happen in an inflammatory milieu, where superoxide is abundant. Addition of superoxide to urate free radical generates urate hydroperoxide (HOOU) [26,72], a two-electron oxidant that, by oxidizing biomolecules, is reduced to hydroxyisourate and allantoin. Therefore, independent of the oxidation mechanism, allantoin is the common end-product (Figure 2).

Allantoin seems to be the predominant stable end-product in the biological environment [70]. The level of allantoin in body fluids is purported to be a reliable biomarker for *in vitro* and *in vivo* monitoring of oxidative stress [73]. Elevated plasma allantoin is associated with rheumatoid arthritis, type 2 diabetes, gout, and cystic fibrosis [74–76]. A substantial amount of allantoin was found in human atherosclerotic plaques [77,78] and the degree of atherosclerosis in knockout mice prone to atherosclerosis was positively associated with urinary excretion of allantoin [79]. Of relevance, a systematic review and meta-analysis from 19 studies revealed a significant increase of allantoin, but not UA, in patients with rheumatoid arthritis compared to healthy controls [80]. Likewise, our group found that allantoin was positively correlated with carotid intima-media thickness (c-IMT) from patients with subclinical atherosclerosis.

In this patient cohort, allantoin correlation with c-IMT was stronger than that for UA and it was independent of classical risk markers of atherosclerosis [81]. In one hand, allantoin is being proposed as a marker of oxidative stress where UA donates electrons, protecting other biomolecules from oxidation. Indeed, Nieto et al. showed that, in atherosclerotic patients, there was an increase in serum total antioxidant capacity when compared to controls [82]. This antioxidant capacity was correlated almost entirely by serum UA. Based on this and in other experimental evidence [5,83] it has been purposed that hyperuricemia could be a compensatory mechanism to mitigate oxidative damage in humans and that elevated UA levels in cardiovascular disease would reflect a beneficial response to the oxidative stress commonly associated with this condition. On the other hand, intermediates of urate oxidation are not inert, raising the question whether urate oxidation would be protective or deleterious.

Reaction of UA with oxidizing species

A well-documented antioxidant effect of UA is the capacity to react with peroxynitrite [80,83–87]. UA scavenges nitrogen dioxide radical ($NO_2^{\bullet}$), a product from peroxynitrite decomposition [88–90]. The kinetics of $NO_2^{\bullet}$ scavenging by UA was monitored by spectrophotometry at pH 7.4, with a second-order rate constant of 2×10^7 $M^{-1}s^{-1}$ and the formation of urate free radical [91]. In the presence of CO_2, peroxynitrite decomposes to $NO_2^{\bullet}$ and $CO_3^{\bullet-}$ [92,93] and the latter could also quickly react with UA [92], whose structure and redox potential are similar to the known $CO_3^{\bullet-}$ scavenger, 8-oxo-7,8-dihydro-29-deoxyguanosine (8-oxo-dG) [94]. However, two independent studies demonstrating divergent effects of urate against peroxynitrite-induced nitration in the presence of CO_2 open the question of whether urate would be a relevant scavenger for $CO_3^{\bullet-}$ *in vivo*. Whereas urate effectively inhibited peroxynitrite-mediated 4-hydroxyphenylacetic acid nitration (IC_{50} 100 μM) in the presence of CO_2 [92], it was ineffective against tyrosine nitration in bovine serum albumin [95], suggesting that the protective effect of urate depends on the competing substrate for nitration. The reaction of UA with peroxynitrite involves the consumption of oxygen to form allantoin, alloxan and urate-derived radicals. The main radical identified, aminocarbonyl radical, was likely responsible for amplifying peroxynitrite-mediated oxidation of liposomes and LDL [96].

Later, triuret was proposed as a specific biomarker of urate oxidation by peroxynitrite [97,98]. In the presence of peroxynitrite and its decomposition products, the one-electron abstraction from urate forms a urate free radical. Dismutation of two urate radical yields one urate and a dehydrourate. The latter is susceptible to a nucleophilic attack from H_2O to yield hydroxyisourate. Further hydrolysis of hydroxyisourate generates 2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline (OHCU), which equilibrates with H_2O. Decarboxylation of OHCU leads to the formation of a first intermediate (intermediate A), followed by the addition of an HO^- from H_2O to produce a second intermediate (intermediate B). The latter readily undergoes further decarboxylation to generate triuret [97]. The formation of triuret occurs in plasma and *in vivo* under pathological conditions. However, triuret is not inert and it has been described as the inhibitor agent of CD26/dipeptidyl peptidase (DPP)IV, suggesting a role for urate oxidation products in endothelial dysfunction [99]. In addition, the reaction of UA with peroxynitrite in the presence of alcohols leads to irreversible alkylation, which can accumulate [98]. Indeed, despite the fact that UA reacted 16 times faster with peroxynitrite than ascorbate, the latter was necessary to protect endothelial $NO^{\bullet}$ synthase (eNOS) from the uncoupling caused by the products of the reaction between UA and peroxynitrite, proving that the antioxidant effect of UA is only complete when paired with another electron donor with lower reduction potential to neutralize free radical intermediates of this reaction [86].

A paradoxical effect of UA has also been described in the availability of $NO^{\bullet}$. An early study proposed that the reaction between UA and peroxynitrite resulted in spontaneous release of $NO^{\bullet}$ [100]. However, urate can decrease $NO^{\bullet}$ availability in inflammatory situations by promoting $NO^{\bullet}$ oxidase activity of MPO [72] as does tyrosine [101]. In addition, Gersch et al. showed that $NO^{\bullet}$ is inactivated by UA in human plasma and cell lysates and 6-aminouracil was the most stable product of this reaction. This product was also found in urine from patients with hypertension and preeclampsia, underscoring the biological significance of the reaction between UA and $NO^{\bullet}$. In the presence of peroxynitrite or hydrogen peroxide, UA reacted

preferentially with $NO^{\bullet}$ and this reaction was partially blocked by glutathione [102]. Depletion of $NO^{\bullet}$ contributes to endothelial dysfunction by increasing vascular toned and is likely one of the mechanisms of how UA disrupts endothelial function.

UA either quenches or reacts with singlet oxygen ($^1O_2$) at a rate constant of 3.6×10^8 $M^{-1}s^{-1}$ [103] and 2.3×10^6 $M^{-1}s^{-1}$ [104], respectively. $^1O_2$, a very strong oxidant, is usually produced by photosensitization, but two-electron oxidation of hydrogen peroxide also can generate $^1O_2$ [105]. This prominent oxidant has an important role as a bactericide [106] but can lead to oxidative damage to host lipids [107], proteins [108] and DNA [109]. In this sense, the capacity of UA quenching $^1O_2$ can offer a beneficial role in cases of oxidative stress. However, in conditions of infection or inflammation, UA could damper $^1O_2$ physiological effects. Based on this, it is of great relevance to know how UA acts in a particular context. Oxidation of UA by $^1O_2$ generates mostly parabanic acid (Figure 3) and it has been purposed as a specific product for this reaction [69], offering the possibility to clarify the real impact of the reaction between UA and $^1O_2$ in a specific situation. UA and parabanic acid were found in skin lavage samples, and the levels were increased upon sunlight exposure [69].

Photo-oxidation of UA could simultaneously occur through Type I and Type II reaction mechanisms. In the former, there is the production of free radicals intermediates and, in the latter, the oxidation occurs through the formation of $^1O_2$. Parabanic acid was also elevated in aneurysmal subarachnoid haemorrhage [110] and in arteries pre-treated with IFN-γ, a condition that mimics inflammation [111]. The proposed mechanism for parabanic acid formation is depicted in Figure 3. Addition of $^1O_2$ to UA generates an endoperoxide that rearranges into a hydroperoxide. The following hydration – dehydration generates (2,5-dioxioimidazolidin-4-ylidene) aminocarbonylcarbamic acid (DIAA) that can subsequently undergo a hydration to form the 4-hydroxy-DIAA. The final formation of parabanic acid occurs by the leaving of N-carboxyurea (Figure 3). Alternatively, DIAA can be decarboxylated to form dehydroallantoin (DHA), and 4-hydroxyallantoin (4-HAL) and parabanic acid by the leaving of urea [112]. Although parabanic acid is proposed as a specific product from $^1O_2$ oxidation, the decomposition pathway that generates parabanic acid is common for HOOU and so, it can also be a product of superoxide addition to urate free radical. In fact, Type I photo-oxidation of UA, which involves the riboflavin-catalysed one-electron transference from UA to oxygen, with HOOU as the main intermediate, also had DIAA as a putative spontaneous decomposition product. The identity of the product was not investigated in this study, but it presented a *m/z* $[M - H]^+$ 199 and maximal absorption at $\lambda =$ 221 nm, which is very much expected for DIAA [29].

UA also scavenges hypochlorous acid (HOCl). The estimated rate constant for this reaction at pH 7.0 is 3×10^5 $M^{-1}s^{-1}$ [113–115]. This oxidant is produced from the oxidation of Cl^- by H_2O_2 catalysed by myeloperoxidase (MPO), playing a central role in host defence [116]. Nonetheless, HOCl can oxidize different residues in proteins [117,118], beside DNA and lipids [115], altering their function. Therefore, urate is supposed to have a protective effect against HOCl-induced biomolecule oxidation/degradation. However, the true antioxidant role of urate against HOCl in plasma has been controversial [119,120]. *In vitro*, the deprotonate ClO^-/HOCl (pKa = 7.5) chlorinates UA to generate 5-*N*-carboxyimino-6-*N*- chloroaminopyrimidine- 2,4(3*H*)-dione (CCPD) (Figure 4), whereas allantoin is a minor product [121]. CCPD is considered a specific product of urate oxidation by ClO^-/HOCl and, therefore, has been proposed as a marker for HOCl production. However, a more recent study demonstrated that CCPD was unstable in human plasma, limiting its use as a marker. Matsubara et al. found that CCPD

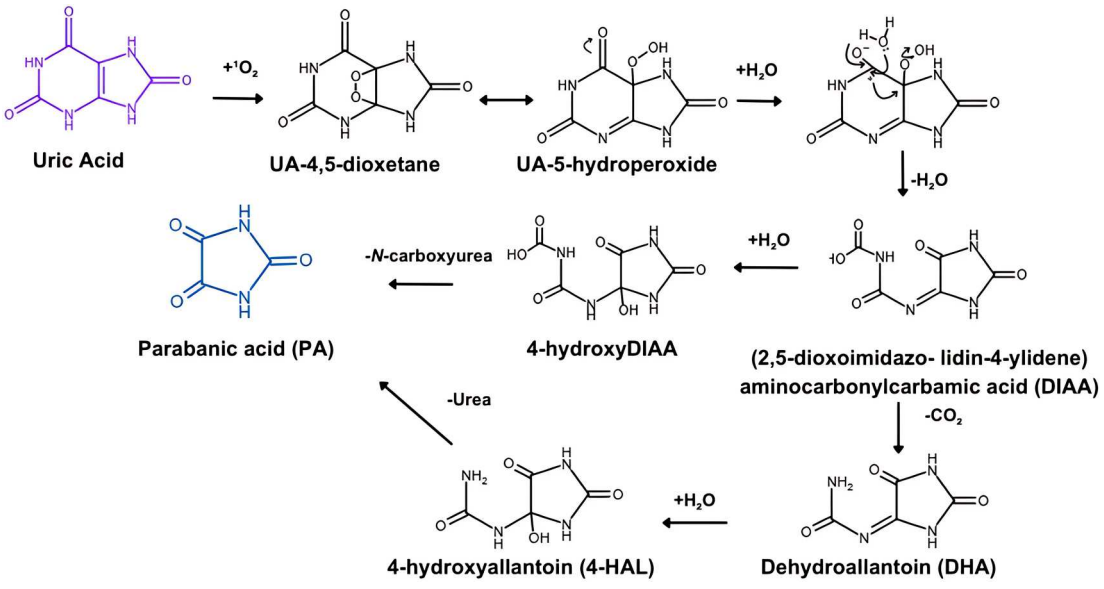

Figure 3. $^1O_2$ incorporates uric acid (UA), producing UA-4,5-dioxetane. The 4,5-dioxetane is converted to UA-5-hydroperoxide. The nucleophilic addition of H_2O on the C6 carbonyl carbon of UA-5-hydroperoxide outcomes in cleavage of the peroxide and the C5—C6 bond to yield (2,5-dioxoimidazo- lidin-4-ylidene) aminocarbonylcarbamic acid (DIAA). This scheme suggests that an oxygen atom from $^1O_2$ is incorporated into the 5-oxo- group and another oxygen atom from water is situated on the carboxylic group of DIAA. DIAA is then converted either to dehydroallantoin (DHA) by decarboxylation or to (4-hydroxy-2,5- dioxoimidazolidin-4-yl)aminocarbonylcarbamicacid (4-hydroxyDIAA) by hydration. DHA is hydrolysed to 4-hydroxyallantoin (4-HAL), which decomposes to parabanic acid and urea. HDAA is rapidly converted to parabanic acid and N-carboxyurea.

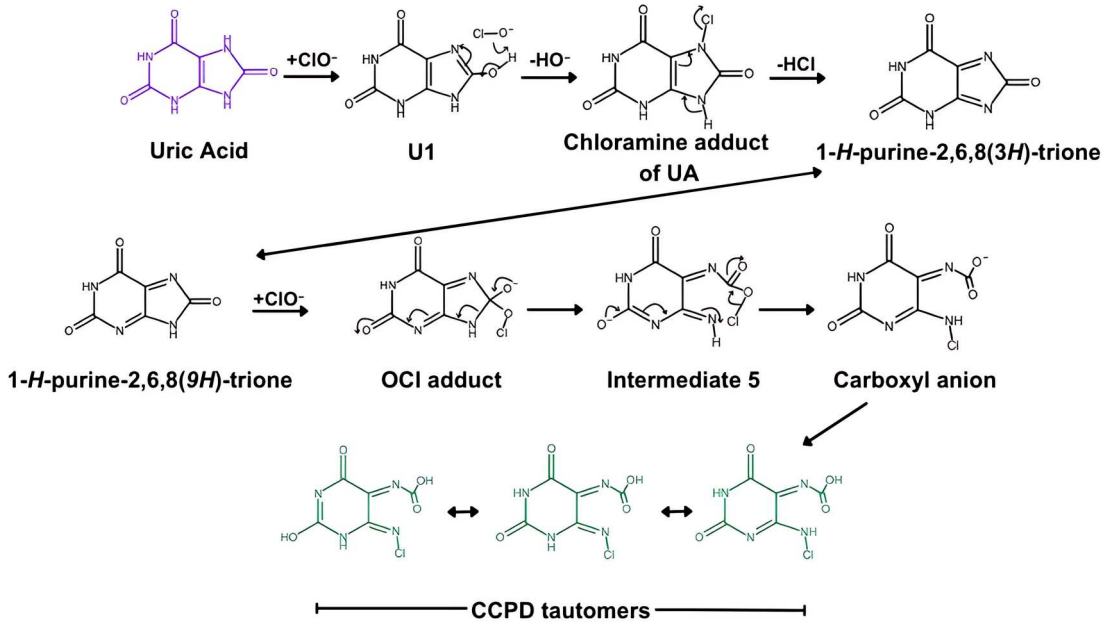

Figure 4. 5-*N*-carboxyimino-6-*N*- chloroaminopyrimidine- 2,4(3*H*)-dione (CCPD) is produced from the reaction of one molecule of UA with two molecules of ClO^-. HO^- and Cl^- are eliminated from the reaction product. The N = C–O–H of UA and ClO^- form a 6-membered ring releasing HO^- to yield the chloramine adduct of UA. This adduct subsequently releases HCl, resulting in the formation of 1-*H*-purine-2,6,8(3*H*)-trione. 1-*H*-purine-2,6,8(3*H*)-trione is tautomerized to 1-*H*-purine-2,6,8(9*H*)-trione. Nucleophilic attack on the C8 carbonyl carbon by a second ClO^- gives rise to an OCl adduct. Cleavage of the C8-N9 bond produces intermediate 5, which is isomerized to a carboxyl anion and subsequently protonated to form CCPD.

promptly reacts with thiol compounds such as glutathione and cysteine to yield 5-*N*-carboxyimino-6-aminopyrimidine-2,4(3*H*)-dione (CAPD) (Figure 5), which remained stable in human plasma [122]. CAPD was generated by MPO-induced UA oxidation and lipopolysaccharide-induced pseudo-inflammation in collected human blood. Thus, CAPD may act as a novel biomarker for ClO^- production *in vivo* [122].

Clearly, UA exerts a dual role depending on its chemical microenvironment. Measurements of UA signature metabolites such as allantoin, triuret, 6-aminouracil, CAPD and parabanic acid in different contexts, would provide insights into the relevance of each of these pathways in a determinate condition and/or disease.

Reaction of UA with heme-peroxidases

UA has been recognized as the primary organic substrate for peroxidases in human plasma [123]. It reacts with myeloperoxidase (MPO) [26,72], lactoperoxidase (LPO) [124], and peroxidasin (PXDN) [28,125] at rate constants that are depicted in Table 1. These heme-peroxidases are in the ferric form (Fe^{III}) when resting and react with hydrogen peroxide to form an oxyferril radical ($Fe^{IV}O \ldots Por^{+\bullet}$) denominated Compound I. Compound I can react with halides or pseudo-halides to yield the corresponding hypohalous or pseudo-hypohalous acid. This regenerates the native Fe^{III} enzyme in a halogenation cycle [126–128]. Alternatively, Compound I can react with other substrates, including UA, abstracting one electron to form the intermediate Compound II ($Fe^{IV}O$), Compound II abstracts a second electron from a second substrate to regenerate the Fe^{III} form of the enzyme in a peroxidatic cycle mechanism (Figure 6) [126–128].

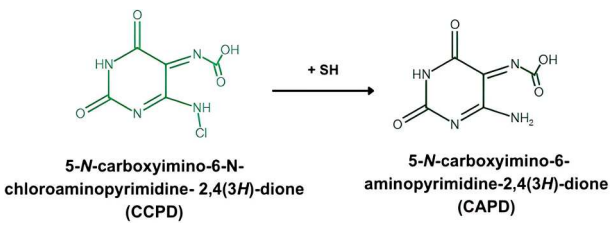

Figure 5. Carboxyimino-6-*N*-chloroaminopyrimidine-2,4(3*H*)-dione (CAPD) formation: The reaction between uric acid and ClO^- produces 5-*N*-carboxyimino-6-*N*- chloroaminopyrimidine- 2,4(3*H*)-dione (CCPD), an unstable product that reacts with thiol groups to form CAPD, a stable marker of UA oxidation.

The product of UA oxidation by heme-peroxidases, urate free radical, can combine with superoxide to produce the oxidant HOOU [72] which decomposes mainly into hydroxyisourate and allantoin (Figure 2). Our research group characterized [29] and identified, for the first time, the production of HOOU by isolated peripheral neutrophils [26]. Using HL-60 cells differentiated into neutrophils (dHL-60), we quantified urate oxidation by measuring a more stable product, hydroxyisourate (Figure 2). We found that 1.2 µM hydroxyisourate was produced per million cells incubated with 100 µM urate within 30 min. This is 10-fold lower than HOCl production (14 µM per million cells in 1 h at 140 mM chloride). However, urate was able to compete with chloride by MPO since, at 500 µM, it significantly decreased HOCl from 14 to 9 µM (~ to 60%). Urate further increased oxygen consumption, superoxide production and glutathione oxidation in cultured dHL-60 treated with PMA, demonstrating that, despite decreasing HOCl, UA still increases the overall oxidative status [26].

Although HOOU is not the sole source of hydroxyisourate, the study by Silva et al. irrefutably proved HOOU formation

Table 1. Second-order rate constants for the oxidation of urate by heme-peroxidases.

Enzyme	Compound I ($M^{-1}s^{-1}$)	Compound II ($M^{-1}s^{-1}$)	Reference
MPO	4.6×10^5	1.7×10^4	[76]
LPO	1.1×10^7	8.5×10^3	[128]
PXDN	1.9×10^4	5.8×10^2	[129]

Myeloperoxidase (MPO); lactoperoxidase (LPO), and peroxidasin (PXDN).

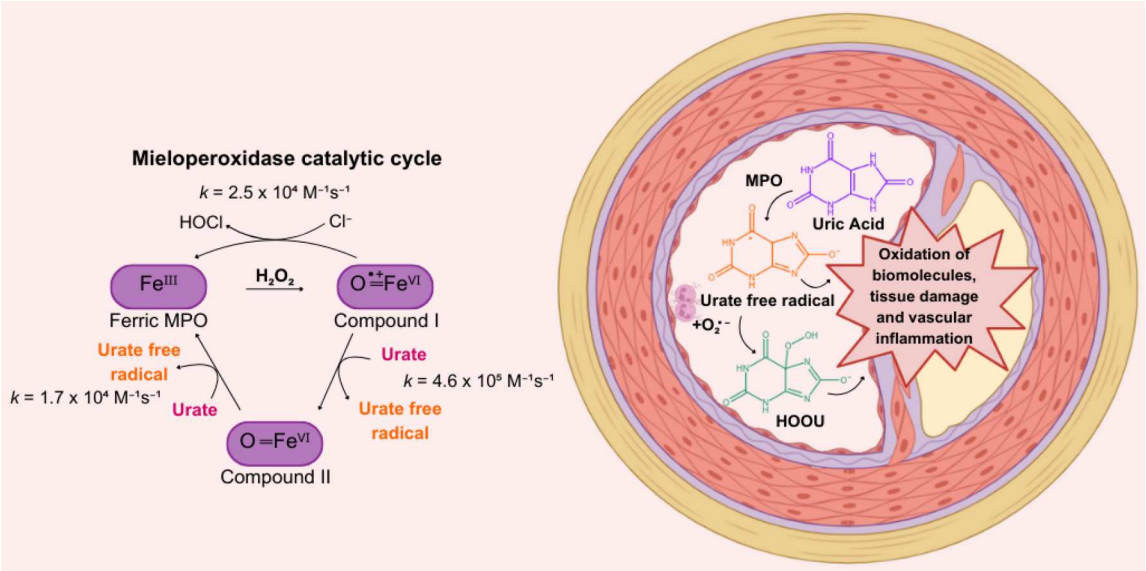

Figure 6. Catalytic cycle of myeloperoxidase (MPO) in chloride (Cl^-) and uric acid oxidation. The native ferric MPO reacts with hydrogen peroxide (H_2O_2) to produce Compound I. In the halogenation cycle, chloride is oxidized to generate hypochlorous acid (HOCl), returning MPO to its native ferric state. Alternatively, in the peroxidatic cycle, Compounds I and II oxidize urate by removing a single electron, forming the urate free radical. Superoxide can be mainly provided by NADPH oxidase or xanthine oxidase to combine with urate radical to produce the oxidant HOOU. Urate free radical and HOOU can lead to the oxidation of biomolecules, corroborating with tissue damage and inflammation.

during the cellular oxidative burst [26]. This peroxide reacts with sulphur-containing molecules like methionine, cysteine, and glutathione and might affect cellular and tissue function. The rate constant for the reaction with glutathione is 13.7 $M^{-1}s^{-1}$, this suggests that HOOU can oxidize glutathione in the biological system independent of enzymatic catalysis [29]. Nevertheless, reactive protein thiols are likely more susceptible to oxidation by HOOU. In fact, HOOU oxidized peroxiredoxins 1 and 2 at rate constants of 4.5×10^5 and 2.6×10^6 $M^{-1}s^{-1}$, respectively [129].

Peroxiredoxins are ubiquitous cysteine-dependent peroxidases, which the main function is to reduce hydrogen peroxide [130–132]. Peroxiredoxins 1 and 2 are typical 2-Cys proteins present in cytosol [133]. Their enzymatic cycle involves oxidation of the peroxidatic cysteine to a sulfenic acid, followed by disulphide-bond formation with the resolving cysteine of another subunit, resulting in a head-to-tail covalent dimer. Their basic functional unit is a homodimer during all the catalytic cycle and they can assemble into high molecular weight species in cells [134]. The intermolecular disulphide bond is mainly reduced by the thioredoxin-thioredoxin reductase system (Trx-TrxR) [133] and in some cases by glutaredoxin [135], since these proteins can form mixed disulphide with glutathione [136]. Peroxiredoxins also reduce peroxynitrite and other organic peroxides. However, it occurs at lower rates than for hydrogen peroxide [129,137137]. Nevertheless, HOOU oxidized peroxiredoxin 1 and 2 in cultured monocytes (THP-1) at the same extent as hydrogen peroxide (Figure 7). Alternatively to the reduction by the reductase systems, peroxiredoxins can transfer their oxidizing equivalents to another signalling protein through thiol-disulfide exchange reactions[138–140]. For instance, peroxiredoxin 2 forms a redox relay with STAT3 (Signal transducer and activator of transcription 3), inhibiting STAT3 migration to the nucleus [139]. Peroxiredoxin 1, on the other hand, can transfer their oxidizing equivalents to the ASK1 (Apoptosis-regulating kinase-1 signalling), resulting in the phosphorylation of p38 and activation of apoptosis [140]. Therefore, the reaction of peroxiredoxins with peroxides can affect cell response through redox signalling. Although redox signalling is a well-described event in the oxidation of peroxiredoxins by hydrogen peroxide, much less is known when the enzyme is oxidized by other peroxides and, therefore, the true role of organic peroxides and peroxynitrite in redox signalling dependent on peroxiredoxins should be investigated.

This is even more relevant when we consider the lower specificity of organic peroxides and peroxynitrite toward thiol proteins. As these peroxides are usually more powerful oxidants than hydrogen peroxide, they may indiscriminately oxidize different thiol proteins dissipating what could otherwise be a focused signal. In the case of HOOU, it also oxidizes thioredoxin 1 and this could limit peroxiredoxin turnover. When pre-reduced thioredoxin 1 (~3.2 µmol SH/µmol protein) was incubated with sub-stoichiometric HOOU, we detected a 1:1 oxidation of SH, with an incomplete oxidation at supra-stoichiometric HOOU, likely due to buried thiols in protein (Figure 8). However, the rate reaction of HOOU with thioredoxin 1 is four orders of magnitude lower than for peroxiredoxin (Table 2). Therefore, a direct oxidation of thioredoxin 1 by HOOU may not be relevant in cells, where more reactive thiol proteins are present.

HOOU also oxidized thiols in glyceraldehyde phosphate dehydrogenase, inhibiting enzyme activity [142] and protein disulfide isomerase (PDI) [27]. Oxidation of PDI catalytic cysteine by HOOU occurs at a lower rate (6×10^3 $M^{-1}s^{-1}$) than the oxidation of peroxiredoxins. However, it was at a much higher rate than the oxidation of PDI by hydrogen peroxide (17.3 $M^{-1}s^{-1}$) and glutathione disulfide (188 $M^{-1}s^{-1}$) [143,144]. PDI is a ubiquitous enzyme predominantly located in the endoplasmic reticulum where it catalyses the oxidative folding of nascent proteins [145,146]. A relatively minor fraction of PDI has been well-documented in the extracellular milieu and this pool is closely involved in vascular remodelling, mechano-response organization [147–149], platelet activation, aggregation, thrombosis[150–154] and

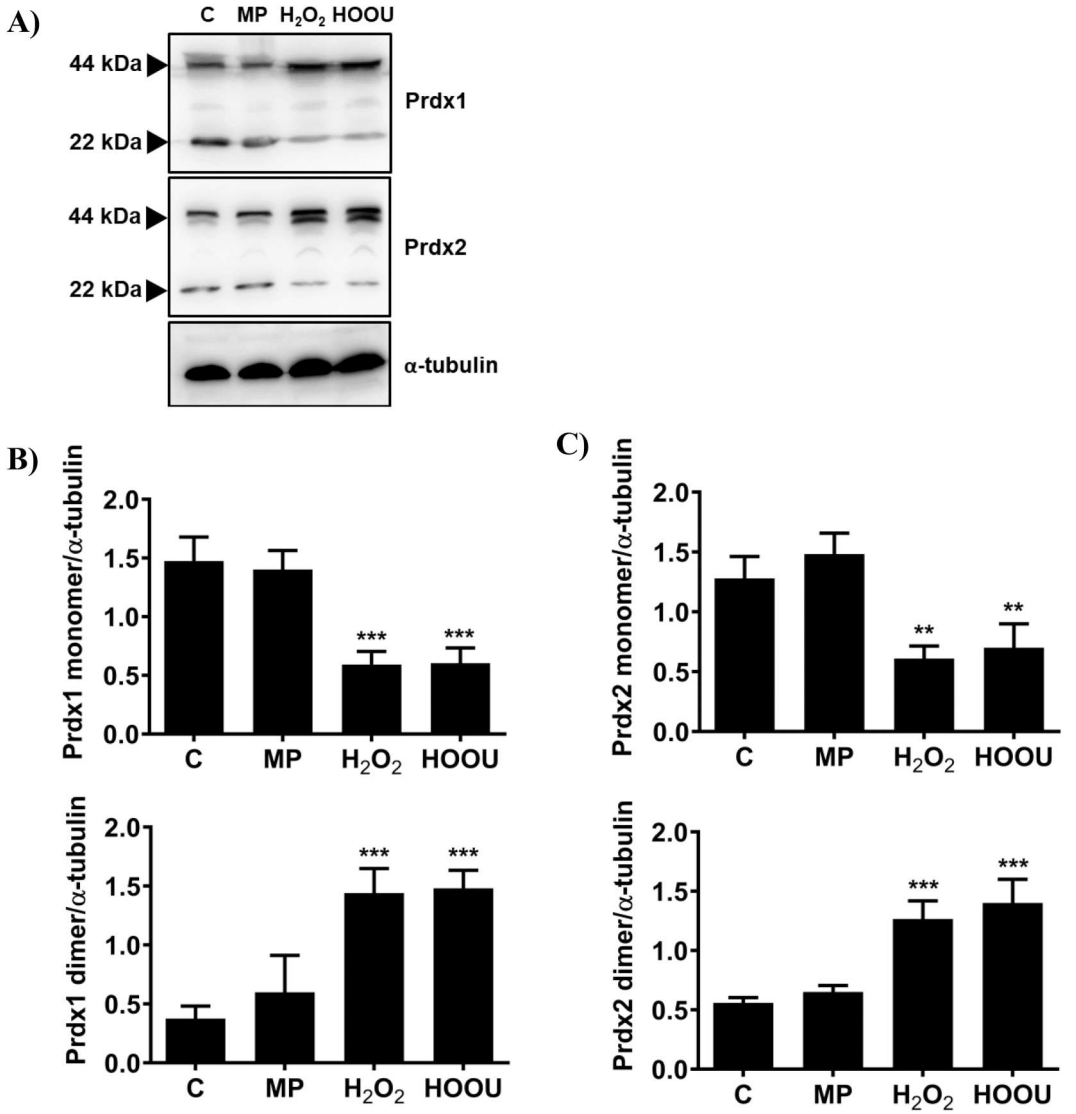

Figure 7. Oxidation of peroxiredoxins (Prdx1) and (Prdx2) in macrophage differentiated THP-1 cells by urate hydroperoxide (HOOU) and hydrogen peroxide (H_2O_2). Cells (2×10^6) were incubated in 10 mM PBS (pH 7.4) with 5 mM glucose with: mobile phase (MP), 200 µM H2O2, or 200 µM HOOU for 10 min at 37° C. Western blot analysis of (A) Prdx1 and Prdx2 from macrophage differentiatedTHP-1 lysate (100 µg/well) separated in a non-reducing SDS-PAGE. Semi-quantitative band intensity of Prdx1 monomer (upper panel B) and dimer (lower panel B) and Prdx2 monomer (upper panel C) and dimer lower panel (B) normalized by α-tubulin. Each bar represents the mean ± standard error of three independent experiments. Statistical analysis was performed by one-way analysis of variance (ANOVA); followed by Bonferroni's test, ***$p < 0.001$ when compared to the control group. C: control, MP: mobile phase.

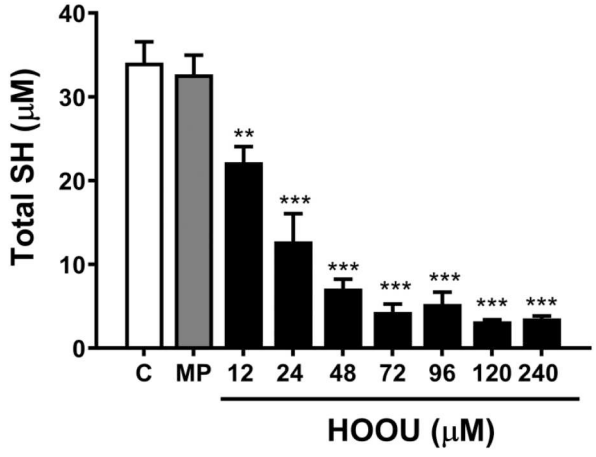

Figure 8. Urate hydroperoxide (HOOU) oxidizes thiols in thioredoxin 1. Pre-reduced thioredoxin 1 (12 µM; ~3.2 µmol SH/µmol protein) was incubated with increasing concentrations of HOOU or vehicle (mobile phase, MP) for 10 min at 37°C. Remaining protein thiols were quantified after reaction with 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) and generation of TNB, which has a maximum absorption coefficient at $\lambda_{412nm} = 14{,}100\ M^{-1}.cm^{-1}$. Each bar represents the mean ± standard error of three independent experiments. Statistical analysis was performed by one-way analysis of variance (ANOVA); followed by Bonferroni's test, **$p < 0.01$ and ***$p < 0.001$ when compared to the control group. C: control, MP: mobile phase.

integrin signalling [155–157]. Beside to an *in vitro* oxidation, HOOU oxidized extracellular cell surface PDI in endothelial cell impairing cell migration and adhesion [27]. The extracellular production of HOOU during the inflammatory oxidative burst would make thiols from PDI a preferential target for this oxidant and this effect could be related to the endothelial dysfunction attributed to UA.

In the vascular milieu, UA can also be oxidized by PXDN (Table 1) to produce urate free radical and hydroxyisourate

Table 2. Second-order rate constants for the oxidation of GSH and thiol proteins by HOOU.

Thiol	Rate constant ($M^{-1}s^{-1}$)	Reference
GSH	13.7	[29]
Prdx1	4.5×10^5	[129]
Prdx2	2.6×10^6	[129]
Trx1	2.8×10^2	[141][a]
Albumin	19.9	[141][a]
PDI	6×10^3	[27]

[a]PhD thesis published only in Portuguese [141].
GSH, glutathione; Prdx, peroxiredoxin; Trx, thioredoxin; PDI, protein disulfide isomerase.

[28,125]. Although we have not specifically evidenced HOOU in this situation, the formation of this peroxide cannot be excluded since sources of vascular superoxide, as xanthine oxidase, are also present in this environment. PXDN is a ubiquitous heme-peroxidase involved in vascular homeostasis, tissue genesis and cancer metastasis [158,159]. The enzyme catalyses the production of hypobromous acid (HOBr) from hydrogen peroxide and bromide to form sulfilimine covalent bonds in collagen IV cross-link, supporting basement membrane and extracellular matrix (ECM) [159,160]. UA decreased Tyr-bromination in both HEK-293 T overexpressing PXDN cells and in immortalized HUVECs. Decrease in HOBr can result from the competition between UA and Br^- by PXND oxidation [125], but can also occur by a direct reaction between UA and HOBr (3.4×10^6 $M^{-1}s^{-1}$) [161]. Despite the mechanism, the decrease in HOBr by UA was accompanied by disruption in collagen IV crosslinks in isolated ECM from PFHR9 cells and impairment in migration and adhesion of endothelial cells [28]. Therefore, oxidation of UA by PXDN and HOBr is likely a relevant mechanism in endothelial dysfunction. Of relevance, oxidation of UA by PXDN was the main responsible by the pro-oxidant effect of UA in endothelial cells. Exogenous addition of UA to HUVECs encoding the intracellular Grx1-roGFP2 probe led to a time-dependent oxidation of the sensor. This pro-oxidant effect was markedly inhibited in PXDN knockdown HUVECs and in cells treated with the heme-peroxidase inhibitor phloroglucinol [39]. This reveals that, despite the fact that UA diminishes HOBr, either by preventing its formation or by scavenging it, UA oxidation promotes an intracellular oxidative status.

The main antioxidant protein from plasma, albumin, can also be a target for HOOU oxidation. Albumin contains 35 cysteine and 34 of them are involved in intramolecular disulphide bridge. Therefore, Cys34 in human serum albumin is the only that is free for the oxidation by hydrogen peroxide (2.2 $M^{-1}s^{-1}$) [161]. HOOU oxidized free thiols in pre-reduced bovine serum albumin (~18 µmol SH/µmol BSA) and non-reduced (~0.8 µmol SH/µmol BSA) (Figure 9). Using non-reduced BSA, we found that HOOU oxidized it nearly 10-fold faster (19.9 $M^{-1}s^{-1}$) [141] than hydrogen peroxide.

Despite the low reactivity of albumin cysteine towards hydrogen peroxide, the high abundance of this protein in plasma makes it the main target for several oxidants, including xanthine oxidase generated superoxide, hydroxyl radical [162], peroxynitrite and lipid hydroperoxides [163,164]. Albumin can be oxidized at other sites besides cysteine and ε-amine from lysine is a main target for non-enzymatic post-translational modification, especially glycation, in this protein [165]. This modification can alter physical–chemical properties of the protein, and, in the case of albumin, it has been associated with ongoing increase in endothelial damage, production of inflammatory mediators and monocyte migration, due to an enhanced expression of endothelial cell adhesion molecules [166,167], and cardiovascular morbidity [168–170].

Albumin adducts with products from urate oxidation are detected in plasma of healthy patients and increased markedly when plasma was incubated with activated-neutrophils. A mass addition of 140 Da in lysine residues was found in different peptides from albumin. The exact intermediate that is reacting with the amine group was not identified, but a mechanism of the reaction has been purposed [142]. This post-translational modification, now termed uratylation,

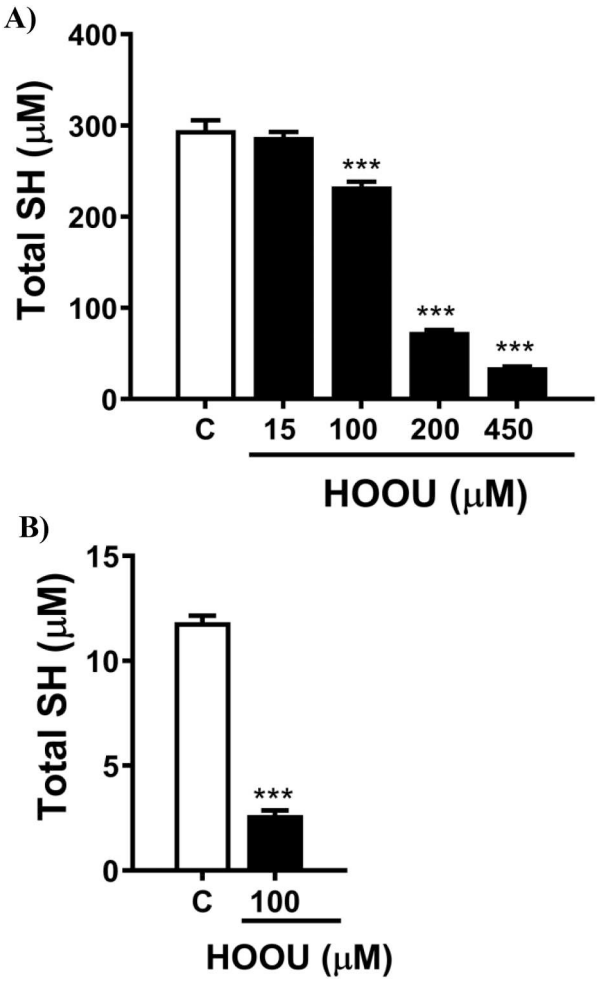

Figure 9. Urate hydroperoxide (HOOU) oxidizes thiols from albumin. (A) Pre-reduced BSA (15 µM, ~18 SH/BSA) was incubated with increasing concentrations of HOOU (15, 100, 200, and 450 µM) for 10 min at 37°C and (B) unreduced BSA (15 µM, ~0.8 SH/BSA) was incubated with 100 µM HOOU for 10 min at 37°C. Thiols were quantified after reaction with 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) by spectrophotometry $\varepsilon_{412nm} = 14{,}100$ $M^{-1}.cm^{-1}$. Each bar represents the mean ± standard error of three independent experiments. Statistical analysis was performed by one-way analysis of variance (ANOVA); followed by Bonferroni's test, $^{**}p < 0.01$ and $^{***}p < 0.001$ or unpaired t-test (right panel) when compared to the control group.

was also elevated in plasma and synovial fluid from individuals with gout and rheumatoid arthritis [142].

Since UA is mostly present in aqueous environments, it is expected that surrounding water soluble molecules will be the main targets for UA oxidation products. It is also important to consider the reactivity of each oxidation product and the reactivity of the putative target. For instance, a direct oxidation by HOOU will preferentially occur on thiols in proteins or low-molecular water soluble thiols. However, as hydrogen peroxide, HOOU is susceptible to Fenton reaction and the corresponding free radical product will react with unspecific biomolecules. Similarly, the urate free radical generated by the one-electron oxidation of UA, or the aminocarbonyl radical that is a product of UA oxidation by peroxynitrite, can more indiscriminately target proteins, lipids and nuclei acids.

As mentioned above, urate as an alternative substrate for heme-peroxidases has additional consequences beyond the production of urate free radical, HOOU and adducts in proteins. Whereas oxidized by PXDN, urate disturbs collagen IV cross-link and ECM formation and when oxidized by MPO or LPO it deviates production of HOCl and hypothiocyanite

(˙OSCN), respectively, impairing the killing activity towards *Pseudomonas aeruginosa* [128,171]. Our group demonstrated that UA, at physiological concentrations, decreased HOCl production both by isolated MPO and by neutrophils but increased the overall oxidative status as measured by GSH/GSSG and further increased superoxide production induced by *P. aeruginosa*, proving that, despite the decrease in HOCl, urate oxidation increases the overall oxidant status. This is likely a sum of a direct pro-oxidant effect by urate-oxidative intermediates and an indirect effect by inducing superoxide production [171]. In fact, hyperuricemia accelerated the death of mice that were instilled with intranasal *P. aeruginosa* [172]. In clinical a study, oxidation of UA was positively correlated with bronchiectasis, MPO, neutrophil elastase, chlorotyrosine, glutathione sulphonamide, and inflammatory cytokines in bronchoalveolar lavage of children suffering of pulmonary infection by *P. aeruginosa*. This strengthened the link between UA oxidation and worse outcome in patients with *P. aeruginosa* infection and suggests the investigation of oxidized glutathione and UA as biomarkers of early cystic fibrosis lung disease [173]. A correlation between plasma UA levels and worse outcomes has also been found in sepsis [174]. Additionally, a positive correlation between blood xanthine dehydrogenase and the sequential organ failure assessment (SOFA) score, an indicator of sepsis severity, was described [175], suggesting that the synthesis of UA is more prominent in sepsis. Together, these data revealed that oxidation of urate may damper the physiological function of proteins, cells and tissues not only by producing oxidizing intermediates but also by diverting the production of important biomolecules. In this context, it is crucial to underscore the importance of considering UA levels as a biochemical marker in a broader spectrum of conditions, including CVD, sepsis, and cystic fibrosis lung disease, rather than restricting its evaluation to traditional conditions such as gout.

Conclusion

UA can paradoxically act as an anti or pro-oxidant. The two electron oxidation of UA by oxygen or nitrogen-reactive species can efficiently remove such oxidants. However, depending on the intermediate that is formed, the reaction can be detrimental, promoting tissue damage, inflammation and oxidative damage. The one-electron oxidation of UA could only be protective whether two urate free radicals dismutate to dehydrourate, then allantoin, restoring one UA, but by inferring it, we are ignoring the possible reactivity of the intermediate hydroxyisourate. One-electron oxidation of UA can also be protective whether ascorbate is present, as it efficiently restores UA from urate free radicals. In all other situations, urate free radical can either abstract one-electron from biomolecules, propagating the free radical-chain reaction or combine with superoxide to form the also oxidizing agent HOOU. The innovation or novelty in this review is that it focuses on the descriptionof such reactive urate oxidation intermediate species and that their formation may explain how UA causes cell damage and propagates inflammation. By knowing the oxidation pathways of UA and detection of its metabolic products, such as, allantoin, hydroxyisourate, triuret, 6-aminouracil, parabanic acid, carboxyimino-6-*N*-chloroaminopyrimidine-2,4(3*H*)-dione (CAPD) and adducts in lysine residues (+140 Da) we may elucidate UA role in specific conditions. The main limitations for some conclusions in this area are: (1) the reaction of UA with some oxidants can produce common (no specific) oxidation products, hampering the conclusion on which oxidation pathway is predominating; (2) the limitation of using rodent models to explore *in vivo* UA mechanisms on diseases, since these species express uricase, (3) the restricted availability of research addressing the role of UA oxidation products, underscoring the importance of further explore this field. In summary, investigating the antioxidant and pro-oxidant paradox of UA, alongside its enzymatic interactions, is vital for identifying potential therapeutic applications and strategies to minimize its harmful effects, fostering a balanced approach to health and disease management.

Author contributions

CRediT: **Mikaela Peglow Pinz:** Conceptualization, Data curation, Investigation, Writing – original draft; **Isadora Medeiros:** Data curation, Writing – original draft; **Larissa Anastácio da Costa Carvalho:** Data curation, Investigation, Methodology.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Availability of data and materials

All data and resources used in the paper have been cited and indicated.

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