Xanthine oxidoreductase in respiratory and cardiovascular disorders

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XANTHINE OXIDOREDUCTASE (XOR) has been the subject of extensive biochemical characterization as the prototypical member of the family of molybdo-flavoenzymes. Because of its ability to generate reactive oxygen species (ROS), XOR has been thoroughly investigated in diseases where oxidative stress prevails. This review will focus essentially on the potential pathogenic role of XOR in respiratory and cardiovascular disorders. Other comprehensive reviews related to the general biology of XOR have been published elsewhere (23, 36).

Biology of XOR
XOR (EC 1.17.1.4) has long been recognized as the key enzyme in the catabolism of purines, oxidizing hypoxanthine into xanthine and xanthine into uric acid (Fig. 1). It exists in two forms: xanthine oxidase (XO) and xanthine dehydrogenase (XDH), XO being derived from XDH (the original translational product of the XOR gene) by posttranslational modification.

XDH can be the target of oxidation and proteolysis, modifications that change the binding specificity of the enzyme and lead to the formation of XO. Both XDH and XO catalyze the terminal steps in the metabolism of purines. However, in the case of XDH, which binds NAD+, the electrons deriving from the oxidation of hypoxanthine or xanthine reduce NAD+ to NADH. In contrast, XO, being no longer able to bind NAD+, generates electrons that are transferred directly to molecular oxygen, leading to the formation of the ROS superoxide. The alteration in binding specificity for NAD+ is irreversible in the case of proteolysis but can be reversed by reducing agents in the case of oxidation. Nevertheless, it is important to point out that the dehydrogenase form of XOR can also use molecular oxygen and produce ROS, although less efficiently than the oxidase form.

In addition, in certain conditions, XOR can function as an NADH oxidase, generating ROS without metabolizing xanthine or hypoxanthine. The catalysis in this case occurs at the FAD site and does not involve the molybdenum (Mo) cofactor and therefore cannot be prevented by XOR inhibitors such as allopurinol, which target the Mo cofactor (27). Moreover, XO can generate nitric oxide (NO) by catalyzing the reduction of nitrate to nitrite and nitrite to NO in the presence of NADH as electron donor. These reactions can be completely blocked by the Mo-directed XOR inhibitor, allopurinol, or the FAD-directed inhibitor, diphenyliodonium (DPI). Therefore, nitrate reduction to nitrite as well as nitrite reduction to NO occur at the Mo center, whereas NADH is oxidized at the FAD site (Fig. 2). Interestingly, this NO generation pathway is oxygen independent and, as a result, may participate in the redistribution of blood flow to ischemic areas where nitric oxide synthase (NOS) activity is limited by hypoxia (118). The exact relevance of this particular XOR function is unclear at this time since the $K_m$ of the NO-mediated nitrite reduction is fairly high (66).

Structural discoveries followed the biochemical characterization of XOR and largely supported the model derived from biochemical characterization. It is believed that both reversible and irreversible conversions of XDH to XO are due to conformational changes in the molecule, resulting in an alteration of the electrostatic charge at the opening of the FAD site, thus blocking access of NAD+ to that site (20).

Regulation of XOR Activity
XOR purified from bovine milk, as well as from rat liver and human milk, exists largely in an inactive state. The significant
increase in XOR activity on exposure to cytokines cannot solely be explained by the increase in XOR mRNA and protein levels observed in this setting (82). Furthermore, hypoxia increases XOR mRNA and protein levels only after days of exposure (38), whereas the enzymatic activity is upregulated as early as 4 h after the initiation of the hypoxic stimulus (51). Taken together, these results suggest that XOR is regulated both transcriptionally and posttranslationally (36), the post-translational modifications accounting, for instance, for the acute enzymatic activation in response to brief exposures to inciting stimuli.

XOR activity has been detected in all species and almost all organs tested. In mammals, it is highest in the liver and intestine and to a lesser degree in other organs such as the lung. XOR mRNA and protein levels follow a similar pattern to that of XOR activity in terms of organ distribution. Under physiological conditions, the concentration of circulating XOR is low, but it increases dramatically in certain disease states such as acute viral hepatitis where it can reach levels 1,000-fold higher than normal. Most of the circulating XOR exists in the oxidase form, likely due to conversion of XDH to XO by serum proteases. Once in the circulation, XOR has the ability to translocate to intracellular compartments (42).

XOR activity can be reduced by removal of the Mo cofactor or sulfate moieties from the XOR protein (36). In that regard, increasing concentrations of hydrogen peroxide \((H_2O_2)\) progressively decrease XOR activity and induce a concomitant loss of its ability to transfer electrons from xanthine to 2,6-dichlorophenolindophenol (DCPIP). Given the fact that DCPIP directly accepts electrons from reduced Mo, this observation suggests that \(H_2O_2\) inhibits XOR activity by deactivating the Mo center (68). Hence, preventing the deletion or deactivation of such critical components of the XOR protein has been proposed as potential mechanisms of post-translational modification leading to enzymatic activation.

Another mechanism of posttranslational modification of XOR is achieved by phosphorylation, leading to acute enzymatic activation (51). XOR phosphorylation appears to be modulated in part by casein kinase II (CK2) and p38 MAPK signaling as inhibitors of CK2 and p38 MAPK prevent hypoxia-mediated XOR phosphorylation as well as activation. However, phosphorylation of p38 alone (e.g., in response to hyperosmolar stress or arsenite exposure) is not enough to phosphorylate XOR. Taken together, these observations suggest that activation of p38 kinase may be necessary but not sufficient to induce the phosphorylation and consequently the activation of XOR in response to hypoxia. The exact site(s) of phosphorylation are yet to be elucidated.

The only commercially available pharmacological inhibitor of XOR is allopurinol. Its inhibitory activity is largely a result of the metabolite oxypurinol, a noncompetitive inhibitor of XO that prevents oxidation of xanthine to uric acid by acting at the Mo site. However, allopurinol and oxypurinol are relatively nonselective inhibitors as they are structurally similar to other purine and pyrimidine compounds. Febuxostat, a new nonpurine selective agent, inhibits the oxidized and reduced forms of XOR without affecting other enzymes of purine and pyrimidine metabolism (107). Data regarding this new XO inhibitor are still emerging.

**XOR and Respiratory Diseases**

The respiratory system is subject to many exogenous and endogenous stimuli that alter its homeostatic physiology. Acute lung injury (ALI), a complex inflammatory syndrome affecting various components of the respiratory system, is a devastating illness with an annual incidence of 200,000 and a mortality ranging in the order of 40% in the United States (94). Most commonly seen in the setting of sepsis, ALI is marked by increased vascular permeability resulting in tissue edema and subsequent profound hypoxia (112). Therapy for this syndrome is largely supportive and includes mechanical ventilation during the acute period to allow the lung parenchyma to recover from the overwhelming insult. Although considered to be the mainstay of therapy for ALI and its more severe form, acute respiratory distress syndrome (ARDS), mechanical ventilation...
is now recognized as a contributor to alveolar overdistension, thereby worsening pulmonary capillary leakage and inflammation and leading to ventilator-associated lung injury (VALI) (69). Other than ventilating at lower tidal volumes, which presumably imparts lower mechanical stress, so far no directed therapies have proven effective and there is little understanding of the pathophysiology of this syndrome.

Oxidant injury related to the formation of ROS (possibly generated by XOR) has been recently postulated in the pathogenesis of ALI/VALI (Fig. 3). In animal models of ALI, XOR is increased (~400-fold) in lung parenchyma and bronchoalveolar lavage (BAL). In humans, increased hypoxanthine and XOR levels have been demonstrated in the epithelial lining fluid of premature neonates with bronchopulmonary dysplasia (95) and in the serum of patients with ARDS compared with normal controls or critically ill patients with other organ diseases. Furthermore, plasma hypoxanthine levels are highest in nonsurvivors of ARDS, implicating oxidative damage (and presumably XOR) as a determinant of mortality in these patients (91).

In vitro, XO has been shown to decrease double-stranded DNA of murine lung epithelial type II cells and de novo phosphatidyl choline synthesis linking XO to abnormal surfactant metabolism and eventual surfactant deficiency leading to propagation of lung injury (13). It is also possible that, in ARDS and ALI, XOR-derived superoxide and NO generated from inducible NOS (iNOS) react to form the highly toxic oxidant peroxynitrite, resulting in lung protein nitrotyrosine formation and oxidative damage. Peroxynitrite has been shown to inhibit pulmonary surfactant activity and promote its degradation (34, 60). In further support of oxidant-mediated toxicity by peroxynitrite is the demonstration of nitrotyrosine residues in the vascular endothelium and subendothelial tissues in patients with sepsis-induced ALI (60) and in the BAL of patients with ARDS (63).

The following sections will explore the role of stimuli specifically implicated in the initiation or perpetuation of ALI [e.g., hypoxia, endotoxin challenge, cytokine challenge, mechanical ventilation, hypoxia, and lung ischemia-reperfusion (IR) injury] in the regulation of lung XOR. Included also is a list of others conditions where XOR has also been incriminated: chronic obstructive pulmonary disorder (COPD), obstructive sleep apnea (OSA), pulmonary manifestations of sickle cell disease (SCD), interstitial pneumonitis, as well as rejection of heart-lung transplant (Table 1).

### ALI

**Hypoxia.** Hypoxia is a sine qua non feature in patients with ALI. It has a significant impact on respiratory physiology as a causative stimulus and is often prevalent as a consequence of insults to the lung parenchyma.

Multiple groups have shown that hypoxia upregulates XOR activity. For example, exposure of bovine aortic endothelial cells to anoxia or various degrees of hypoxia significantly increases XOR activity (53, 89). In vivo data also supports these observations. A 24-h exposure of Sprague-Dawley rats to hypoxia causes a significant (2.7-fold) increase in lung XOR activity compared with normoxic controls. This is accompanied by an increase in the wet-to-dry lung weight ratio, a marker of lung injury, in the hypoxia-exposed animals. Pretreatment of these animals with a tungsten-rich, Mo-free diet leads to complete suppression of XO activity and prevention of ALI (37).

Hypoxia-inducible factor-1 (HIF-1) is a key regulator of oxygen homeostasis. In hypoxic conditions, HIF-1 mediates the induction of a variety of oxygen-responsive genes involved in diverse functions such as angiogenesis, hormonal regulation, energy metabolism, cellular transport, growth, and apoptosis (98). A putative binding site for HIF-1 has been identified in the human XOR gene (40), but evidence of HIF-mediated regulation of XOR has not been published. Conversely, XO seems to be a critical regulator of HIF-1 in cells having a glycolytic-dependent phenotype. In these cells, XO-derived ROS lead to accumulation of HIF-1 protein, and XO inhibition significantly attenuates this response (30).

Therefore, there is compelling evidence for a causal role of XOR in hypoxia-induced ALI. The underlying mechanisms are...
incompletely understood. However, HIF-1 may be an important downstream effector of XOR in this setting.

Endotoxin challenge. There is overwhelming evidence for induction of ALI with instillation of LPS via intravenous, intraperitoneal, or intratracheal routes (37, 45, 50). Furthermore, there is strong evidence that free radicals, including ROS, contribute significantly to endotoxin-induced ALI in experimental models (22, 67).

Exposure of pulmonary microvascular endothelial cells to LPS and IL-1β causes a dramatic upregulation of XOR mRNA and XOR activity (14). Similarly, a single intraperitoneal injection of LPS and IL-1β in Sprague-Dawley rats produces a significant increase in lung XOR mRNA and activity and prominent lung injury, which is successfully prevented by XOR inhibition. Furthermore, XOR mRNA levels and enzymatic activity as well as markers of lung injury are significantly more pronounced when animals are exposed to a combination of LPS/IL-1β and hypoxia compared with animals subjected to LPS or IL-1β alone (37).

LPS administration alone is also associated with an upregulation of XO. In fact, a single intravenous administration of LPS in heifers increased plasma XO activity after 3 h compared with saline administration (49). No measurements of lung injury were performed in this study, although the authors (49) noted that the animals experienced respiratory symptoms such as labored breathing and cough. In another study, intraperitoneal LPS injection in mice caused marked XO induction such as labored breathing and cough. In another study, intra-

Cytokine challenge. ALI is not only characterized by physiological derangements such as hypoxia and loss of pulmonary compliance, but also by an inflammatory state defined by enhanced cytokine expression and release. There is compelling evidence indicating that inflammatory cytokines are involved in the initiation and propagation of oxidant-mediated lung injury in various disease states (8, 27, 51, 57, 72).

TNF-α, IL-1β, and IFN-γ, when added individually or in combination to human mammary epithelial cell lines (HB-4a) for 24 h, significantly increase XOR activity and mRNA levels over control conditions in a dose-dependent and synergistic fashion (82). Similar findings were noted in Sprague-Dawley rats after intratracheal instillation of IL-1 and/or IFN-γ. In conjunction, lung tissue sections demonstrated increased numbers of airway and perivascular inflammatory cells (in rats treated with IL-1 and IFN-γ compared with animals treated with saline, IL-1, or IFN-γ alone), which were significantly attenuated by tungsten and allopurinol treatment (116). Such observations suggest that XOR mediates lung inflammatory processes resulting from cytokine exposure.

Interestingly, unlike renal tubular epithelial cells, which clearly show a considerable induction of XOR activity on exposure to IL-6 (87), HB-4a cells fail to do so (82). These experiments suggest a tissue-specific and/or cytokine-specific regulation of XOR activity.

Mechanical stress. The cell signaling pathways mechanismi-

cally implicated in VALI are not entirely understood. Never-
thess, there is emerging evidence for a putative pathogenic role of XOR in mediating VALI.

Exposure of bovine aortic endothelial cells to oscillatory shear stress causes a threefold increase in superoxide production compared with control cells exposed to laminar shear stress or static conditions, which is abrogated with XOR inhibition by oxypurinol or tungstic acid (71). Similarly, exposure of rat pulmonary endothelial cells to pathological, high-grade cyclic stretch for 1–2 h causes a significant upregulation of XOR activity, however, with no associated increase in XOR mRNA and protein levels. Pathological, but not physiological, cyclic stretch also causes significant activation of several MAP kinases (p38, ERK5, and ERK1/2, but not JNK), which, when pharmacologically inhibited, effectively prevent stretch-induced XOR activation (1). Furthermore, the authors (1) demonstrated that mice ventilated at high tidal volumes (HV, 20 ml/kg), as opposed to low tidal volume (LV, 7

Table 1. Effects of xanthine oxidoreductase inhibition in various respiratory disorders in animal models and humans

| Animal studies | LPS and IL-1β causes a dramatic upregulation of XOR mRNA and XOR activity (14). Similarly, a single intraperitoneal injection of LPS and IL-1β in Sprague-Dawley rats produces a significant increase in lung XOR mRNA and activity and prominent lung injury, which is successfully prevented by XOR inhibition. Furthermore, XOR mRNA levels and enzymatic activity as well as markers of lung injury are significantly more pronounced when animals are exposed to a combination of LPS/IL-1β and hypoxia compared with animals subjected to LPS or IL-1β alone (37).
| Mechanical stress (1, 71) | No change in BAL protein and wet-to-dry ratios | Pulmonary filtration coefficient (Kf) |
| Hyperoxia (78) | No change in BAL protein and wet-to-dry ratios | Pulmonary filtration coefficient (Kf) |
| Ischemia-reperfusion (2, 31) | No change in lung lymph flow, lymph-to-plasma protein ratio, polymorphonuclear cells, extravascular lung water, histological evidence of tissue edema, and destruction | No change in airflow obstruction |
| Human studies (43) | No change in BAL protein and wet-to-dry ratios | Pulmonary filtration coefficient (Kf) |
| Animal studies (75) | No change in lung lymph flow, lymph-to-plasma protein ratio, polymorphonuclear cells, extravascular lung water, histological evidence of tissue edema, and destruction | No change in airflow obstruction |
| Human studies (18, 100) | No change in lung lymph flow, lymph-to-plasma protein ratio, polymorphonuclear cells, extravascular lung water, histological evidence of tissue edema, and destruction | No change in airflow obstruction |

References are noted in parentheses. BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disorder; OSA, obstructive sleep apnea.

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ml/kg) and spontaneous breathing, had a significant increase in lung XOR enzymatic activity at 2 h. Similar to the in vitro findings, there was no difference in lung XOR mRNA and protein levels, and there was significant activation of the MAPK pathway (i.e., p38, ERK5, and ERK1/2, but not JNK) in response to HV(T) but not LV(T) ventilation. Furthermore, parameters of lung injury (e.g., BAL protein concentration and pulmonary leakage of Evans blue dye) were increased in response to 2-h HV(T) ventilation compared with spontaneous breathing but were significantly reduced when mice were pretreated with allopurinol (1). Overall, these results indicate that XOR is activated in response to mechanical stress in vitro and in vivo through upregulation of the MAPK signaling system and contributes to lung injury in a mouse model of VALI.

Hyperoxia. Supplemental oxygen is a significant component of the supportive care provided to patients with ALI. Although necessary to maintain adequate peripheral oxygenation, therapeutic hyperoxia can contribute to and exacerbate lung injury. In rat pulmonary endothelial cells, 80% hyperoxia resulted in a time-dependent decrease in XOR activity and mRNA copies to completely undetectable levels at 16 h (38). In parallel, Moores et al. (78) had demonstrated that adult male Fischer rats exposed to 100% hyperoxia had an increased BAL fluid albumin concentration and neutrophils as well as an increase in wet-to-dry lung weight ratios compared with animals exposed to normoxic conditions. XOR inhibition decreased BAL fluid neutrophil count (but not albumin concentration) and had no effect on wet-to-dry lung weight ratios (78). These results suggest that XOR may be involved in lung neutrophil recruitment and inflammation but does not contribute directly to lung injury during exposure to hyperoxia. Clearly, a better understanding of the interaction between XOR, neutrophils, and lung injury is required to optimize treatment strategies against hyperoxic lung injury.

Lung IR injury. Despite the presence of a dual pulmonary and bronchial circulation, the lung microvasculature is known to be susceptible to IR injury. Such injury is the result of ROS generated by the ischemic tissue on reperfusion.

In isolated perfused lung preparations, XOR inhibition prevents IR-induced increase in pulmonary filtration coefficient (Kf) (2). Interestingly, there are segmental differences in the microvascular leakage patterns: the postalveolar segments represent 52% of the total Kf, whereas the prealveolar and alveolar segments account only for 27% and 23%, respectively (55). These differences are possibly explained by the finding that endothelial cells from pulmonary veins are more susceptible to XO-dependent mitochondrial DNA damage than endothelial cells from pulmonary arteries or capillaries (31). Thus XOR may mediate lung IR injury preferentially in the postalveolar segments. However, this segmental pattern of injury appears to be stimulus-specific since capillary (rather than postalveolar) segments display the largest increase in Kf in response to high peak inflation pressures (83).

Other Respiratory Diseases

Although the role of XOR has been highlighted thus far in ALI/VALI, this enzyme seems also to play a role in other disorders affecting the respiratory system. For instance, in COPD, a substantial imbalance between oxidant and antioxi-

XOR and Cardiovascular Diseases

Cardiovascular diseases result in high mortality rates worldwide. However, successful therapies related to recent advances in our understanding of the pathogenesis of this group of disorders have resulted in a downward trend in overall cardiovascular mortality (93). Among the described mechanisms, there is substantial evidence supporting the involvement of oxidative stress in a variety of pathophysiological states such as atherogenesis (69), systemic hypertension (29), myocardial IR injury (81), atrial fibrillation (61), pulmonary hypertension (41, 46), ventricular hypertrophy (25), cardiomyopathy, and heart failure (Ref. 109; Table 2).

The major sources of ROS in the cardiovascular system include XOR, NAD(P)H oxidase, NOS, and, perhaps to a lesser extent, mitochondrial cytochromes, lipoxygenases, cyclooxygenases, and hemoglobin (35). The contribution of XOR to cardiac oxidative injury was initially a matter of debate, mainly due to the low activity of XOR initially detected in animal and human hearts. However, it is now generally accepted that under pathological conditions, there is a significant increase in cellular XOR level and activity in the cardiovascular system, even though these changes may not be easily detectable using whole tissues assays (36). The
role of XOR in cardiac oxidative stress has been largely based experimentally on the demonstration of attenuation of tissue damage following inactivation of XOR by allopurinol or oxypurinol. It is also recognized that in addition to their antioxidant effects such as free radical scavenging, inhibition of lipid peroxidation, heat shock factor expression, and calcium sensitization (81). However, elevated serum levels of uric acid, an end product of XOR, in heart failure patients supports a causative role of XOR in cardiac oxidative stress (65). A cross talk between different enzymatic systems may contribute to ROS generation. In this regard, McNally et al. (71) have shown that the conversion of XDH to XO in endothelial cells when the growth medium is supplemented with hypoxanthine or uric acid (80). Based on these experimental data and clinical observations, XOR to hypertension via its effect on COX-2 has been postulated. XOR is a major source of vascular oxidative stress as evidenced by several experimental and clinical data. For instance, diet-induced atherosclerosis has been attributed to increased oxidative stress via XOR activation since oxypurinol normalizes superoxide production in the vessels of hypercholesterolemic rabbits but has no effect on the vasculature of control animals (79). In patients with coronary artery disease or carotid stenosis, endothelial XO activity and protein levels are substantially increased (32, 85, 101) and inversely related to endothelium-dependent vasodilation (101). The mechanisms leading to the activation of endothelial XO in this setting are not yet entirely defined. Nevertheless, Ang II has been recently shown to markedly increase XO activity and protein levels in cultured endothelial cells (64). In patients with coronary disease, losartan, an angiotensin receptor blocker, is able to reduce endothelium-bound XO activity. In addition, endothelium-dependent vasodilation can be improved by oxypurinol only if administered before, but not after, losartan (64). These findings suggest that Ang II contributes to XO-induced endothelial dysfunction.

In regard to hypertension, the involvement of XOR has been convincingly demonstrated in numerous animal models (11) including spontaneously hypertensive rats (104), hypertensive salt-sensitive rats (105), and in hypertension induced by high salt intake (62) or dexamethasone administration (111). In these models, elevation of the mean arterial pressure was normalized with XOR inhibition. Data in humans are, however, quite limited. XOR may be an endogenous regulator of cyclooxygenase-2 (COX-2). Indeed, in the first month of life, a period of rapid renal development, wild-type mice exhibit a significant increase in renal XOR activity and COX-2 expression. In contrast, during the same period, no similar COX-2 induction is observed in XOR knockout mice (80). A comparable increase in COX-2 expression is also seen in cultured cells when the growth medium is supplemented with hypoxanthine or uric acid (80). Based on these experimental data and on epidemiological evidence of an association between uric acid and systemic hypertension (4, 47, 113), the contribution of XOR to hypertension via its effect on COX-2 has been postulated.

### Atherosclerosis and Systemic Hypertension

It is now increasingly evident that XOR-generated ROS are key mediators in atherogenesis and hypertension. In atherogenesis, ROS participate in the initiation of endothelial dysfunction, fatty streak development, lesion progression, and eventual plaque rupture (44). The mechanisms involved in this process include direct ROS injury to cell membranes and nuclei (44), reduction of the bioavailability of endothelium-derived vasoactive mediators such as NO (117), and production of cytoxic compounds responsible for platelet aggregability and vasoconstriction (74). ROS can also induce adhesion molecules and inflammatory reactions, leading to endothelial dysfunction (117), formation of oxidized low-density lipoproteins (44), and activation of matrix metalloproteinases (76), ultimately resulting in excessive collagen destruction and fibrous cap thinning. XOR is a major source of vascular oxidative stress as evidenced by several experimental and clinical data. For instance, diet-induced atherosclerosis has been attributed to increased oxidative stress via XOR activation since oxypurinol normalizes superoxide production in the vessels of hypercholesterolemic rabbits but has no effect on the vasculature of control animals (79). In patients with coronary artery disease or carotid stenosis, endothelial XO activity and protein levels are substantially increased (32, 85, 101) and inversely related to endothelium-dependent vasodilation (101). The mechanisms leading to the activation of endothelial XO in this setting are not yet entirely defined. Nevertheless, Ang II has been recently shown to markedly increase XO activity and protein levels in cultured endothelial cells (64). In patients with coronary disease, losartan, an angiotensin receptor blocker, is able to reduce endothelium-bound XO activity. In addition, endothelium-dependent vasodilation can be improved by oxypurinol only if administered before, but not after, losartan (64). These findings suggest that Ang II contributes to XO-induced endothelial dysfunction.

### Myocardial Infarction

In the setting of acute myocardial infarction, ROS are thought to play a significant role in tissue necrosis, pathogenesis of myocardial stunning, and myocardial remodeling pro-

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**Table 2. Beneficial effects of xanthine oxidoreductase inhibition shown so far in various cardiovascular disorders**

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<tr>
<th>Atherosclerosis and Hypertension</th>
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<tr>
<td>Animal studies (61, 102,104)</td>
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<tr>
<td>Vascular free radical production</td>
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<tr>
<td>HTN</td>
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<td>HTN-induced LV hypertrophy</td>
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<td>Endothelial-dependent vasodilation</td>
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<th>Myocardial Ischemia-Reperfusion Injury</th>
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<tr>
<td>Animal studies (69, 76)</td>
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<tr>
<td>Myocardial infarct size</td>
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<td>Reperfusion arrhythmias</td>
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<td>Human studies (47, 82)</td>
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<td>Mortality rate</td>
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<td>Need for post-CABG inotropic and mechanical support</td>
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<td>CI</td>
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<td>Incidence of infarct extensions</td>
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<th>Pulmonary Hypertension</th>
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<tr>
<td>Animal studies (41, 46)</td>
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<td>Pulmonary hypertensive changes</td>
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<th>Chronic Heart Failure</th>
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<tr>
<td>Animal studies (5, 72)</td>
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<tr>
<td>LV cavity dilation</td>
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<td>Myocardial hypertrophy</td>
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<td>Myocardial fibrosis</td>
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<td>Afterload</td>
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<td>LV contractile function</td>
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<td>Human studies (24, 100)</td>
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<td>Survival (high dose allopurinol)</td>
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<td>Mortality (low dose allopurinol)</td>
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<tr>
<td>BNP</td>
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<td>No effect on exercise capacity</td>
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References are noted in parentheses for each set of studies. HTN, hypertension; LV, left ventricle; CABG, coronary artery bypass graft; CI, cardiac index; ROS, reactive oxygen species; BNP, brain natriuretic peptide.
explained by possible regional differences since, in the animal study, the NAD(P)H oxidase activity in the left atrium and left atrial appendage was examined. Because of lower metabolic reserve and increased wall stress, it is speculated that the left atrium may be more sensitive to oxidative injury compared with the right atrium.

**Pulmonary Hypertension**

XOR is also recognized as an important effector in pulmonary vascular pathology. XOR-derived free radicals cause a dose-dependent contraction of rabbit pulmonary arterial rings (103). In addition, Hoshikawa et al. (41) demonstrated elevated lung XOR activity in a model of hypoxia-induced pulmonary hypertension in which the associated pulmonary hypertensive changes were significantly attenuated by allopurinol. In a recent study, Jankov et al. (46) reported that, in the lungs of neonatal rats exposed to chronic hypoxia, the major sources of ROS may differ with time. An increase in XOR activity and expression is an early and time-limited event since daily administration of allopurinol or oxypurinol completely attenuates the increase in ROS production at day 4, but not at day 14 of hypoxia. Conversely, DPI, a NAD(P)H inhibitor, abolishes ROS formation after a 14-day, but not 4-day, exposure to hypoxia (46).

**Ventricular Hypertrophy, Cardiomyopathy, and Heart Failure**

A fundamental response to increased biomechanical stress on the heart is cardiomyocyte and cardiac chamber hypertrophy. The expression and activity of XOR were studied by de Jong et al. (15) in different rat models of myocardial hypertrophy including monocrotaline-induced pulmonary hypertension and cor pulmonale, coronary artery ligation-induced heart failure with compensatory right ventricular hypertrophy, and spontaneously hypertensive rats. In this study, ventricular wet weight ratios were used as surrogates for chamber hypertrophy, and the diagnosis of heart failure was based on the presence of clinical signs of decompensation, postmortem detection of pleural and peritoneal effusions, along with increased plasma levels of atrial natriuretic peptide. XOR activity was highest in the failing heart: right ventricle in the case of monocrotaline injury or left ventricle in the setting of coronary artery ligation. However, there was no significant increase in XOR activity in the right ventricle with compensatory hypertrophy at 8 wk following coronary ligation (15). Furthermore, spontaneously hypertensive rats exhibited only a slight increase in hypertrophic heart XOR activity compared with normotensive rats (9). Taken together, these observations suggest that XOR activity may play a pathological role essentially in the failing ventricles, whereas the enzyme is quiescent in compensatory hypertrophy.

The role of XOR and XOR-related oxidant species is also well-documented in the pathogenesis of ischemic (19), pacing-induced (5), and idiopathic, dilated cardiomyopathic (73) animal models of heart failure. In these models, significant increases in myocardial XOR have been observed, and allopurinol therapy resulted in improved cardiac contractility, mechanical efficiency at rest, as well as during exercise and dobutamine-induced β-adrenergic stimulation.
XOR was shown to impact myocardial function through the development of cardiac hypertrophy, postmyocardial infarction remodeling, inhibition of the sarcoleptosomal reticulum calcium pump in the cardiac contraction-relaxation cycle (114), myocyte apoptosis, and β-adrenergic downregulation (73). In addition, inhibiting neuronal NOS (nNOS) abrogates the beneficial effects of allopurinol in dogs with heart failure (96). Conversely, in normal dogs, nNOS inhibition causes mechanoenergetic uncoupling that can be reversed following XOR inhibition (54). These findings suggest that an interaction between cardiac NO and XOR signaling pathways plays a role in the pathogenesis of heart failure, with nNOS being an important regulator of XOR activity. In fact, there is a direct protein-protein interaction between XOR and nNOS in the sarcolemmal reticulum of cardiomyocytes (54). On the other hand, increased NO production via induction of NOS has been suggested as a major mechanism by which cytokines mediate cardiac contractile dysfunction (97, 99, 115). Thus NO has both beneficial and harmful effects on the cardiovascular system. Current understanding suggests that the ultimate effect of NO depends on its bioavailability, which is dictated by a delicate balance between NOS, ROS, and antioxidants, with NO being inactivated by ROS and protected by antioxidants.

Beyond XOR-dependent free radical accumulation, recent data have linked uric acid itself to a multitude of detrimental processes that occur in heart failure, including cytokine production, cell apoptosis, and endothelial dysfunction (16). Epidemiological studies have revealed that elevated levels of uric acid independently predict mortality in patients with congestive heart failure (6). Paradoxically, uric acid is an effective inhibitor of radicals derived from the by-products of peroxynitrite (26), a strong oxidizing agent resulting from the reaction of NO with superoxide anion. Therefore, as a source of uric acid, XOR seems to encompass a combination of beneficial and deleterious potential effects in the cardiovascular system. Also, paradoxically, recent studies have emphasized the central role of free radicals in “ischemic preconditioning,” a protective process providing increased tissue tolerance to IR damage (28, 86).

Despite the plethora of evidence for a pivotal role of XOR in the pathophysiology of cardiovascular diseases and animal studies supporting the role of allopurinol in preventing serious cardiovascular outcomes, clinical human trials have so far failed to demonstrate any conclusive results (12, 24, 102, 106).

Conclusion and Future Perspectives

The data presented herein collectively suggest that, as well as its established function in the catabolism of purines, an essential biochemical pathway, it is increasingly evident that XOR initiates a variety of oxidative stress-related processes in respiratory and cardiovascular disorders. Significant progress has been made in the identification and structural characterization of this enigmatic enzyme. However, our understanding of the extent of its involvement in disease processes and the exact signaling pathways involved in the regulation of its expression and activity remains limited. In addition, there has been little progress in terms of translating the positive findings of the currently abundant in vitro and animal investigations into human trials. This disconnect may arise from the fact that, thus far, clinical trials had small sample sizes, and no large scale study has been published to date. Also, most if not all patients in these trials have already had substantial burden of oxidant-dependent disease before enrollment, and the XOR-targeted therapy might not have been administered early enough to have a significant impact on the outcome. Furthermore, we currently lack accurate tools for the identification of high-risk patients with potentially more severe oxidative stress who may preferentially benefit from the intervention. Moreover, many of the drugs currently used to treat common comorbid conditions (e.g., angiotensin-converting enzyme inhibitors, statins, and β-blockers) have themselves important antioxidant properties that may contribute to their therapeutic effects, thus diminishing the apparent benefit of anti-XOR therapy (29). Another reason may relate to the fact that conversion of allopurinol to its active metabolite oxypurinol is known to be catalyzed by XOR itself resulting in a paradoxical generation of ROS (69).

The relative nonspecificity of the currently available inhibitors of XOR, allopurinol and oxypurinol, may be liable as well. Febuxostat, a new nonpurine selective agent, inhibits the oxidized and reduced forms of XOR without affecting other enzymes of purine and pyrimidine metabolism (107). Recently, in a large phase III clinical trial, this drug was shown to be twice as effective in reducing uric acid levels in patients with gout compared with allopurinol (10). Whether febuxostat will have more clear-cut beneficial effects than allopurinol on human respiratory and cardiovascular disorders remains to be determined.

In conclusion, because of the wealth of information about its basic biology, its involvement in various disease states, and the availability of reasonably safe inhibitors, XOR is now primed as a unique focus of interest for translational research studies. The results of well-designed clinical trials exploring the role of specific and potent XOR inhibitors, given in an optimal dosing regimen to an adequately selected large patient population, are awaited with interest. This will provide an effective addition to the treatment arsenal for the all too common and often deadly cardiac and respiratory conditions discussed above.

REFERENCES


