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Review

Pathophysiology of circulating xanthine oxidoreductase: New emerging roles for a multi-tasking enzyme

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ABSTRACT

The enzyme xanthine oxidoreductase (XOR) catalyses the last step of purine degradation in the highest uricotelic primates as a rate-limiting enzyme in nucleic acid catabolism. Although XOR has been studied for more than a century, this enzyme continues to arouse interest because its involvement in many pathological conditions is not completely known. XOR is highly evolutionarily conserved; moreover, its activity is very versatile and tuneable at multiple-levels and generates both oxidant and anti-oxidant products. This review covers the basic information on XOR biology that is essential to understand its enzymatic role in human pathophysiology and provides a comprehensive catalogue of the experimental and human pathologies associated with increased serum XOR levels. The production of radical species by XOR oxidase activity has been intensively studied and evaluated in recent decades in conjunction with the cytotoxic consequences and tissue injuries of various pathological conditions. More recently, a role has emerged for the activity of endothelium-bound enzymes in inducing the vascular response to oxidative stress, which includes the regulation of pro-inflammatory and pro-thrombotic activities of endothelial cells. The possible physiological functions of circulating XOR and the products of its enzyme activity are presented here together with their implications in cardiovascular and metabolic diseases.

1. More than a century of studies

The enzyme xanthine oxidoreductase (XOR) catalyses the oxidation of hypoxanthine to xanthine and the latter to uric acid as the terminal steps of purine degradation in the highest primates [1]. The activity of XOR has been known since the end of the 1800s and has been the object of a large number of studies, mainly because of its stability and high concentration in a widely available source, bovine milk (for historical reviews, see [2–4]).

1.1. Functions of xanthine oxidoreductase

In addition to being a housekeeping enzyme, XOR is known as the rate-limiting enzyme in purine catabolism. Indeed, it has a regulatory effect on the turnover of nucleic acids because its activity produces irreversible metabolites, thus blocking the recovery of nucleotides through the purine salvage pathway [5]. This activity could also interfere with the purinergic activity of adenosine accumulation, as suggested by the anti-nociceptive effect of the XOR inhibitor allopurinol in mice [6]. However, other functions have been proposed for XOR, explaining the constant interest in this poorly understood enzyme.

XOR can oxidise a variety of endogenous metabolites, including aldehydes, purines, pyrimidines, pteridines, azopurines, heterocyclic compounds [7] (reviewed in [8]), and retinol [9]. Additionally, different xenobiotics can be catalysed by XOR [10], including antiviral and anticancer agents (reviewed in [11]), thus contributing to liver detoxification. XOR can also reduce oxygen, methylene blue, ferricyanide, and NAD^+ (reviewed in [12]), implying a low specificity towards both oxidising and reducing substrates. When oxygen is the electron acceptor, the reaction occurs via a one-electron reduction, producing superoxide ions [13], and a two-electron reduction, generating hydrogen peroxide (reviewed in [12]). These reactive oxygen species (ROS) may be produced by XOR and are responsible for cytotoxicity in physiological and pathological conditions due to the formation of hydroxyl radicals in the presence of iron via the Haber–Weiss reaction [14], as is the case in acute iron intoxication [15]. In particular, XOR-derived oxygen radicals have been implicated in reperfusion injury, including during organ preservation and transplantation [16].

In addition to cytotoxicity and tissue damage, XOR-generated ROS are responsible for many biological activities, including iron mobilisation from ferritin in the liver [17], iron absorption in the intestinal mucosal [18], and the induction of proliferation [19–21] as well as defence against infectious diseases by contributing to leukocyte–

Abbreviations: de-Mo, demolybdo form; de-S, desulpho form; Mo, molybdenum; NO, nitric oxide; ROS, reactive oxygen species; RyR2, ryanodine receptor; XDH, xanthine dehydrogenase; XDH/XO, intermediate form; XO, xanthine oxidase; XOR, xanthine oxidoreductase; *XOR*, gene for XOR

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endothelial cell adhesive interactions. Indeed, these oxidants may play a role in the cytotoxic activity of phagocytes either directly [22] by activating a chemotactic factor [23] and pro-collagenase [24], by increasing the adhesion of leukocytes [25] and cytokine production by monocytes [26], or by inducing the accumulation of leukocytes in microvasculature [27]. The contribution of XOR to host defence against ROS-sensitive pathogens was described in a mouse model of chronic granulomatous disease [28]. The bactericidal activity of XOR may be potentiated either by the possibility to act as an NADH oxidase [29–31] or by the ability to produce peroxynitrite [32]. The anti-microbial activity of XOR has been exhaustively reviewed in [33]. However, patients with a defective XOR gene causing xanthinuria do not show impaired immunological responses, suggesting that XOR activity is not essential to host defences (reviewed in [34]), although it may be responsible for tissue damage.

However, it should be noted that the final product of purine catabolism, uric acid, has an important role in vivo as an antioxidant (reviewed in [35]). The protection against oxidative stress provided by uricaemia has been proposed as the reason for increased life span in humans; therefore, the lack of uricase activity could represent an evolutionary advantage for uricotelic primates over ureotelic mammals [36] (reviewed in [37]). Collectively, the above considerations and others that will be put forth later have generated many hypotheses on the biological roles of XOR, which are not yet completely understood (reviewed in [34]).

1.2. Species and organ distribution

XOR has a widespread distribution among different species and has been identified in prokaryotic organisms as well as in plants and animals (reviewed in [2,38,39]), although with some variation in its catalytic activity; early studies established XOR in lower organisms as a dehydrogenase, whereas mammalian XOR was thought to be an oxidase (reviewed in [3]) until almost half a century ago, when mammalian XOR was shown to be a dehydrogenase in its native state [40,41]. XOR has a high level of phylogenetic conservation, with 70% and 53% sequence homology between the mammalian and avian or *Drosophila* enzymes, respectively (reviewed in [42]).

In mammals, the presence of XOR has been demonstrated in many organs, albeit at different levels; however, most cells show little enzyme activity (reviewed in [39,43]). Accordingly, XOR mRNA has been detected in most tissues, and the highest transcript levels are found in the organs with the highest enzyme levels, i.e., liver and intestine in mice [44] and humans [45]. This tissue distribution shows a considerable species variation; in particular, a wide range of activity levels has been reported in biological fluids, such as blood [46] (reviewed in [47]) and milk (reviewed in [39]). XOR has mostly been purified from cytosolic fractions, although it has also been associated with the cellular membrane as well as with intracellular organelles, such as peroxisomes [48] (reviewed in [49]).

Based on its levels of mRNA and protein expression, the highest activity levels of human XOR are present in the intestine [50,51] and liver [52–54], while a very low activity has been detected in other human organs [55,56]. XOR can be found in human milk, although with a lower activity compared with cow milk [57,58]. High serum XOR levels have been reported in several species, in particular in rodents and bovines, whereas in humans, it is very low in physiological conditions (reviewed in [39,48]). Interestingly, human endothelial cells from the microvasculature of several tissues have been identified as having high levels in XOR activity. Moreover, XOR has been detected not only in the cytoplasm of endothelial cells but also on the outer surface of the plasma membrane (reviewed in [11]). Later, the implications of these findings will be discussed together with the increase of XOR serum levels in pathological conditions.

XOR activity determination is usually performed by a spectrophotometric test to measure the accumulation of uric acid at 292 nm and NADH formation at 340 nm. The detection of XOR in tissues and fluids containing low levels of XOR requires particularly sensitive assays, such as radiometry [59,60], fluorometry [61,62], HPLC [63], enzyme immunoassays, and immunohistochemistry [64–66]. Obviously, immunological methods cannot determine the quantity or quality of enzyme activity; however, such methods provide an opportunity for measuring the amount of total XOR protein, both active and inactive.

1.3. Molecular and catalytic structure

The kinetics and molecular structures of the dehydrogenase and oxidase activities have been detailed, and a domain structure common to all xanthine oxidising enzymes has been proposed (reviewed in [42]). The XOR molecule belongs to the metalloflavoprotein family (reviewed in [8]) and is a homodimer consisting of two chains with a molecular mass of approximately 145 kDa (reviewed in [12]). Each subunit corresponds to one catalytic centre that includes a molybdopterin cofactor containing one atom of molybdenum, one molecule of FAD, and two unequal iron–sulphur redox centres (Fig. 1). These cofactors are contained in three domains of approximately 85, 40, and 20 kDa, respectively (Fig. 1a). The oxidation of a fully reduced XOR molecule includes the transfer of its six electrons to O_2, with the generation of two hydrogen peroxide and two superoxide anion species (reviewed in [42]). Usually, the substrate binds to the molybdopterin domain, and the electrons are released to the acceptor by the FAD-containing domain (Fig. 1b), except in the case of NADH oxidation, which is catalysed at the FAD-containing domain (reviewed in [34]). As a consequence, competitive XOR inhibitors, such as allopurinol, are ineffective against XOR NADH oxidase activity [29].

2. A multiple-level tuneable enzyme activity

The human gene for XOR (*XOR*) has been cloned and expressed in vitro [68–70]. Human *XOR* is located on chromosome 2, and its expression is under strict regulatory control (reviewed in [8,71]). Gene expression of *XOR* in humans is lower compared with other mammals, and promoter suppression has been hypothesised as the cause [72]. In animal models, the inhibition of protein synthesis by cycloheximide induces an increase of XOR mRNA levels, suggesting the presence of a repressor protein that limits *XOR* expression (reviewed in [37]).

2.1. Transcriptional regulation of xanthine oxidoreductase protein

Based on the above observations, the first level of XOR modulation is gene expression, which may be regulated by nutritional factors, oxygen tension, steroid hormones [73], phorbol esters [74], regenerative

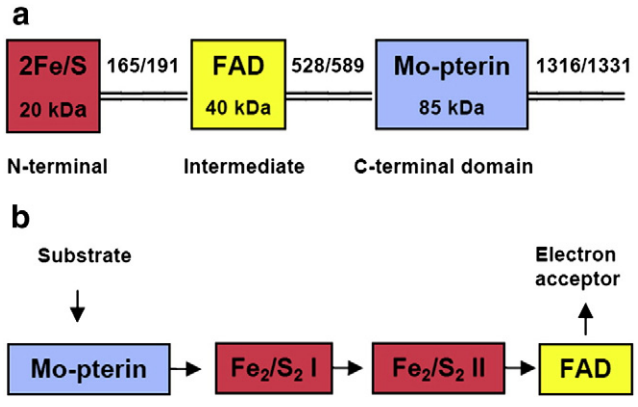

Fig. 1. *Molecular and catalytic structure of the xanthine oxidoreductase (XOR) monomer.* (a) Domains and linker peptides of rat XOR: each XOR monomer includes a 20-kDa N-terminal domain, a 40-kDa intermediate domain and an 85-kDa C-terminal domain (reviewed in [67]). (b) Primary catalytic electron flow: the catalytic centre of XOR includes a molybdopterin cofactor (MO-pterin), two unequal iron–sulphur redox centres, Fe_2/S_2 I, Fe_2/S_2 II and one molecule of FAD (reviewed in [67]).

and hyperplastic stimulus [75,76], different cytokines implicated in inflammation (reviewed in [77]), insulin, growth factors, and differentiation [78]. Additionally, tobacco smoke condensate up-regulated XOR activity in pulmonary endothelial cells by increasing XOR mRNA expression and *XOR* gene promoter activity [79].

Transcriptional regulation of *XOR* has been reported in association with food intake; for instance, *XOR* expression decreased due to the intake of tungsten, which antagonises molybdenum (Mo), and a diet poor in proteins and molybdenum [80]. In contrast, a lack of vitamin E, selenium or folic acid or a lipid-enriched diet increased liver XOR mRNA and protein expression [81,82]. In cell cultures and animal studies, iron depletion resulted in low XOR enzyme activity, which was reversed when the intracellular concentration of iron was increased (reviewed in [71]).

XOR expression was down-regulated in weakly differentiated tumours; in particular, decreased XOR protein is associated with a poorer prognosis in most patients with gastric, colorectal, and non-small cell lung cancer, breast tumours, hepatomas, and serous ovarian cancer (reviewed in [83]).

XOR activity may be subject to transcriptional regulation in mouse mammary epithelial cells, increasing mRNA levels from mid-pregnancy until lactation followed by decreasing levels with mammary involution [84]. The increase in *XOR* expression during pregnancy and lactation has been shown to be due to glucocorticoids and prolactin [85] and has been suggested to involve a glucocorticoid receptor and the STAT3 intracellular transduction pathway [78]. Human mammary epithelial cells increased *XOR* expression and enzyme activity when stimulated by IFNγ and other pro-inflammatory cytokines [86]. In this case, the up-regulation of both *XOR* transcriptional and post-translational activation was implicated because XOR activity increased much more than either XOR mRNA or protein levels alone. Moreover, the expression of *XOR* in the apical plasma membrane of mammary epithelial cells is required for enveloping milk fat droplets, and a role has been envisaged for XOR as a membrane-associated protein in the secretion of milk lipids during lactation in mice [87,88] (reviewed in [89]).

The administration of IFN or IFN-inducers to mice stimulated a marked increase of XOR activity in different organs [90], particularly in the liver [91,92]. Additionally, XOR activity was up-regulated by IFNγ in rat lung endothelial cells [93]. This effect was abolished by cycloheximide, suggesting that new protein synthesis was required. Moreover, IFNγ markedly increased XOR mRNA levels, demonstrating the transcriptional activation of the *XOR* gene. Likewise, various inflammatory cytokines, including IFNγ, IL-1, IL-6, and TNF, and steroids may up-regulate both *XOR* expression and activity in bovine epithelial cells [94]. In this case, new XOR protein synthesis and increased levels of XOR mRNA were also required. These and subsequent similar findings (reviewed in [8]) are in agreement with the reported increase of XOR activity in inflammatory/reparative conditions [60], in particular in viral infections, including lung [95], brain [96], and liver [97] in mouse pathology and liver in human pathology [54]. The infection of mice with *Salmonella typhimurium* induced a significant increase of XOR and nitric oxide (NO) synthase activity in the liver concurrently with the formation of granulomas with neutrophils and macrophages. The inhibition of these enzyme activities resulted in the exacerbation of the infection, indicating their importance as anti-microbial leukocyte defences [98].

2.2. Post-transcriptional regulation of xanthine oxidoreductase activity

The post-translational regulation of XOR activity (Fig. 2) includes conversions between active and inactive forms (Fig. 2a, [58,99] reviewed in [100]) as well as between XOR dehydrogenase and oxidase activities (Fig. 2b, [101] reviewed in [67]).

Studies on XOR from milk have shown that only 2% of the human enzyme is present in an active form, possibly because most XOR is in demolybdo- and/or desulpho-forms (Fig. 2a), both of which also occur

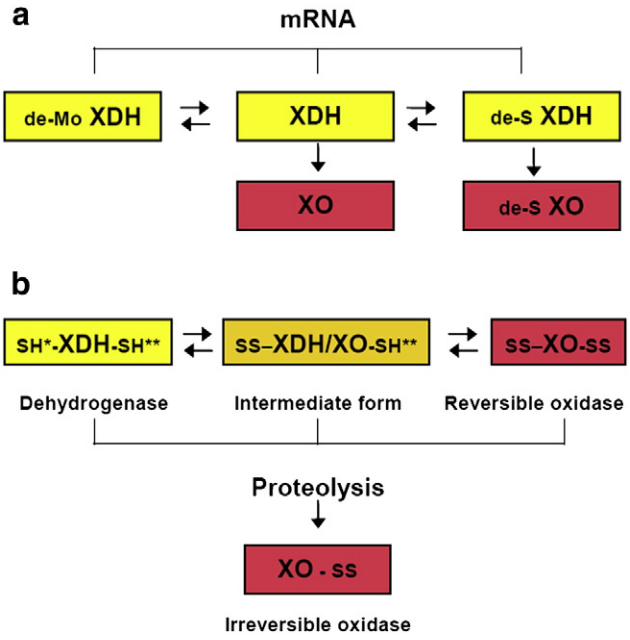

Fig. 2. *Post-transcriptional regulation of enzyme activity.* (a) Active and inactive forms of Xanthine oxidoreductase [58]: inter-convertible active (XDH) and inactive demolybdo-form (de-Mo XDH) [99] or desulpho-form (de-S XDH) of xanthine dehydrogenase (reviewed in [100]). Inter-convertible active (XO) and inactive desulpho-forms (de-S XO) of xanthine oxidase. (b) XOR activities and proposed modalities of conversion [101]: inter-convertible enzyme activities include dehydrogenase (XDH), intermediate (XDH/XO), and reversible oxidase. Thiol groups involved in the transition: fast (SH*) and slow (SH**) reacting (reviewed in [67]). Irreversible oxidase activity is the only result of partial proteolytic cleavage of XOR.

in bovine XOR, although to a lesser extent [58]. XOR commonly contains a small percentage of these forms, which are unable to catalyse xanthine but may still act as an NADH oxidase, generating superoxide and hydrogen peroxide [102,103]. The demolybdo-XOR apoprotein may be converted into the active enzyme in mouse L929 fibroblastic cells by molybdenum salts [99]. The desulpho-XOR could be reactivated in vivo by the XOR molybdenum cofactor sulphurase via the reinsertion of a sulphur atom at the active site (reviewed in [100]). Indeed, the phenotype of patients with molybdenum cofactor sulphurase mutations was that of xanthinuria, i.e., XOR deficiency [104]. Thus, the presence of demolybdo- and desulpho-XOR forms may be considered to be a modality of post-translational control of XOR activity. Accordingly, XOR activity varied widely in human milk, in most cases reaching the highest level during the first two weeks after parturition and falling thereafter, without changes in XOR protein levels [105].

The effect of hypoxia in Swiss 3T3 cells has been reported to increase XOR activity without an increase of mRNA levels and followed by an increase of mRNA levels and XOR activity [106]. These results are in agreement with the increase of XOR activity observed in rat brain slices in simulated ischaemia/reperfusion conditions [107]. Hypoxia, alone or associated with LPS and IL-1, up-regulated both *XOR* expression and activity in rat lung and induced pulmonary oedema, which was caused by XOR activity because it was prevented by treating the animals with tungsten [108]. Moreover, hyperoxia was shown to be a negative regulator of XOR activity in rat pulmonary endothelial cells [109]. Thus, the control of XOR activity by oxygen tension is likely to involve several points of adaptation at pre- and post-translational levels. In cells, hypoxia is able to induce numerous metabolic alterations and the activation of transcriptional factors, including hypoxia-inducible factor-1 (HIF-1), nuclear factor-κB (NF-κB), and protein kinases, such as mitogen-activated protein kinase (MAPK) (reviewed in [110]). Although the mechanism of regulating *XOR* is still unclear, some evidence points to the phosphorylation of STATs by Janus kinases followed by the

translocation of these transcriptional factors to the nucleus. Indeed, XOR activity is increased in cultured endothelial cells during hypoxia, inducing the production of IL-6 and the sequential activation of JAK-STAT signal transducers and transcription activators [111].

A different interpretation has been envisaged to explain the hypoxia-dependent increase of XOR activity instead of a higher level of either gene expression or post-translational activation induced by hypoxic conditions. This alternative hypothesis suggested that the molybdenum centre of XOR may be inactivated by the atmospheric oxygen in normoxic conditions, and the lack of such inactivation in hypoxia could explain the apparent induction of XOR activity. Accordingly, the increased XOR activity observed in response to hypoxia or hypoxia and reoxygenation is due to the post-translational modulation of XOR in cultured bovine aortic endothelial cells [112]. Additionally, the increase of enzyme activity in human bronchial epithelial cells cultured in hypoxic conditions was not accompanied with any change in the level of mRNA or enzyme protein, and the apparent activation was reversed upon return to normoxic conditions [113].

2.3. Dehydrogenase and oxidase enzyme activities

Mammalian XOR has the peculiarity of being found in two interconvertible forms: constitutively expressed in vivo NAD^+-dependent dehydrogenase (XDH, EC 1.1.1.204) and post-transcriptionally modified oxidase (XO, EC 1.1.3.22). The conversion may occur either irreversibly, by limited proteolysis [40,114], or reversibly, by chemical [101] or enzymatic [115] oxidation of XDH thiol groups [116,117].

It was reported that four Cys residues were modified to form two disulphide bonds during the conversion of XDH to XO from rat liver and bovine milk (Fig. 2b). The oxidation of two Cys residues forms a disulphide bond in the first rapid phase of the reversible XDH to XO transition followed by a second, much slower, phase associated with the modification of two more Cys residues (reviewed in [67]). These findings are in agreement with previous observations suggesting the presence of an intermediate form of XOR that is able to react with O_2 and NAD^+ [101,118,119]. The proteolytic nicking of the crucial Cys residues in the rapid-phase formation of the disulphide bond is responsible for the irreversible XDH to XO conversion, as already proposed [120]. The cleaved peptide connects the molybdenum- and FAD-containing domains. Either disulphide formation or proteolytic cleavage occurs at the same linker peptide in the reversible or irreversible transition, respectively (reviewed in [67]).

The ability of some XOR forms to react with O_2 and to act as NADH oxidases, inducing oxidative stress, has prompted much research to identify pathological situations in which the conversion from XDH to XO occurs. Such a transition can be shown by an elevated XO/XDH activity ratio and has been the object of numerous studies in the last forty years. The increase of ROS-producing enzyme forms has been reported in burn injury [121]; haematoporphyrin derivative-mediated cutaneous photosensitisation [122]; in vivo and in vitro ethanol intoxication [119,123,124]; glutathione depletion [125]; endothelial stimulation with C5a, TNF, a chemotactic N-formyl peptide [126] or activated neutrophils [127,128]; kainic acid [60] or glutamate neurotoxicity [129]; ricin hepatotoxicity [130]; in a rat model of nephropathy [131]; ultraviolet B radiation [132]; irradiation with gamma rays [133]; hepatocellular injury induced by iron accumulation [134] or cholestasis [135]; in gut mucosal lining of experimental cirrhotic rats [136]; and in rat brain following in vivo acute ammonia intoxication [137].

Additionally, reversible or irreversible XDH/XO conversion was observed in a variety of hypoxic/ischaemic conditions in rat tissues, including the liver, kidney, heart, and lung [138], liver and kidney [139], intestine [140], liver [141], and brain [106,142–144]. The transition from the dehydrogenase to the oxidase form of XOR proceeded slowly in rat brain during complete ischaemia [143]. Cell death preceded the conversion of XDH to XO during the reoxygenation injury of isolated rat hepatocytes. Furthermore, XOR was released from the severely injured cells before the XDH/XO conversion occurred [145]. The production of ROS by XO and by other cellular systems has been reported in many experimental hypoxic conditions with different cell types (reviewed in [110]). However, whether the increase in the XO/XDH activity ratio and the production of ROS precedes or is a consequence of cellular injury is not yet clear.

In particular, attention has been focused on the role of XOR-derived ROS in the pathogenesis of tissue lesions induced by reperfusion after ischaemia (reviewed in [16,146]). The presumed sequence of events was as follows: first, ischaemia induced the Ca^{++}-dependent proteolytic conversion of XDH to XO and the increased level of substrate, which was derived from the degradation of nucleic acids; second, the reperfusion supplied the molecular oxygen needed for ROS production (reviewed in [146]). The hypothesis concerning the post-ischaemia/reperfusion damage induced by XOR-generated ROS was supported by the results of many investigations, which have been carried out in the small intestine, stomach, pancreas, liver, skin, skeletal muscle, heart, lung, kidney, and central nervous system (reviewed in [147]). Reoxygenation-dependent oxidative stress has been reported to cause injury in a variety of cell cultures, showing an intracellular generation of such oxidants and demonstrating that ROS production by activated neutrophils, as proposed for vascular reperfusion injury, is not required (reviewed in [148]). XOR has been indicated as the major cause of oxidative stress after hypoxia/reoxygenation and ischaemia/reperfusion; however, the XOR activity level was highly variable in the different experimental models, suggesting that more than one source of ROS may be implicated (reviewed in [110]).

The relevance of XOR activity on tissue injury after reperfusion was investigated in animal models by pre-treatment with tungsten, which inactivates XOR, to prevent organ damage (reviewed in [149]). For the same purpose, the XOR inhibitor allopurinol has been used in many experimental and clinical studies (reviewed in [150]). For instance, patients with chronic heart failure due to dilated cardiomyopathy showed an up-regulated expression of *XOR*, and the inhibition of enzyme activity by allopurinol administration resulted in a significant improvement in myocardial efficiency [151]. Additionally, ischaemic pre-conditioning has been used to protect organs from ischaemia reperfusion damage, and oxidative stress is reduced as a consequence of the diminished conversion of XDH to XO in rat small intestine subjected to pre-conditioning procedures [152]. Despite the protective roles of tungsten and allopurinol treatment and ischaemic pre-conditioning in ischaemia/reperfusion injury, the impact of XOR-derived ROS is still a point of contention. However, ROS could at least contribute to the amplification of tissue damage (reviewed in [153]) based on the three-zone-model of liver injury due to hypoxia and reoxygenation (reviewed in [154]).

Tissue damage due to ROS generated during reperfusion after ischaemia has been considered to be a key problem in organ preservation for transplantation, particularly for XOR-rich organs, such as the intestine and liver. Therefore, the University of Wisconsin preservation solution includes allopurinol [155], although XOR activity was not definitively shown to be the main source of oxidative stress in tissue grafting. However, enhanced levels of XOR protein and activity were observed in rat renal allografts at day 9 post-transplantation, and XOR-generated ROS were associated with histological signs of acute renal allograft rejection [156].

A marked XDH to XO conversion occurred as a consequence of cold ischaemia in rat liver transplantation [157–159]. This conversion was prevented by ischaemic pre-conditioning of the graft through a 10-min interruption of blood flow of the donor liver followed by reflow for another 10 min. Additionally, the pre-conditioning was able to reduce liver damage and injurious effects in the lung following liver transplantation. Similar protective effects were obtained by inhibiting XOR with allopurinol and were abolished by the administration of xanthine and XOR to pre-conditioned rats.

3. Increased xanthine oxidoreductase serum levels in experimental and human pathology

As highlighted above, high serum XOR levels are present in healthy individuals of some animal species, although the purpose of this presence has not been clarified. However, the amount of circulating enzyme may become more elevated in some pathological conditions, such as liver pathology, viral infectious diseases, and tissue injury, due to hypoxia, as reported for hypoxic new-born pigs [160], or reoxygenation after hypoxia.

3.1. Experimental pathology

Hepatic damage caused by a variety of toxic agents was associated with elevated serum XOR levels, particularly after treatment with CCl_4 or thioacetamide in sheep [161] and after treatment with CCl_4 [162,163], colchicine [164], halothane [165], ethanol [166], saporin [130] or aluminium [167] in rats. Cobra venom factor injection in rats and the consequent intravascular activation of complement resulted in a rapid increase of XOR activity in plasma, which was partially related to the appearance of histamine in plasma [168]. Additionally, in rat pups treated with selenite to induce cataract formation, XOR serum levels were significantly higher than those in control animals, whereas antioxidant enzymes were lower than those in controls, suggesting a pathogenic role of XOR-produced ROS in this model of eye damage [169].

A higher XOR level in blood serum has been described in association with inflammation, as reported in mice treated with the IFN inducer [90] or infected with influenza virus [170–172], in rats after thermal skin injury [173–175], in cattle receiving LPS [176], in hypercholesterolaemic rabbits [177], in streptozotocin-induced diabetic rats [178] and mice [179], and in fructose-induced hyperuricaemic mice [180].

Ischaemia/reperfusion was apparently the reason for serum XOR elevation after artery ligation in cats [181], rabbits [182–186], and rats [27,157,187,188] as well as after haemorrhagic shock in mice [26] and rats [189,190]. XOR was also released in the perfusate of ischaemic/reperfused rat liver [191], and the XOR activity level determined in the perfusate was similar to the paralleled decrease in endogenous tissue enzyme [192]. Similarly, in rat liver/lung preparations, XOR was released from the liver after ischaemia/reperfusion, in which XOR activity decreased in hepatic tissue and increased in the effluent perfusate [193]. Moreover, an increase in XOR activity was demonstrated in the effluent of ischaemic/reperfused rat pancreas, whereas it decreased by half in the organs that underwent ischaemia [194].

These ex-vivo experiments implicated the XOR that leaked out from the liver in ischaemia/reperfusion injury and in vascular endothelium damage because, in these conditions, the absence of blood allows the exclusion of neutrophil components from the generation of ROS. However, the contribution of leukocyte-derived ROS to the pathogenesis of ischaemia/reperfusion damage in vivo was confirmed by the observation that neutrophil depletion attenuates human intestinal reperfusion injury [195]. In an in vivo rabbit model of lung ischaemia/reperfusion injury, XOR increased together with malondialdehyde content in serum and with the activity of myeloperoxidase and the expression of intercellular adhesion molecule-1 in lung tissue, indicating oxidative stress and inflammation [196].

XOR activity increased in the venous effluent of reperfused human muscle flaps subjected to experimental warm ischaemia. Such an increase was statistically correlated with the duration of ischaemia and biochemical markers of cellular injury [197].

3.2. Human pathology

The activity of serum XOR in healthy humans is very low, corresponding to the production of less than 4 nmol O_2/ml of plasma/minute when calculated as the reduction of ferricytochrome c by free radicals [198]. However, its increase has been reported in several pathological conditions. Increased plasma XOR levels were first observed in patients with viral hepatitis [199] in which two main situations were identified, inflammation and liver pathology, possibly explaining the sizable amount of circulating enzyme in humans. Elevated XOR levels in human serum were found in patients with acute and chronic viral hepatitis [61,62,163,200–203], infectious mononucleosis [200], rheumatic and autoimmune diseases [204], pneumonia [205], irritable bowel syndrome [206], schizophrenia [207], and type 2 diabetes [208,209]. In the latter pathology, a correlation was observed between the serum XOR activity level and lipid peroxidation in diabetic lens injury and senile cataract formation. Additionally, the blood concentration of glycated haemoglobin was positively correlated with lens and serum XOR levels, suggesting that inadequate glycaemic control may upregulate XOR activity, possibly contributing to an earlier onset of cataracts by inducing lens oxidative stress [210].

Among human hepatopathies that induce increased plasma XOR levels, we can count massive toxic necrosis [200] as well as the liver damage due to halothane anaesthesia [211], alcoholism [212], or cholestatic disorders [203]. An elevation of serum XOR was observed in patients after surgical operations [213]. In particular, elevated serum enzyme activity followed biliary tract and gastric surgery, possibly reflecting hepatocellular damage caused by surgical trauma to the liver [214]. Additionally, a significant elevation in serum XOR has been reported in children affected by falciparum malaria with varying severity based on parasitaemia. In the severe malaria group, a significant correlation, based on biochemical parameters, was observed between serum XOR levels and liver function impairment [215].

A significant increase in plasma XOR activity occurred in sickle cell disease patients, possibly because this pathology is associated with intrahepatic hypoxia/reoxygenation, which can induce XOR release into the circulation from an injured liver [216]. Sickle cell disease was reproduced in a knockout-transgenic mouse model. Mice demonstrated decreased liver XOR, with XOR increased on and in vascular luminal cells, suggesting that circulating XOR can bind to endothelial cells and impair vascular function. A similar enhancement of XOR was observed in the plasma of hypoxic subjects, such as patients with adult respiratory distress syndrome [217] and preterm newborn babies [218]. XOR serum levels were greater in the spermatic vein of varicocele patients compared with the peripheral vein, possibly because of the hypoxia due to reduced blood flow in the dilated varicocele vein [219].

XOR activity increased after tourniquet release (reperfusion) in local and systemic blood in patients undergoing tourniquet-induced exsanguination for limb surgery [220–222]. Additionally, in one patient, an elevated circulating XOR level was detected that was caused by ischaemia/reperfusion injury during an aortic cross-clamp procedure, and it was proposed that XOR-induced ROS may explain the damage to the lung or heart observed after ischaemia in human liver and intestine [223].

Maternal and foetal plasma XOR levels were higher than normal in pre-eclampsia [224]. Indeed, pre-eclampsia is characterised by hyperuricaemia and signs of increased formation of ROS as well as by endothelial dysfunction and inflammatory cytokine production. Moreover, the serum XOR level significantly correlated to the severity of the pre-eclamptic condition [225]. However, the results of these studies were not sufficient to clarify if the elevation of circulating XOR is the main pathogenic mechanism of ROS generation and the resulting vascular dysfunction or just a consequence of this syndrome.

Increased XOR serum levels were reported in patients undergoing liver [226–228] and kidney [229,230] transplantation. Elevated plasma concentrations of XOR were observed during reperfusion after liver transplantation compared with preoperative levels [226]. XOR was released into blood from liver grafts but not from recipient bowels [227,228]. XOR was transformed from XDH to reversible XO in the circulation. The serum XOR level was higher in the liver effluent of patients with moderate dysfunction compared with those with slight primary graft dysfunction [227]. The elevation of serum XOR persisted during the three weeks following liver transplantation [228].

The serum XOR activity level in the recipients of kidney allografts was significantly increased compared with the pre-grafting level 1 and 5 min after transplantation [229]. Additionally, the XOR level was inversely related to the early, slow, and delayed graft function activation. Moreover, a higher XDH to XO conversion was observed in the patient group, showing delayed functional graft recovery compared with the other two groups. A 6-month follow-up after transplantation showed that the average serum XOR level was constantly growing in kidney-transplanted patients starting from the first day after surgery [230]. It has been suggested that the XOR level in plasma on the first day after transplantation reflects the extent of damage caused by an ischaemia/reperfusion insult of the transplanted organ and that this may influence the grafting outcome. Further increases of XOR levels could be explained by immunosuppressive therapy, which includes steroids.

3.3. Origin and fate of circulating xanthine oxidoreductase

XOR is primarily produced by epithelial cells, although antibodies recognising the bovine milk enzyme detected the XOR molecule in capillary endothelial cells of many human tissues [231]. XOR leakage out of cells may occur as a result of physiologic cell turnover or as a consequence of pathological cell conditions. The intestine and liver are the main sources of serum XOR because of the high XOR content of these organs, and these organs may support a strong elevation of circulating XOR only after a wide tissue injury, which induces XOR release into the circulation from damaged cells [130,181] (Fig. 3).

After being released into plasma, XOR is rapidly converted to the XO form [124,227,232]. Circulating XOR may reach remote organs and has the ability to stick to vascular cells because it binds to sulphated glycosaminoglycans on the endothelial cell surface [233]. The binding of XOR to vascular lining was shown to be heparin reversible in studies with cultured endothelial cells [189]. Commercial re-purified XOR bound specifically and with high affinity to sulphated proteoglycans on the surface of bovine aortic endothelial cells in culture. A shift was then observed for XOR, while still conserving the superoxide-production ability, from extracellular binding sites to intracellular compartments (Fig. 4). Finally, endothelium-bound XOR activity was also able to inhibit endothelial NO production, thus impairing vasodilatory reaction [234].

XOR bound to and endocytosed by endothelial cells may explain the discrepancies in the reported organ and cellular distribution of XOR as well as the protection given by enzyme inhibitors to organs with a low XOR activity. Indeed, increased circulating XOR may bind to endothelial cells of distant organs devoid of enzyme activity after hypoxic or ischaemic damage, particularly in the splanchnic system.

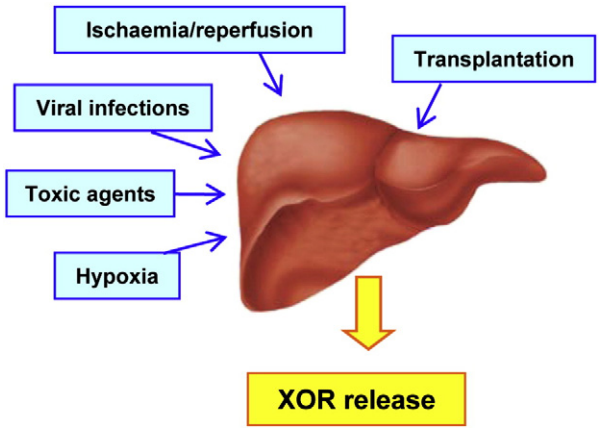

Fig. 3. *Increase of circulating xanthine oxidoreductase (XOR) due to hepatic leakage.* Main aetiological factors causing hepatocellular damage that induce XOR release in blood: hypoxia [216–219], ischaemia/reperfusion [223], transplantation [226–228], toxic agents [200,211,212], and viral infection [61,62,163,200–203].

4. Physiological and pathological roles of circulating xanthine oxidoreductase

4.1. Remote organ injury caused by increased xanthine oxidoreductase activity in serum

Circulating XOR has been implicated in the development of endothelial dysfunction and remote organ injury after different ischaemia/reperfusion protocols, including the ligation and reperfusion of limbs, in experimental and clinical pathology. The XOR level released in the perfusate was sufficient to produce severe vascular endothelial injury in vitro and in a rat model of liver ischaemia/reperfusion. These results suggested that the amount of ROS produced in circulation by XOR, which leaks out from damaged cells after hepatic ischaemia, could produce widespread tissue injury [192].

Hepatic ischaemia/reperfusion in rats not only induced the XOR conversion from XDH to XO in liver tissue but also resulted in increased levels of circulating XOR and microvascular disorders in the lung. Ischaemic pre-conditioning reduced XOR conversion and leakage as well as liver and lung damage, whereas the administration of xanthine and XOR abolished the benefits of pre-conditioning [157]. XOR leaked out of ischaemic/reperfused liver in rat liver/lung preparations, and it was converted from XDH to XO in the hepatic effluent. Subsequently, XO concentrated within the lung, where XOR activity increased significantly, possibly because of its binding to the endothelial lining. Finally, a dramatic increase in pulmonary microvascular and alveolar permeability occurred, suggesting that ROS derived from circulating XO severely impairs alveolar-capillary membrane integrity [193].

Rats subjected to intestinal ischaemia/reperfusion not only had increased plasma XOR activity but also had increased plasma leukotactic activity for neutrophils and lung neutrophil retention compared with sham-treated rats. These alterations decreased in rats administered antisera against XOR, indicating that circulating XOR mediates lung neutrophil sequestration after being released from ischaemia/reperfusion injured intestine [187]. Gut ischaemia/reperfusion is followed in rats by simultaneous liver and lung injury, and neutrophils have been shown to play a critical role in this process, which has been suggested to be mediated by XOR. Indeed, the lung and liver capillary leakage and the hepatic metabolic derangement induced by gut ischaemia/reperfusion were not observed in animals given a tungsten-enriched, molybdenum-depleted diet [235].

In a rat model of gut ischaemia/reperfusion, increased gut and lung myeloperoxidase levels were reported together with increased lung endothelial permeability. XOR inactivation by a tungsten-enriched, molybdenum-depleted diet abolished these alterations, suggesting a XOR-dependent neutrophil mechanism in the pathogenesis of remote organ injury [236]. The increased plasma XOR level induced by haemorrhage in mice is associated with the expression of pro-inflammatory and immune-regulatory cytokines in the lungs. The administration of post-haemorrhage plasma to recipient mice activated the nuclear transcriptional regulatory factor CREB and increased cytokine expression in lung mononuclear cells through an XOR-dependent mechanism [26].

The animal model of occlusion/reperfusion of the descending thoracic aorta has been used to study the remote consequences of hepatoenteric ischaemia/reperfusion induced by circulating XOR. Remote pulmonary injury manifested by increased protein concentrations in bronchoalveolar lavage in rabbits was significantly associated with hepatic ischaemia/reperfusion induced by the occlusion/reperfusion of the descending thoracic aorta, i.e., with liver injury and elevated circulating XOR activity [184,237]. These results are in agreement with the attenuation of post-occlusion shock after descending thoracic aorta occlusion and reperfusion observed in rabbits pre-treated with tungsten, which showed a moderate increase of plasmatic enzyme activity, thus suggesting a contribution of XOR-derived ROS in the pathogenesis of de-clamping shock [183]. Tungsten pre-treatment of rabbits subjected to occlusion/reperfusion of the descending thoracic aorta was also

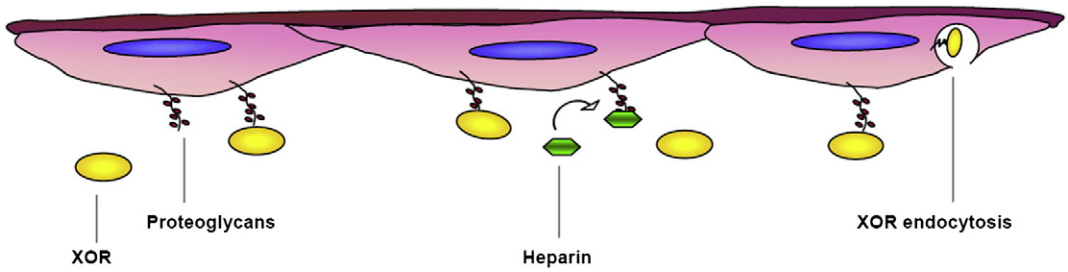

Fig. 4. *Binding of xanthine oxidoreductase (XOR) to endothelial cells.* The binding of circulating XOR to proteoglycans on the surface of the vascular lining is heparin-reversible until XOR is endocytosed by endothelial cells [189,234].

protective towards the decrease of gastric intramucosal pH induced by hepatoenteric ischaemia/reperfusion, which is considered a marker of multiple organ injury [185]. A similar XOR-dependent mechanism has been suggested for remote myocardial injury following hepatoenteric ischaemia/reperfusion induced in rabbits by the occlusion/reperfusion of the descending thoracic aorta because of the protective effect of tungsten pre-treatment in animals [186].

A high circulating XOR level has been observed after the ligation and reperfusion of rat hind limbs, and it has been proposed that the XOR released from the ischaemic limb after reperfusion is able to induce liver damage by activating Kupffer cells and neutrophils, thus contributing to the development of multiple system organ failure [188]. The vascular alterations produced by XOR-released ROS may be responsible for endothelium activation and cytokine production, with possible consequences in distant organs that may lead to systemic inflammatory response syndrome and multiple organ dysfunction syndrome (reviewed in [238]). Ischaemic pre-conditioning protects liver and lung from damage in rat liver transplantation by preventing post-ischaemic ROS generation from hepatic XOR and liver injury as well as lung inflammatory damage, including neutrophil accumulation, oxidative stress, and oedema formation. The role of XOR was demonstrated by the elevation of transaminases to levels similar to those found after reperfusion and by the abolition of the protective effect of pre-conditioning on lung inflammatory damage caused by the administration of xanthine and XOR to pre-conditioned rats [158].

In many cases of XOR leakage from damaged tissues, XOR inhibition by allopurinol reduced remote organ injury, suggesting that XOR may act as a circulating mediator in the induction of tissue damage (reviewed in [150]). Accordingly, intestinal reperfusion after superior mesenteric artery ligation in rats led to an elevation in XOR serum levels, and XOR activity inhibition with febuxostat reduced the degree of local intestinal injury and the consequent remote hepatic and lung impairment [239].

The supposed mechanism of action is based on the binding of circulating XOR to the endothelium, where XOR-produced superoxide can combine with the endothelium-derived NO. The resulting peroxynitrite may activate downstream pathways of cell injury in a variety of pathological conditions, which can lead to endothelial and tissue injury (reviewed in [150]). For example, although the depression of myocardial function induced in dogs by haemorrhagic shock was restored after the reinfusion of shed blood, 50% of the animals suffered complete circulatory collapse and death in a couple of hours. However, the pre-treatment with allopurinol was able to guarantee a 100% survival rate [240].

4.2. Vascular effects of oxidants produced by circulating xanthine oxidoreductase

The direct effect of XOR on endothelial cells was studied by an experimental model in rat brain; oedema and vascular permeability were induced by oxygen-derived free radicals produced by the infusion of XOR, and the consequent brain injury was proportional to the injected enzyme level [241].

The release of ROS in the bloodstream has been implicated in remote damage. For example, the protection from ischaemia/reperfusion injury obtained by direct ischaemic pre-conditioning of an organ may be achieved by short cycles of artery occlusion in a different organ. This effect suggests that low oxidant levels may lead to endothelial cell stimulation and to the formation of protective pre-conditioning triggers in distant organs, whereas high levels of ROS may activate a cascade of ischaemia/reperfusion injury (reviewed in [242]). In addition to the immediate protection against ischaemia/reperfusion injury, pre-conditioning triggers may also induce delayed protection. Redox signalling may be involved during the initial pre-conditioning period and the late pre-conditioning period, thus providing delayed protective effects, possibly through the increased expression of antioxidants (reviewed in [243]).

A biphasic production of ROS was observed after ischaemia/reperfusion; early XOR-dependent oxidative stress was followed by later ROS generation by leukocytes, which were induced to adhere to vasculature lining through the activation of endothelial cells caused by XOR-produced oxidants (reviewed in [148,244]; see also above). Thus, before having cytotoxic effects, the production of ROS may promote inflammatory alterations to vascular endothelium during ischaemia/reperfusion injury (Fig. 5). The consequences of endothelial dysfunction are represented by reduced NO production and the impaired vasodilation of arterioles (Fig. 5a), the increased expression of adhesion molecules in capillaries that induce leukocyte adhesion, protein leakage from post-capillary venules and the formation of tissue oedema (Fig. 5b) (reviewed in [245]).

Because XOR is one of the main sources of ROS in plasma, a role has been suggested for its enzyme activity in orientating the behaviour of endothelial cells and in regulating their pro-inflammatory and pro-thrombotic activities. A variety of therapeutic procedures, such as thrombolytic therapy, organ transplantation, and cardiopulmonary surgery, may induce microvascular dysfunction related to the oxidative stress generated by ischaemia/reperfusion, which overlaps the molecular and biochemical changes that are characteristic of an acute inflammatory response. For instance, enhanced fluid filtration and leukocyte plugging were observed in capillaries and in neutrophils and plasma protein extravasation in post-capillary venules (reviewed in [238]). In this frame, the post-translational conversion of XDH to the ROS-producing form of XOR in vivo could imply its well-studied pathological role of inducing tissue damage and the physiological function of triggering a microvascular inflammatory response through an oxidant-mediated signal transduction (reviewed in [246]).

Data that demonstrate the role of XOR activity as a transducer of inflammatory signals and the implications related to vascular dysfunction continue to accumulate. XOR in mononuclear phagocytes contributed to acute cytokine-induced lung injury in rats, and the reduction of enzyme activity obtained either by allopurinol's administration or by feeding the animals with tungsten resulted in a decrease of inflammatory cell infiltration and alveolar cell apoptosis [247]. Moreover, XOR activity was increased in mononuclear phagocytes isolated from inflamed rat lungs and promoted the inflammatory state of these cells through the

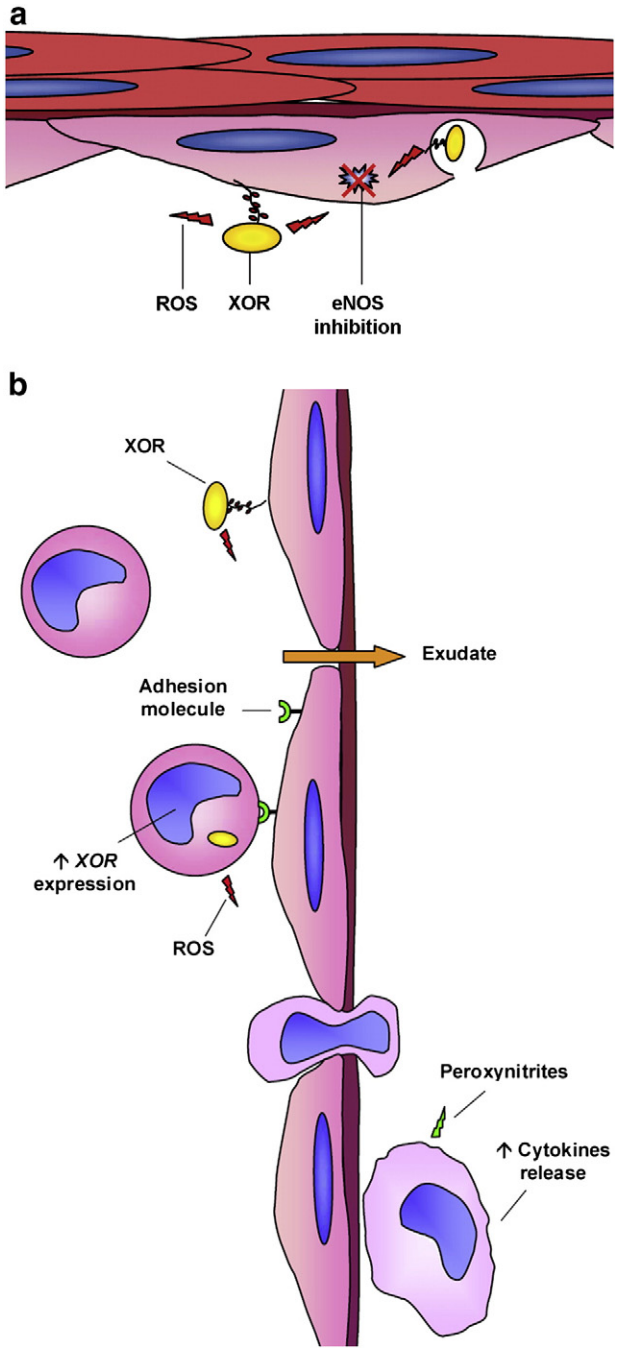

Fig. 5. *Vascular effects of oxidants produced by xanthine oxidoreductase (XOR).* (a) Impaired vasodilation of arterioles: XOR activity causes the endothelial dysfunction that is responsible for reduced nitric oxide production ending in the impaired vasodilation of arterioles [234]. (b) Pro-inflammatory action: the pro-inflammatory action of ROS derived from XOR activity causes (i) the induction of adhesion molecules and the permeabilisation of endothelial cells, (ii) exudate formation and mononuclear cell adhesion and diapedesis (reviewed in [244]), and (iii) the consequent over-expression of *XOR* by mononuclear cells and the production of ROS, peroxynitrites, and cytokines [247,248]. ROS, reactive oxygen species; eNOs, endothelial nitric oxide synthase.

modulation of chemokine expression [248]. In virus-infected mice, the endosomal Toll-like receptors 7 and 8 stimulated the expression of hypoxia-inducible factor 1, possibly inducing XOR transcription, which in turn may activate inflammasome-complex formation through the production of uric acid and ROS, as suggested by the effect of allopurinol in attenuating caspase 1 activation and IL-1β release [249].

The supposed role of XOR-produced ROS as a modulator of endothelial function suggests that the evolution of the *XOR* gene in mammals led to the acquisition of the post-translational modification of the activity from XDH to XO, thus allowing the generation of oxidants with the function of mediators on the surface of the vascular lining.

4.3. Role of xanthine oxidoreductase in cardiovascular disease

The microvascular alterations elicited by ischaemia/reperfusion, including the impaired endothelium-dependent dilation in arterioles, are similar to those induced by hypercholesterolaemia, hypertension, and diabetes, which are well known risk factors for cardiovascular disease (reviewed in [238]). Indeed, the inhibition of NO production by vascular XOR activity could explain how XOR released into plasma during diverse pathological processes may alter vascular functions via oxidative mechanisms by reducing vasodilatory responses (reviewed in [244]).

The enhanced expression and activity of XOR were reported in a rat model of heart failure [250]. Indirect evidence of a role for XOR in cardiovascular disease was provided by the protective effect of allopurinol in dogs with pacing-induced heart failure [251]. In this animal model, an increased expression of *XOR* in cardiac myocytes was shown, which resulted in an imbalance between XOR activity and NO production, contributing to mechanoenergetic uncoupling in heart failure [252]. The up-regulation of XOR at the transcriptional level was associated with elevated circulating XOR in dog heart failure, and XOR inhibition with allopurinol was able to prevent increases in systemic vasoconstriction and was able to improve myocardial contractility [253]. Long-term allopurinol treatment has been shown to prevent left ventricular hypertrophy and to improve cardiac function and structure in a rat model with established chronic heart failure [254]. The functional improvement of cardiac contractility with XOR inhibitors was dependent on the initial XOR activity level, which was more pronounced in failing rat myocardium [255]. Additionally, XOR inhibitors improved energy metabolism and function after infarction in failing mouse heart [256].

XOR serum levels were significantly higher in patients with ischaemic heart disease than those in control subjects, and there was a significant positive correlation between XOR and ischaemia-modified albumin, creatine kinase and other cardiac markers [257]. XOR inhibition with allopurinol had beneficial effects in patients with idiopathic dilated cardiomyopathy [151], and it improved peripheral vasodilator capacity and blood flow locally and systemically in hyperuricaemic patients with chronic heart failure [258,259] and in patients with coronary artery disease [260]. XOR inhibition by oxypurinol was tested for one month in thirty patients with congestive heart failure and compared with placebo-treated controls. The treatment significantly improved the left ventricular ejection fraction only in 21 patients who exhibited this initial parameter lower or equal to 40% [261]. The vascular benefits of allopurinol have been demonstrated in stroke survivors by reducing arterial wave reflection as a measure of vascular function [262]. Allopurinol treatment attenuated the rise in intercellular adhesion molecule-1 levels, thus controlling the expression of inflammatory markers in patients after acute ischaemic stroke [263]. However, in 405 patients with symptomatic heart failure due to systolic dysfunction, clinical outcomes did not differ in a 24-week clinical trial with oxypurinol treatment versus placebo, although a sub-group of patients with high levels of serum uric acid had some benefit from XOR inhibition in a manner correlating with the degree of serum uric acid reduction [264].

A further possible link between circulatory impairment and XOR activity has been suggested by the ability of angiotensin II to increase XOR protein levels and superoxide production activity in cultured endothelial cells. In patients with coronary disease, treatment with the AT1-receptor blocker losartan reduced endothelium-bound XOR activity and endothelial dysfunction [265]. These observations indicate a regulatory function of the vascular tone for endothelium-bound XOR through its activity products, i.e., ROS and uric acid (Fig. 6).

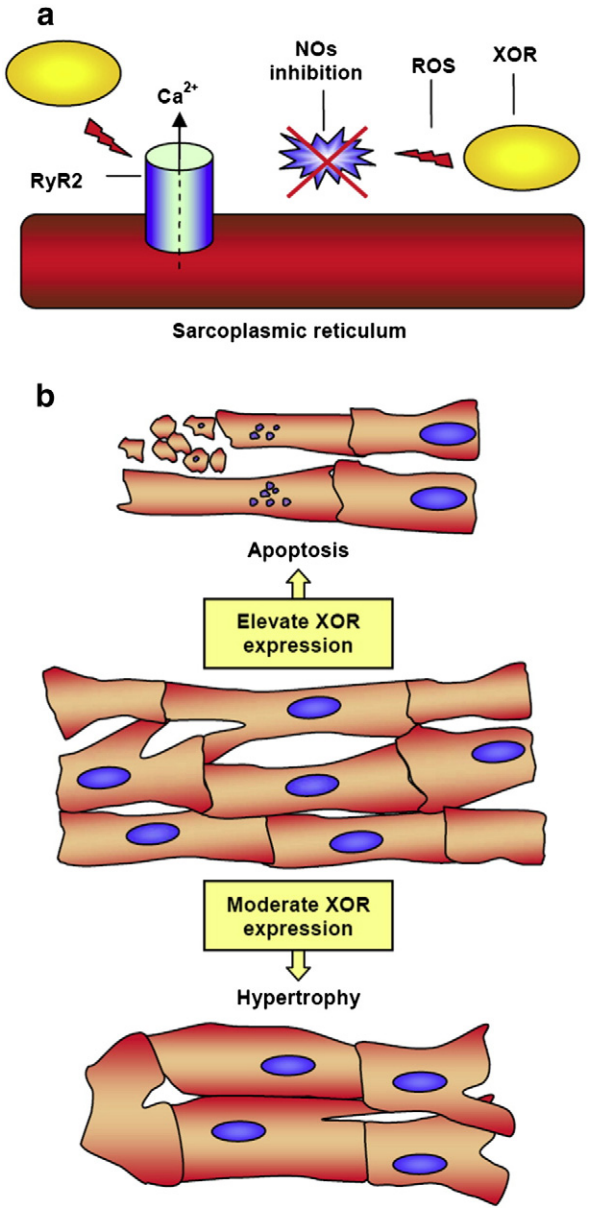

Fig. 6. *Effects of redox imbalance on cardiac cells.* (a) Hyperactivity of the ryanodine receptor (RyR2) in failing myocardium: the reactive oxygen species (ROS) produced by xanthine oxidoreductase (XOR) inhibit nitric oxide synthase (NOs), thus blocking the regular nitrosylation of RyR2, and induce the abnormal oxidation of RyR2, causing a diastolic leak and a consequent reduction of CA^{2+} content in the sarcoplasmic reticulum that impairs sarcomere contraction (reviewed in [266]). (b) Hypertrophic/apoptotic response of myocardiocytes: the reactive oxygen species (ROS) produced by angiotensin II-up-regulated *XOR* may activate the gene expression of mitogen activated protein kinase (MAPK) if present at low levels or may trigger the mitochondrial apoptotic pathway if present at high levels (reviewed in [243,267]).

Increased *XOR* expression was detected by immunostaining heart sections of rats with spontaneous-hypertensive heart failure. XOR up-regulation was associated with increased superoxide production and with decreased S-nitrosylation of the ryanodine receptor. As a consequence, increased diastolic calcium leakage from the sarcoplasmic reticulum and decreased sarcomere shortening in failing myocytes were observed (Fig. 6a). With XOR inhibition, these alterations were reduced to the levels of non-failing myocytes. Collectively, these findings indicate that nitroso-redox disequilibrium at the sarcoplasmic reticulum level is due to oxidative stress, which is possibly dependent on XOR up-regulation [266].

It has been proposed that XOR activity is part of an oxidant/anti-oxidant balance, the disequilibrium of which, leading to increased ROS production, may favour either hypertrophic or apoptotic effects in cardiac myocytes (reviewed in [267]). Moreover, the implication of superoxide anion and hydrogen peroxide has been suggested through redox-sensitive pathways in cardiac outcomes (Fig. 6b), including detrimental effects, such as pathological hypertrophy or apoptosis, and protective effects, such as adaptive hypertrophy and angiogenesis (reviewed in [243]).

4.4. Role of uric acid in vascular alterations

It has been proposed that the reduced vasodilator capacity and impaired peripheral blood flow observed in association with hyperuricaemia in patients with chronic heart failure could be ascribed to the endothelial dysfunction induced by the products of XOR activity [258]. Indeed, the serum concentration of uric acid was correlated with a reliable marker of endothelial dysfunction, albuminuria, in patients with heart failure [268].

The pathophysiological role of uric acid is controversial because it appears to be responsible for both antioxidant (primarily in plasma) and pro-oxidant (primarily within the cell) effects and pro-inflammatory effects, the latter function being exerted by the products of uric acid oxidation in the presence of peroxide [269]. Hyperuricaemia is associated not only with gout, urate nephrolithiasis, and tumour lyses syndrome but also with vascular diseases (reviewed in [270,271]), which are considered an adverse prognostic marker for metabolic syndrome (reviewed in [272]), including hypertension, visceral obesity, dyslipidaemia, insulin resistance, and type 2 diabetes, as well as for several consequences of atherosclerosis, such as stroke, myocardial infarction, and cardiovascular death (reviewed in [273,274]).

Estimated cardiac output and stroke volume were inversely related to uricaemia in a large sample of pharmacologically untreated subjects [275]. Moreover, the serum concentration of uric acid was an independent predictor of all-cause mortality in patients at high risk of cardiovascular disease [276]. This study was conducted between 1998 and 2004 on a total of 1300 patients, taking into account age, sex, smoking status, alcohol consumption, weight, body mass index, waist circumference, blood pressure, history of cardiovascular disease, estimated glomerular filtration rate, cholesterol fraction levels, and plasma glucose levels. Clinical studies with XOR inhibition or therapies with uricosuric agents indicated that uric acid reduction may contribute to the reduction of risk (reviewed in [277,278]).

The possibility of predicting clinical outcomes in heart failure from the elevation of uric acid in serum indicates a pathogenic role either for the oxidative stress produced by the activity of circulating XOR or for the impairment of NO signalling directly by uric acid (reviewed in [273]). Uric acid-derived free radicals can have a pathogenic role in atherosclerosis development by activating dysfunctional responses, such as up-regulating the angiotensin system in cultured endothelial cells and lowering the availability of NO by directly scavenging and inducing the degradation of NO as well as by stimulating the proliferation and migration of arterial smooth muscle cells and inducing them to produce monocyte chemoattractant protein-1 (Fig. 7, reviewed in [274,279]).

Increased serum uric acid levels may be a consequence of metabolic derangement due to the hypertension developed in many pathological conditions or may just reflect the increased activity of XOR, which can result in high blood pressure through the production of ROS. However, a direct pathogenic role of hyperuricaemia in hypertension has been suggested by its frequent emergence before blood pressure rises and because serum uric acid level is an independent predicting factor for the development of hypertension. The supposed mechanism of action is mediated by the intracellular production of uric acid-derived radicals and the consequent oxidative stress on endothelial cells, which activates the renin/angiotensin pathway, thus developing renal arteriolar disease (reviewed in [280]). This hypothesis was supported by the

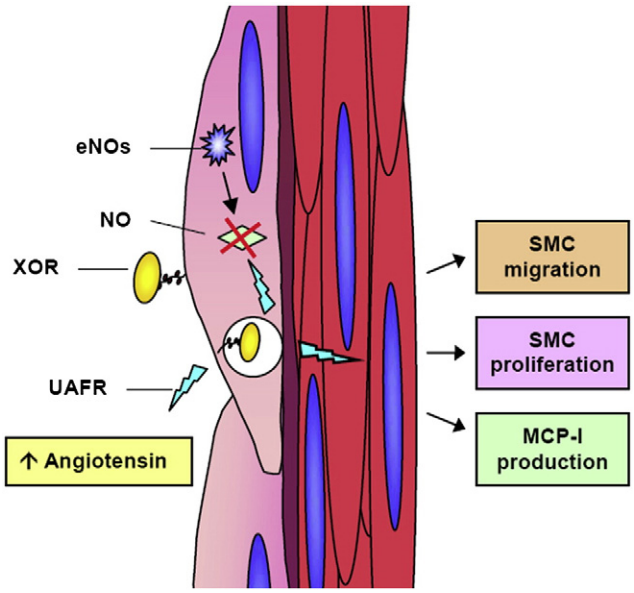

Fig. 7. *Role of uric acid-derived free radicals (UAFR) in hypertension and atherosclerosis.* UAFR may cause endothelial dysfunction, leading to systemic and renal vasoconstriction (i) by decreasing nitric oxide (NO) availability and (ii) by stimulating the up-regulation of the renin/angiotensin pathway. Moreover, UAFR may activate arteriolar smooth muscle cells (SMC) by inducing migration, proliferation, and the production of monocyte chemotactic protein 1 (MCP-1) (reviewed in [274,279]). XOR, xanthine oxidoreductase; eNOs, endothelial nitric oxide synthase.

observation in a rat model that oxonic acid-induced hyperuricaemia is associated with hypertension and that the latter was prevented either by inhibiting XOR activity, which produces uric acid, or by inducing uricosuria, which eliminates uric acid [281,282]. A high-fructose diet was shown to induce obesity and hyperuricaemia [283], which, by impairing the NO production of endothelial cells, may not only cause hypertension but also stimulate hyperinsulinaemia and hypertriglyceridaemia. These features of metabolic syndrome were prevented with allopurinol or reversed with a uricosuric agent [284] (reviewed in [272,285]).

Plasma levels of uric acid were significantly higher in spontaneously hypertensive rats than in control animals, and blood pressure was decreased markedly by XOR inhibitors, suggesting that enzyme-generated oxidants may have a critical role in the pathogenesis of hypertension [286]. Mean arterial pressure positively correlated with uric acid, cholesterol, and XOR activity in the serum of normotensive subjects [287]. Moreover, allopurinol treatment resulted in blood pressure reduction in adolescents with newly diagnosed hypertension [288], although it is not clear if the benefit was due to XOR activity inhibition, uricaemia reduction, or the ROS scavenging activity of allopurinol (reviewed in [289]). XOR activity inhibition with polydatin reduced enzyme activity in serum and renal injury by attenuating hyperuricaemia and related inflammatory responses in fructose-induced urate nephropathic mice [180]. Moreover, epidemiological evidence indicates the advantage of lowering the serum concentration of uric acid by XOR inhibition, which supports this clinical strategy (reviewed in [277]).

4.5. New emerging roles for xanthine oxidoreductase activity

In the second half of the past century, a large body of evidence was focused on the cytotoxic effect and consequent tissue damage of oxidative stress, while more recently, much attention has focused on the signal transmission and regulation of vascular function exerted by ROS. The main molecular sources of such oxidants in endothelial cells have been identified in NAD(P)H oxidase, xanthine oxidase, and NO synthase, which are involved in the dysregulation of the vascular redox environment that is associated with impaired vascular function and cardiovascular diseases (reviewed in [290]). In aging rats, the increased ROS-associated elevation of systolic blood pressure appeared to be dependent on vascular XOR but not on NAD(P)H oxidase activity [291]. However, XOR may be converted by NAD(P)H oxidase to the ROS-producing form of the enzyme and XOR-derived ROS may activate NAD(P)H oxidase activity. Moreover, the increased *XOR* expression induced by angiotensin II is prevented by NAD(P)H oxidase inhibition. Therefore, it is not simple to identify the specific role of these two enzymes in cardiovascular diseases (reviewed in [292]).

The pro-inflammatory action of XOR products has been enriched by new details that link this enzyme with pathologies resulting from endothelial dysfunction. Increased plasma XOR activity was found significantly correlated with NF-kB activation, high levels of inflammatory markers and insulin resistance in familial combined hyperlipidaemia [293]. In patients with metabolic syndrome and in patients with or at risk of cardiovascular disease, XOR inhibition by means of allopurinol treatment (1) had beneficial effects on endothelial function, thus improving brachial artery flow-mediated dilation and (2) reduced oxidative stress by decreasing myeloperoxidase levels [294] (reviewed in [295]).

Collectively, these findings suggest that XOR-induced oxidative stress is implicated in the pathogenesis of endothelial dysfunction, which is associated with smoking, hypercholesterolaemia, hypertension, and diabetes, i.e., the main risk factors for atherosclerosis (reviewed in [244]). The consequences of such vascular dysfunction are platelet aggregation, the loss of vasodilatation and inflammation, which are responsible for cardiovascular diseases (reviewed in [296]).

Recently, the effects of XOR activity inhibition on heart failure, chronic kidney diseases, and other pathologies were analysed by comparing the protective results obtained with allopurinol and febuxostat treatments (reviewed in [297]). Febuxostat is a non-competitive, specific XOR inhibitor and is more effective against the endothelium-associated enzyme than allopurinol. In animal models, febuxostat has displayed a protective role in pathological conditions with an underlying inflammatory process. In particular, febuxostat attenuates systolic overload-induced left ventricular hypertrophy and dysfunction in mice [298]. Additionally, febuxostat treatment corrected hyperuricaemia, hypertension, and hypertriglyceridaemia and prevented an increase in fasting plasma insulin in rats with fructose-induced metabolic syndrome [282].

The protection given by XOR inhibition, either with allopurinol or febuxostat, to damaged hearts was effective only if initiated shortly after myocardial infarction in rabbits, suggesting the need to consider the differences between species in choosing the appropriate therapy against the vascular consequences of oxidative stress [299].

Controversial results have been obtained in both experimental and clinical investigations addressing the pathological role of XOR in coronary diseases, cardiomyopathy and heart failure. Thus far, no conclusive results have been reached regarding the suggested protection given by XOR inhibition against adverse cardiovascular outcomes, possibly because these effects result from the balance and interactions between the activities of XOR and the other ROS producing enzymes NAD(P)H oxidase and NO synthase (reviewed in [292]).

XOR inhibitor treatment efficiently lowers hyperuricaemia and alleviates problems associated with urate accumulation, such as gout and tumour lyses syndrome. Such treatment could possibly be beneficial for metabolic syndrome with diet-derived obesity, in which the level of serum uricaemia is increased by alimentary fructose. However, a protective effect against hypertension or chronic renal disease induced by XOR inhibition is not yet demonstrated, although hyperuricaemia has been suggested to cause these pathologies through the down-regulation of NO production (reviewed in [300]).

An opposite role for XOR activity was proposed in a rat model of myocardial ischemia/reperfusion injury because nitrite-induced cardio protection against infarction was abolished by inhibiting XOR or NADPH oxidase but not by inhibiting NO synthase activity [301]. Accordingly, a recent study showed that erythrocytic XOR may have a

blood pressure-lowering action because of its nitrite reductase activity in an experimental model of hypertension and possibly in hypertensive patients. Thus, allopurinol treatment could suppress the blood pressure-lowering effects of dietary nitrates [302].

As for the future perspectives, despite the lack of a definitive demonstration, the pathogenic role of XOR in cardiovascular diseases seems to be the most promising field for further investigations. The experience from previous clinical trials suggests designing future studies with large numbers of patients, and it also indicates an essential role of a more stringent selection process of the cohort of patients to include in each trial to better identify the pathological conditions that are sensitive to the inhibition of enzyme activity. The availability and efficacy of inhibitors of the enzyme, in part already used for a long time in therapy, guarantee the opportunity to safely perform these studies and also suggest that the exploration of new drugs that inhibit XOR activity is a promising topic for future research.

5. Conclusions

This review underlines the new emerging role of XOR as a multitasking enzyme and the possible biological meaning of the fine tuning of its action at the transcriptional and post-transcriptional levels. The evidence collected and analysed here indicates the involvement of circulating XOR as one of the main mechanisms for the regulation of endothelial functions. Indeed, XOR action is responsible for the production of ROS and uric acid with pathophysiological consequences, including the induction of pro-inflammatory and pro-thrombotic activities of endothelial cells. Additionally, the increased activity of circulating XOR is associated with hypertension, dyslipidaemia, diabetes, and cigarette smoking, and it may contribute to the pathogenesis of atherosclerosis. Moreover, epidemiological studies continue to accumulate evidence on the beneficial effects of XOR inhibition in metabolic syndrome. However, the beneficial versus detrimental outcomes of XOR activity and XOR-derived products are hardly predictable because they depend on the balance between the actions of various enzymes involved in generating and/or controlling oxidative stress.

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Conflict of interest disclosure statement

Potential conflicts of interest do not exist for any of the authors, including any financial, personal or other relationships with other people or organisations within three years of beginning the submitted work that could inappropriately influence or could be perceived to influence their work.

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References

[1] E.J. Morgan, C.P. Stewart, F.G. Hopkins, On the anaerobic and aerobic oxidation of xanthin and hypoxanthin by tissues and by milk, Proc. R. Soc. Lond. B 94 (1922) 109–131.
[2] E.C. De Renzo, Chemistry and biochemistry of xanthine oxidase, Adv. Enzymol. Relat. Subj. Biochem. 17 (1956) 293–328.
[3] A. Kooij, A re-evaluation of the tissue distribution and physiology of xanthine oxidoreductase, Histochem. J. 26 (1994) 889–915.
[4] V. Massey, C.M. Harris, Milk xanthine oxidoreductase: the first one hundred years, Biochem. Soc. Trans. 25 (1997) 750–755.
[5] F.A. Mateos, J.G. Puig, M.L. Jiménez, I.H. Fox, Hereditary xanthinuria. Evidence for enhanced hypoxanthine salvage, J. Clin. Invest. 79 (1987) 847–852.
[6] A.P. Schmidt, A.E. Böhmer, C. Antunes, C. Schallenberger, L.O. Porciúncula, E. Elisabetsky, D.R. Lara, D.O. Souza, Anti-nociceptive properties of the xanthine oxidase inhibitor allopurinol in mice: role of A1 adenosine receptors, Br. J. Pharmacol. 156 (2009) 163–172.
[7] T.A. Krenitsky, S.M. Neil, G.B. Elion, G.H. Hitchings, A comparison of the specificities of xanthine oxidase and aldehyde oxidase, Arch. Biochem. Biophys. 150 (1972) 585–599.
[8] C. Beedham, Xanthine oxidoreductase and aldehyde oxidase, in: C.A. McQueen (Ed.), Comprehensive toxicology, 4, Elsevier, Oxford, 2010, pp. 185–205.
[9] G. Taibi, F. Di Gaudio, C.M. Nicotra, Xanthine dehydrogenase processes retinol to retinoic acid in human mammary epithelial cells, J. Enzyme Inhib. Med. Chem. 23 (2008) 317–327.
[10] F. Bergmann, L. Levene, I. Tamir, M. Rahat, Oxidation of methyl derivatives of pteridin-4-one, lumazine and related pteridines by bovine milk xanthine oxidase, Biochim. Biophys. Acta 480 (1977) 21–38.
[11] C.A. Pritsos, Cellular distribution, metabolism and regulation of the xanthine oxidoreductase enzyme system, Chem. Biol. Interact. 129 (2000) 195–208.
[12] R.C. Bray, Molybdenum iron–sulfur flavin hydroxylases and related enzymes, in: P.D. Boyer (Ed.), The enzymes, 12, Academic Press, New York, 1975, pp. 299–419.
[13] J.M. McCord, I. Fridovich, The reduction of cytochrome c by milk xanthine oxidase, J. Biol. Chem. 243 (1968) 5753–5760.
[14] P. Kuppusamy, J.L. Zweier, Characterization of free radical generation by xanthine oxidase. Evidence for hydroxyl radical generation, J. Biol. Chem. 264 (1989) 9880–9884.
[15] D.Z. Hsu, K.T. Chen, S.P. Chien, Y.H. Li, B.M. Huang, Y.C. Chuang, M.Y. Liu, Sesame oil attenuates acute iron-induced lipid peroxidation-associated hepatic damage in mice, Shock 26 (2006) 625–630.
[16] D.A. Parks, G.B. Bulkley, D.N. Granger, Role of oxygen free radicals in shock, ischemia, and organ preservation, Surgery 94 (1983) 428–432.
[17] R.W. Topham, M.C. Walker, M.P. Calisch, Liver xanthine dehydrogenase and iron mobilization, Biochem. Biophys. Res. Commun. 109 (1982) 1240–1246.
[18] R.W. Topham, M.C. Walker, M.P. Calisch, R.W. Williams, Evidence for the participation of intestinal xanthine oxidase in the mucosal processing of iron, Biochemistry 21 (1982) 4529–4535.
[19] F. Stirpe, T. Higgins, P.L. Tazzari, E. Rozengurt, Stimulation by xanthine oxidase of 3T3 Swiss fibroblasts and human lymphocytes, Exp. Cell Res. 192 (1991) 635–638.
[20] G.N. Rao, B.C. Berk, Active oxygen species stimulate vascular smooth muscle cell growth and proto-oncogene expression, Circ. Res. 70 (1992) 593–599.
[21] A. Galli, G. Svegliati-Baroni, E. Ceni, S. Milani, F. Ridolfi, R. Salzano, M. Tarocchi, C. Grappone, G. Pellegrini, A. Benedetti, C. Surrenti, A. Casini, Oxidative stress stimulates proliferation and invasiveness of hepatic stellate cells via a MMP2-mediated mechanism, Hepatology 41 (2005) 1074–1084.
[22] E. Tubaro, B. Lotti, C. Santiangeli, G. Cavallo, Xanthine oxidase: an enzyme playing a role in the killing mechanism of polymorphonuclear leucocytes, Biochem. Pharmacol. 29 (1980) 3018–3020.
[23] W.F. Petrone, D.K. English, K. Wong, J.M. McCord, Free radicals and inflammation: superoxide-dependent activation of a neutrophil chemotactic factor in plasma, Proc. Natl. Acad. Sci. U. S. A. 77 (1980) 1159–1163.
[24] W. Ehrlich, H. Huser, H. Kroger, Induction and activation of procollagenase in rabbit synovial fibroblasts after treatment with active oxygen released by xanthine/xanthine oxidase, Rheumatol. Int. 15 (1995) 131–136.
[25] M. Suzuki, M.B. Grisham, D.N. Granger, Leukocyte–endothelial cell adhesive interactions—role of xanthine oxidase-derived oxidants, J. Leukoc. Biol. 50 (1991) 488–494.
[26] R. Shenkar, E. Abraham, Plasma from hemorrhaged mice activates CREB and increases cytokine expression in lung mononuclear cells through a xanthine oxidase-dependent mechanism, Am. J. Respir. Cell Mol. Biol. 14 (1996) 198–206.
[27] M.J. Müller, B. Vollmar, H.P. Friedl, M.D. Menger, Xanthine oxidase and superoxide radicals in portal triad crossclamping-induced microvascular reperfusion injury in the liver, Free Radic. Biol. Med. 21 (1996) 189–197.
[28] B.H. Segal, N. Sakamoto, M. Patel, K. Maemura, A.S. Klein, S.M. Holland, G.B. Bulkley, Xanthine oxidase contributes to host defense against *Burkholderia cepacia* in the p47phox−/− mouse model of chronic granulomatous disease, Infect. Immun. 68 (2000) 2374–2378.
[29] S.A. Sanders, R. Eisenthal, R. Harrison, NADH oxidase activity of human xanthine oxidoreductase—generation of superoxide anion, Eur. J. Biochem. 245 (1997) 541–548.
[30] Z. Zhang, D.R. Blake, C.R. Stevens, J.M. Kanczler, P.G. Winyard, M.C. Symons, M. Benboubetra, R. Harrison, A reappraisal of xanthine dehydrogenase and oxidase in hypoxic reperfusion injury: the role of NADH as an electron donor, Free Radic. Res. 28 (1998) 151–164.
[31] L. Maia, R.O. Duarte, A. Ponces-Freire, J.J. Moura, L. Mira, NADH oxidase activity of rat and human liver xanthine oxidoreductase: potential role in superoxide production, J. Biol. Inorg. Chem. 12 (2007) 777–787.
[32] B.L.J. Godber, J.J. Doel, J. Durgan, R. Eisenthal, R. Harrison, A new route to peroxynitrite: a role for xanthine oxidoreductase, FEBS Lett. 475 (2000) 93–96.
[33] H.M. Martin, J.T. Hancock, V. Salisbury, R. Harrison, Role of xanthine oxidoreductase as an antimicrobial agent, Infect. Immun. 72 (2004) 4933–4939.
[34] R. Harrison, Structure and function of xanthine oxidoreductase: where are we now? Free Radic. Biol. Med. 33 (2002) 774–797.

[35] G.K. Glantzounis, E.C. Tsimoyiannis, A.M. Kappas, D.A. Galaris, Uric acid and oxidative stress, Curr. Pharm. Des. 11 (2005) 4145–4151.
[36] B.N. Ames, R. Cathcart, E. Schwiers, P. Hochstein, Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis, Proc. Natl. Acad. Sci. U. S. A. 78 (1981) 6858–6862.
[37] C.E. Berry, J.M. Hare, Xanthine oxidoreductase and cardiovascular disease: molecular mechanisms and pathophysiological implications, J. Physiol. 555 (2004) 589–606.
[38] J. Nguyen, Plant xanthine dehydrogenase: its distribution, properties and function, Physiol. Vég. 24 (1986) 263–281.
[39] D.A. Parks, D.N. Granger, Xanthine oxidase: biochemistry, distribution and physiology, Acta Physiol. Scand. Suppl. 548 (1986) 87–99.
[40] E. Della Corte, F. Stirpe, The regulation of rat liver xanthine oxidase: activation by proteolytic enzymes, FEBS Lett. 2 (1968) 83–84.
[41] M.G. Battelli, E. Della Corte, F. Stirpe, Xanthine oxidase type D (dehydrogenase) in the intestine and other organs of the rat, Biochem. J. 126 (1972) 747–749.
[42] R. Hille, T. Nishino, Xanthine oxidase and xanthine dehydrogenase, FASEB J. 9 (1995) 995–1003.
[43] H.Y. Chung, B.S. Baek, S.H. Song, M.S. Kim, J.I. Huh, K.H. Shim, K.W. Kim, K.H. Lee, Xanthine dehydrogenase/xanthine oxidase and oxidative stress, Age (Omaha) 20 (1997) 127–140.
[44] M. Kurosaki, M. Li Calzi, E. Scanziani, E. Garattini, M. Terao, Tissue and cell-specific expression of mouse xanthine oxidoreductase gene in vivo: regulation by bacterial lipopolysaccharide, Biochem. J. 306 (1995) 225–234.
[45] M. Saksela, R. Lapatto, K.O. Raivio, Xanthine oxidoreductase gene expression and enzyme activity in developing human tissues, Biol. Neonate 74 (1998) 274–280.
[46] U.A.S. Al-Khalidi, T.H. Chaglassian, The species distribution of xanthine oxidase, Biochem. J. 97 (1965) 318–320.
[47] J. Wang, A. Van Praagh, E. Hamilton, Q. Wang, B. Zou, M. Muranjan, N.B. Murphy, S.J. Black, Serum xanthine oxidase: origin, regulation, and contribution to control of trypanosome parasitemia, Antioxid. Redox Signal. 4 (2002) 161–178.
[48] A. Kooij, M. Schijns, W.M. Frederiks, C.J.F. Van Noorden, J. James, Distribution of xanthine oxidoreductase activity in human tissues — a histochemical and biochemical study, Virchows Arch. B Cell Pathol. 63 (1992) 17–23.
[49] S. Angermüller, M. Islinger, A. Völkl, Peroxisomes and reactive oxygen species, a lasting challenge, Histochem. Cell Biol. 131 (2009) 459–463.
[50] G. Riario-Sforza, A. Carcassi, P.F. Bayeli, R. Marcolongo, E. Marinello, M. Montagnani, Xanthine oxidase activity in the jejunal mucosa of patients with gout, Boll. Soc. Ital. Biol. Sper. 45 (1969) 785–786.
[51] C. Auscher, N. Amory, P. van der Kemp, F. Delbarre, Xanthine oxidase activity in human intestines. Histochemical and radiochemical study, Adv. Exp. Med. Biol. 122B (1979) 197–201.
[52] E. Della Corte, G. Gozzetti, F. Novello, F. Stirpe, Properties of the xanthine oxidase from human liver, Biochim. Biophys. Acta 191 (1969) 164–166.
[53] R. Guerciolini, C. Szumlanski, R.M. Weinshilboum, Human liver xanthine oxidase: nature and extent of individual variation, Clin. Pharmacol. Ther. 50 (1991) 663–672.
[54] F. Stirpe, M. Ravaioli, M.G. Battelli, S. Musiani, G.L. Grazi, Xanthine oxidoreductase activity in human liver disease, Am. J. Gastroenterol. 97 (2002) 2079–2085.
[55] R.W. Watts, J.E. Watts, J.E. Seegmiller, Xanthine oxidase activity in human tissues and its inhibition by allopurinol (4-hydroxypyrazolo[3,4-d] pyrimidine), J. Lab. Clin. Med. 66 (1965) 688–697.
[56] A. Sarnesto, N. Linder, K.O. Raivio, Organ distribution and molecular forms of human xanthine dehydrogenase/xanthine oxidase protein, Lab. Invest. 74 (1996) 48–56.
[57] F.L. Rodkey, E.G. Ball, A rapid test for distinguishing human from cow's milk based upon a difference in their xanthine oxidase content, J. Lab. Clin. Med. 31 (1946) 354–356.
[58] S. Abadeh, J. Killacky, M. Benboubetra, R. Harrison, Purification and partial characterization of xanthine oxidase from human milk, Biochim. Biophys. Acta 1117 (1992) 25–32.
[59] U.A.S. Al-Khalidi, S. Nasrallah, A.K. Khachadurian, M.H. Shammaa, A sensitive method for the determination of xanthine oxidase activity, Clin. Chim. Acta 11 (1965) 72–77.
[60] M.G. Battelli, L. Buonamici, A. Abbondanza, M. Virgili, A. Contestabile, F. Stirpe, Excitotoxic increase of xanthine dehydrogenase and xanthine oxidase in the rat olfactory cortex, Dev. Brain Res. 86 (1995) 340–344.
[61] A. McHale, H. Grimes, M.P. Coughlan, Human serum xanthine oxidase: fluorometric assay applicable to the investigation of liver disorders, Int. J. Biochem. 10 (1979) 317–319.
[62] T. Yamamoto, Y. Moriwaki, S. Takahashi, Z. Tsutsumi, J. Yamakita, Y. Nasako, K. Hiroishi, K. Higashino, Determination of human plasma xanthine oxidase activity by high-performance liquid chromatography, J. Chromatogr. B Biomed. Appl. 681 (1996) 395–400.
[63] X. Liu, W.M. Lin, X.H. Yan, X.H. Chen, J.R. Hoidal, P. Xu, Improved method for measurement of human plasma xanthine oxidoreductase activity, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 785 (2003) 101–114.
[64] C.R. Stevens, M. Benboubetra, R. Harrison, T. Sahinoglu, E.C. Smith, D.R. Blake, Localisation of xanthine oxidase to synovial endothelium, Ann. Rheum. Dis. 50 (1991) 760–762.
[65] L.J. Price, R. Harrison, Sensitive enzyme linked immunosorbent assay (ELISA) for xanthine oxidase, Biochem. Soc. Trans. 21 (1993) 102S.
[66] M.G. Battelli, A. Abbondanza, S. Musiani, L. Buonamici, P. Strocchi, P.L. Tazzari, L. Gramantieri, F. Stirpe, Determination of xanthine oxidase in human serum by a competitive enzyme-linked immunosorbent assay (ELISA), Clin. Chim. Acta 281 (1999) 147–158.
[67] T. Nishino, K. Okamoto, B.T. Eger, E.F. Pai, T. Nishino, Mammalian xanthine oxidoreductase — mechanism of transition from xanthine dehydrogenase to xanthine oxidase, FEBS J. 275 (2008) 3278–3289.
[68] K. Ichida, Y. Amaya, K. Noda, S. Minoshima, T. Hosoya, O. Sakai, N. Shimizu, T. Nishino, Cloning of the cDNA encoding human xanthine dehydrogenase (oxidase): structural analysis of the protein and chromosomal location of the gene, Gene 133 (1993) 279–284.
[69] R.M. Wright, G.M. Vaitaitis, C.M. Wilson, T.B. Repine, L.S. Terada, J.E. Repine, cDNA cloning, characterization, and tissue-specific expression of human xanthine dehydrogenase/xanthine oxidase, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 10690–10694.
[70] M. Saksela, K.O. Raivio, Cloning and expression in vitro of human xanthine dehydrogenase/oxidase, Biochem. J. 315 (1996) 235–239.
[71] J. Hewinson, C.R. Stevens, T.M. Millar, Vascular physiology and pathology of circulating xanthine oxidoreductase: from nucleotide sequence to functional enzyme, Redox Rep. 9 (2004) 71–79.
[72] P. Xu, P. LaVallee, J.R. Hoidal, Repressed expression of the human xanthine oxidoreductase gene. E-box and TATA-like elements restrict ground state transcriptional activity, J. Biol. Chem. 275 (2000) 5918–5926.
[73] C.J. Wallwork, D.A. Parks, G.W. Schmid-Schönbein, Xanthine oxidase activity in the dexamethasone-induced hypertensive rat, Microvasc. Res. 66 (2003) 30–37.
[74] J.J. Reiners Jr., B.C. Pence, M.C. Barcus, A.R. Cantu, 12-O-tetradecanoylphorbol-13-acetate-dependent induction of xanthine dehydrogenase and conversion to xanthine oxidase in murine epidermis, Cancer Res. 47 (1987) 1775–1779.
[75] M. Feigelson, P. Feigelson, P.R. Gross, Xanthine oxidase activity in regenerating liver, J. Gen. Physiol. 41 (1957) 233–242.
[76] B.C. Pence, J.J. Reiners Jr., Murine epidermal xanthine oxidase activity: correlation with degree of hyperplasia induced by tumor promoters, Cancer Res. 47 (1987) 6388–6392.
[77] J.R. Hoidal, P. Xu, T. Hueckstedt, K.A. Sanders, K. Pfeffer, A.B. Sturrock, Lung injury and oxidoreductases, Environ. Health Perspect. 106 (Suppl. 5) (1998) 1235–1239.
[78] L.E. Roberts, M.A. Fini, N. Derkash, R.M. Wright, PD98059 enhanced insulin, cytokine, and growth factor activation of xanthine oxidoreductase in epithelial cells involves STAT3 and the glucocorticoid receptor, J. Cell. Biochem. 101 (2007) 1567–1587.
[79] U.S. Kayyali, R. Budhiraja, C.M. Pennella, S. Cooray, J.J. Lanzillo, R. Chalkley, P.M. Hassoun, Upregulation of xanthine oxidase by tobacco smoke condensate in pulmonary endothelial cells, Toxicol. Appl. Pharmacol. 188 (2003) 59–68.
[80] D. Furth-Walker, N.K. Amy, Regulation of xanthine oxidase activity and immunologically detectable protein in rats in response to dietary protein and iron, J. Nutr. 117 (1987) 1697–1703.
[81] K. Schröder, C. Vecchione, O. Jung, J.G. Schreiber, R. Shiri-Sverdlov, P.J. van Gorp, R. Busse, R.P. Brandes, Xanthine oxidase inhibitor tungsten prevents the development of atherosclerosis in ApoE knockout mice fed a Western-type diet, Free Radic. Biol. Med. 41 (2006) 1353–1360.
[82] Z.W. Zhang, Q.H. Wang, J.L. Zhang, S. Li, X.L. Wang, S.W. Xu, Effects of oxidative stress on immunosuppression induced by selenium deficiency in chickens, Biol. Trace Elem. Res. 149 (2012) 352–361.
[83] N. Linder, R. Bützow, H. Lassus, M. Lundin, J. Lundin, Decreased xanthine oxidoreductase (XOR) is associated with a worse prognosis in patients with serous ovarian carcinoma, Gynecol. Oncol. 124 (2012) 311–318.
[84] J.L. McManaman, C.A. Palmer, R.M. Wright, M.C. Neville, Functional regulation of xanthine oxidoreductase expression and localization in the mouse mammary gland: evidence of a role in lipid secretion, J. Physiol. 545 (2002) 567–579.
[85] M. Kurosaki, S. Zanotta, M. Li Calzi, E. Garattini, M. Terao, Expression of xanthine oxidoreductase in mouse mammary epithelium during pregnancy and lactation: regulation of gene expression by glucocorticoids and prolactin, Biochem. J. 319 (1996) 801–810.
[86] S. Page, D. Powell, M. Benboubetra, C.R. Stevens, D.R. Blake, F. Selase, A.J. Wolstenholme, R. Harrison, Xanthine oxidoreductase in human mammary epithelial cells: activation in response to inflammatory cytokines, Biochim. Biophys. Acta 1381 (1998) 191–202.
[87] C. Vorbach, A. Scriven, M.R. Capecchi, The housekeeping gene xanthine oxidoreductase is necessary for milk fat droplet enveloping and secretion: gene sharing in the lactating mammary gland, Genes Dev. 16 (2002) 3223–3235.
[88] J.L. McManaman, C.A. Palmer, S. Anderson, K. Schwertfeger, M.C. Neville, Regulation of milk lipid formation and secretion in the mouse mammary gland, Adv. Exp. Med. Biol. 554 (2004) 263–279.
[89] N. Aoki, Regulation and functional relevance of milk fat globules and their components in the mammary gland, Biosci. Biotechnol. Biochem. 70 (2006) 2019–2027.
[90] P. Ghezzi, M. Bianchi, A. Mantovani, F. Spreafico, M. Salmona, Enhanced xanthine oxidase activity in mice treated with interferon and interferon inducers, Biochem. Biophys. Res. Commun. 119 (1984) 144–149.
[91] L. Deloria, V. Abbott, N. Gooderham, G.J. Mannering, Induction of xanthine oxidase and depression of cytochrome P-450 by interferon inducers: genetic difference in the responses of mice, Biochem. Biophys. Res. Commun. 131 (1985) 109–114.
[92] M. Terao, G. Cazzaniga, P. Ghezzi, M. Bianchi, F. Falciani, P. Perani, E. Garattini, Molecular cloning of a cDNA coding for mouse liver xanthine dehydrogenase. Regulation of its transcript by interferons in vivo, Biochem. J. 283 (1992) 863–870.
[93] G.P. Dupont, T.P. Hueckstedt, B.C. Marshall, U.S. Ryan, J.R. Michael, J.R. Hoidal, Regulation of xanthine dehydrogenase and xanthine oxidase activity and gene expression in cultured rat pulmonary endothelial cells, J. Clin. Invest. 89 (1992) 197–202.
[94] K.D. Pfeffer, T.P. Hueckstedt, J.R. Hoidal, Xanthine dehydrogenase and xanthine oxidase activity and gene expression in renal epithelial cells. Cytokine and steroid regulation, J. Immunol. 153 (1994) 1789–1797.
[95] M.I. Sellers, Further studies of interrelationship between xanthine oxidase and influenzal pneumonia in mice, Proc. Soc. Exp. Biol. Med. 91 (1956) 457–460.

[96] D.J. Bauer, P.L. Bradley, The xanthine oxidase groups; a phenomenon associated with the multiplication of neurotropic viruses, Br. J. Exp. Pathol. 37 (1956) 447–460.
[97] D.W. Ziegler, H.D. Hutchinson, R.E. Kissling, Induction of xanthine oxidase by virus infections in newborn mice, Infect. Immun. 3 (1971) 237–242.
[98] K. Umezawa, T. Akaike, S. Fujii, M. Suga, K. Setoguchi, A. Ozawa, H. Maeda, Induction of nitric oxide synthesis and xanthine oxidase and their roles in the antimicrobial mechanism against *Salmonella typhimurium* infection in mice, Infect. Immun. 65 (1997) 2932–2940.
[99] F. Falciani, M. Terao, S. Goldwurm, A. Ronchi, A. Gatti, C. Minoia, M. Li Calzi, M. Salmona, G. Cazzaniga, E. Garattini, Molybdenum(VI) salts convert the xanthine oxidoreductase apoprotein into the active enzyme in mouse L929 fibroblastic cells, Biochem. J. 298 (1994) 69–77.
[100] R.R. Mendel, F. Bittner, Cell biology of molybdenum, Biochim. Biophys. Acta 1763 (2006) 621–635.
[101] E. Della Corte, F. Stirpe, The regulation of rat liver xanthine oxidase. Involvement of thiol groups in the conversion of the enzyme activity from dehydrogenase (type D) into oxidase (type O) and purification of the enzyme, Biochem. J. 126 (1972) 739–745.
[102] C.M. Harris, V. Massey, The reaction of reduced xanthine dehydrogenase with molecular oxygen. Reaction kinetics and measurement of superoxide radical, J. Biol. Chem. 272 (1997) 8370–8379.
[103] L. Maia, A. Vala, L. Mira, NADH oxidase activity of rat liver xanthine dehydrogenase and xanthine oxidase — contribution for damage mechanisms, Free Radic. Res. 39 (2005) 979–986.
[104] K. Ichida, T. Matsumura, R. Sakuma, T. Hosoya, T. Nishino, Mutation of human molybdenum cofactor sulfurase gene is responsible for classical xanthinuria type II, Biochem. Biophys. Res. Commun. 282 (2001) 1194–1200.
[105] A.M. Brown, M. Benboubetra, M. Ellison, D. Powell, J.D. Reckless, R. Harrison, Molecular activation–deactivation of xanthine oxidase in human milk, Biochim. Biophys. Acta 1245 (1995) 248–254.
[106] L.S. Terada, D. Piermattei, G.N. Shibao, J.L. McManaman, R.M. Wright, Hypoxia regulates xanthine dehydrogenase activity at pre- and posttranslational levels, Arch. Biochem. Biophys. 348 (1997) 163–168.
[107] M.G. Battelli, L. Buonamici, M. Virgili, A. Abbondanza, A. Contestabile, Simulated ischaemia–reperfusion conditions increase xanthine dehydrogenase and oxidase activities in rat brain slices, Neurochem. Int. 32 (1998) 17–21.
[108] P.M. Hassoun, F.S. Yu, C.G. Cote, J.J. Zulueta, R. Sawhney, K.A. Skinner, H.B. Skinner, D.A. Parks, J.J. Lanzillo, Upregulation of xanthine oxidase by lipopolysaccharide, interleukin-1, and hypoxia. Role in acute lung injury, Am. J. Respir. Crit. Care Med. 158 (1998) 299–305.
[109] J.J. Lanzillo, F.S. Yu, J. Stevens, P.M. Hassoun, Determination of xanthine dehydrogenase mRNA by a reverse transcription-coupled competitive quantitative polymerase chain reaction assay: regulation in rat endothelial cells by hypoxia and hyperoxia, Arch. Biochem. Biophys. 335 (1996) 377–380.
[110] C. Li, R.M. Jackson, Reactive species mechanisms of cellular hypoxia–reoxygenation injury, Am. J. Physiol. Cell Physiol. 282 (2002) C227–C241.
[111] G. Wang, P. Qian, F.R. Jackson, G. Qian, G. Wu, Sequential activation of JAKs, STATs and xanthine dehydrogenase/oxidase by hypoxia in lung microvascular endothelial cells, Int. J. Biochem. Cell Biol. 40 (2008) 461–470.
[112] W.B. Poss, T.P. Huecksteadt, P.C. Panus, B.A. Freeman, J.R. Hoidal, Regulation of xanthine dehydrogenase and xanthine oxidase activity by hypoxia, Am. J. Physiol. 270 (1996) L941–L946.
[113] N. Linder, E. Martelin, R. Lapatto, K.O. Raivio, Posttranslational inactivation of human xanthine oxidoreductase by oxygen under standard cell culture conditions, Am. J. Physiol. Cell Physiol. 285 (2003) C48–C55.
[114] F. Stirpe, Corte E. Della, The regulation of rat liver xanthine oxidase. Conversion in vitro of the enzyme activity from dehydrogenase (type D) to oxidase (type O), J. Biol. Chem. 244 (1969) 3855–3863.
[115] M.G. Battelli, Enzymic conversion of rat liver xanthine oxidase from dehydrogenase (D form) to oxidase (O form), FEBS Lett. 113 (1980) 47–51.
[116] D.A. Clare, B.A. Blakistone, H.E. Swaisgood, H.R. Horton, Sulfhydryl oxidase-catalyzed conversion of xanthine dehydrogenase to xanthine oxidase, Arch. Biochem. Biophys. 211 (1981) 44–47.
[117] M.G. Battelli, E. Lorenzoni, Purification and properties of a new glutathione-dependent thiol:disulphide oxidoreductase from rat liver, Biochem. J. 207 (1982) 133–138.
[118] Z.W. Kamiński, M.M. Jezewska, Intermediate dehydrogenase–oxidase form of xanthine oxidoreductase in rat liver, Biochem. J. 181 (1979) 177–182.
[119] A. Abbondanza, M.G. Battelli, M. Soffritti, C. Cessi, Xanthine oxidase status in ethanol-intoxicated rat liver, Alcohol. Clin. Exp. Res. 13 (1989) 841–844.
[120] M.G. Battelli, E. Lorenzoni, F. Stripe, Milk xanthine oxidase type D (dehydrogenase) and type O (oxidase). Purification, interconversion and some properties, Biochem. J. 131 (1973) 191–198.
[121] J.C. Sáez, P.H. Ward, B. Günther, E. Vivaldi, Superoxide radical involvement in the pathogenesis of burn shock, Circ. Shock. 12 (1984) 229–239.
[122] M. Athar, C.A. Elmets, D.R. Bickers, H. Mukhtar, A novel mechanism for the generation of superoxide anions in hematoporphyrin derivative-mediated cutaneous photosensitization. Activation of the xanthine oxidase pathway, J. Clin. Invest. 83 (1989) 1137–1143.
[123] L.G. Sultatos, Effects of acute ethanol administration on the hepatic xanthine dehydrogenase/oxidase system in the rat, J. Pharmacol. Exp. Ther. 246 (1988) 946–949.
[124] M.G. Battelli, A. Abbondanza, F. Stirpe, Effects of hypoxia and ethanol on the xanthine oxidase of isolated hepatocytes: conversion from D to O form and leakage from cells, Chem. Biol. Interact. 83 (1992) 73–84.
[125] G. Cighetti, S. Debiasi, R. Paroni, Effect of glutathione depletion on the conversion of xanthine dehydrogenase to oxidase in rat liver, Biochem. Pharmacol. 45 (1993) 2359–2361.
[126] H.P. Friedl, G.O. Till, U.S. Ryan, P.A. Ward, Mediator-induced activation of xanthine oxidase in endothelial cells, FASEB J. 3 (1989) 2512–2518.
[127] S.H. Phan, D.E. Gannon, J. Varani, U.S. Ryan, P.A. Ward, Xanthine oxidase activity in rat pulmonary artery endothelial cells and its alteration by activated neutrophils, Am. J. Pathol. 134 (1989) 1201–1211.
[128] Y. Wakabayashi, H. Fujita, I. Morita, H. Kawaguchi, S. Murota, Conversion of xanthine dehydrogenase to xanthine oxidase in bovine carotid artery endothelial cells induced by activated neutrophils: involvement of adhesion molecules, Biochim. Biophys. Acta 1265 (1995) 103–109.
[129] A. Atlante, S. Gagliardi, G.M. Minervini, M.T. Ciotti, E. Marra, P. Calissano, Glutamate neurotoxicity in rat cerebellar granule cells: a major role for xanthine oxidase in oxygen radical formation, J. Neurochem. 68 (1997) 2038–2045.
[130] M.G. Battelli, L. Buonamici, L. Polito, A. Bolognesi, F. Stirpe, Hepatotoxicity of ricin, saporin or a saporin immunotoxin: xanthine oxidase activity in rat liver and blood serum, Virchows Arch. 427 (1996) 529–535.
[131] W. Gwinner, J. Plasger, R.P. Brandes, B. Kubat, M. Schulze, H. Regele, D. Kerjaschki, C.J. Olbricht, K.M. Koch, Role of xanthine oxidase in passive Heymann nephritis in rats, J. Am. Soc. Nephrol. 10 (1999) 538–544.
[132] G. Deliconstantinos, V. Villiotou, J.C. Stavrides, Alterations of nitric oxide synthase and xanthine oxidase activities of human keratinocytes by ultraviolet B radiation. Potential role for peroxynitrite in skin inflammation, Biochem. Pharmacol. 51 (1996) 1727–1738.
[133] M. Srivastava, R.K. Kale, Radiomodfication of xanthine oxidoreductase system in the liver of mice by phenylmethylsulfonyl fluoride and dithiothreitol, Radiat. Res. 154 (2000) 94–103.
[134] N. Sugawara, T. Ohta, Y.R. Lai, C. Sugawara, M. Yuasa, M. Nakamura, M. Tamura, Iron depletion prevents adenine nucleotide decomposition and an increase of xanthine oxidase activity in the liver of the Long Evans Cinnamon (LEC) rat, an animal model of Wilson's disease, Life Sci. 65 (1999) 1423–1431.
[135] G. Schimpl, P. Pesendorfer, A.M. Kuesz, M. Ratschek, M.E. Höllwarth, The impact of hepatic xanthine oxidase and xanthine dehydrogenase activities on liver function in chronic cholestasis, Pediatr. Surg. Int. 16 (2000) 297–301.
[136] A. Ramachandran, R. Prabhu, S. Thomas, J.B. Reddy, A. Pulimood, K.A. Balasubramanian, Intestinal mucosal alterations in experimental cirrhosis in the rat: role of oxygen free radicals, Hepatology 35 (2002) 622–629.
[137] Y. Kaminsky, E. Kosenko, AMP deaminase and adenosine deaminase activities in liver and brain regions in acute ammonia intoxication and subacute toxic hepatitis, Brain Res. 1311 (2010) 175–181.
[138] T.D. Engerson, T.G. McKelvey, D.B. Rhyne, E.B. Boggio, S.J. Snyder, H.P. Jones, Conversion of xanthine dehydrogenase to oxidase in ischemic rat tissues, J. Clin. Invest. 79 (1987) 1564–1570.
[139] T.G. McKelvey, M.E. Höllwarth, D.N. Granger, T.D. Engerson, U. Landler, H.P. Jones, Mechanisms of conversion of xanthine dehydrogenase to xanthine oxidase in ischemic rat liver and kidney, Am. J. Physiol. 254 (1988) G753–G760.
[140] D.A. Parks, T.K. Williams, J.S. Beckman, Conversion of xanthine dehydrogenase to oxidase in ischemic rat intestine: a reevaluation, Am. J. Physiol. 254 (1988) G768–G774.
[141] C.A. Brass, J. Narciso, J.L. Gollan, Enhanced activity of the free radical producing enzyme xanthine oxidase in hypoxic rat liver. Regulation and pathophysiologic significance, J. Clin. Invest. 87 (1991) 424–431.
[142] Y. Kinuta, H. Kikuchi, M. Ishikawa, Ischaemic brain oedema and xanthine–xanthine oxidase system, Acta Neurochir. Suppl. (Wien) 51 (1990) 192–194.
[143] H. Oka, H. Kanemitsu, H. Nihei, H. Nakayama, A. Tamura, K. Sano, Change of xanthine dehydrogenase and xanthine oxidase activities in rat brain following complete ischaemia, Neurol. Res. 14 (1992) 321–324.
[144] S.R. Thom, Dehydrogenase conversion to oxidase and lipid peroxidation in brain after carbon monoxide poisoning, J. Appl. Physiol. 73 (1992) 1584–1589.
[145] H. de Groot, A. Littauer, Reoxygenation injury in isolated hepatocytes: cell death precedes conversion of xanthine dehydrogenase to xanthine oxidase, Biochem. Biophys. Res. Commun. 155 (1988) 278–282.
[146] J.M. McCord, Oxygen-derived free radicals in postischemic tissue injury, N. Engl. J. Med. 312 (1985) 159–163.
[147] G.B. Bulkley, Free radical-mediated reperfusion injury: a selective review, Br. J. Cancer Suppl. 8 (1987) 66–73.
[148] D.N. Granger, Role of xanthine oxidase and granulocytes in ischemia–reperfusion injury, Am. J. Physiol. 255 (1988) H1269–H1275.
[149] V. Fellman, K.O. Raivio, Reperfusion injury as the mechanism of brain damage after perinatal asphyxia, Pediatr. Res. 41 (1997) 599–606.
[150] P. Pacher, A. Nivorozhkin, C. Szabó, Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol, Pharmacol. Rev. 58 (2006) 87–114.
[151] T.P. Cappola, D.A. Kass, G.S. Nelson, R.D. Berger, G.O. Rosas, Z.A. Kobeissi, E. Marbán, J.M. Hare, Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy, Circulation 104 (2001) 2407–2411.
[152] A. Sola, G. Hotter, N. Prats, C. Xaus, E. Gelpi, J. Roselló-Catafau, Modification of oxidative stress in response to intestinal preconditioning, Transplantation 69 (2000) 767–772.
[153] H. Jaeschke, Xanthine oxidase-induced oxidant stress during hepatic ischemia–reperfusion: are we coming full circle after 20 years? Hepatology 36 (2002) 761–763.
[154] H. de Groot, A. Littauer, Hypoxia, reactive oxygen, and cell injury, Free Radic. Biol. Med. 6 (1989) 541–551.
[155] S.J. Ledingham, O. Katayama, D.R. Lachno, M. Yacoub, Prolonged cardiac preservation. Evaluation of the University of Wisconsin preservation solution by

comparison with the St. Thomas' Hospital cardioplegic solutions in the rat, Circulation 82 (1990) IV351–IV358.
[156] K. Sun, E. Kiss, J. Bedke, T. Stojanovic, Y. Li, W. Gwinner, H.J. Gröne, Role of xanthine oxidoreductase in experimental acute renal-allograft rejection, Transplantation 77 (2004) 1683–1692.
[157] C. Peralta, O. Bulbena, C. Xaus, N. Prats, J.C. Cutrin, G. Poli, E. Gelpi, J. Roselló-Catafau, Ischemic preconditioning: a defense mechanism against the reactive oxygen species generated after hepatic ischemia reperfusion, Transplantation 73 (2002) 1203–1211.
[158] L. Fernández, N. Heredia, L. Grande, G. Gómez, A. Rimola, A. Marco, E. Gelpí, J. Roselló-Catafau, C. Peralta, Preconditioning protects liver and lung damage in rat liver transplantation: role of xanthine/xanthine oxidase, Hepatology 36 (2002) 562–572.
[159] L. Fernández, E. Carrasco-Chaumel, A. Serafín, C. Xaus, L. Grande, A. Rimola, J. Roselló-Catafau, C. Peralta, Is ischemic preconditioning a useful strategy in steatotic liver transplantation? Am. J. Transplant. 4 (2004) 888–899.
[160] T. Rootwelt, R. Almaas, S. Øyasæter, A. Moen, O.D. Saugstad, Release of xanthine oxidase to the systemic circulation during resuscitation from severe hypoxemia in newborn pigs, Acta Paediatr. 84 (1995) 507–511.
[161] U.A.S. Al-Khalidi, R.S. Geha, The sensitivity of serum xanthine oxidase and serum glutamic pyruvic transaminase in detecting liver damage, Clin. Chim. Acta 14 (1966) 833–835.
[162] O.R. Affonso, E. Mitidieri, L.P. Ribeiro, G.G. Villela, Blood serum xanthine oxidase of rats poisoned with carbon tetrachloride, Proc. Soc. Exp. Biol. Med. 90 (1955) 527–529.
[163] C. Ramboer, F. Piessens, J. de Groote, Serum xanthine oxidase and liver disease, Digestion 7 (1972) 183–195.
[164] O.R. Affonso, E. Mitidieri, G.G. Villela, Effect of colchicine on rat blood serum xanthine oxidase, Nature 193 (1962) 64.
[165] S. Giler, E. Ventura, E. Levy, I. Urca, O. Sperling, A. de Vries, Elevation of serum xanthine oxidase following halothane anesthesia in the rat, Experientia 32 (1976) 620–621.
[166] T. Zima, L. Novák, S. Stípek, Plasma xanthine oxidase level and alcohol administration, Alcohol Alcohol. 28 (1993) 693–694.
[167] R. Moumen, N. Ait-Oukhatar, F. Bureau, C. Fleury, D. Bouglé, P. Arhan, D. Neuville, F. Viader, Aluminium increases xanthine oxidase activity and disturbs antioxidant status in the rat, J. Trace Elem. Med. Biol. 15 (2001) 89–93.
[168] G.O. Till, H.P. Friedl, P.A. Ward, Lung injury and complement activation: role of neutrophils and xanthine oxidase, Free Radic. Biol. Med. 10 (1991) 379–386.
[169] R. Yagci, B. Aydin, M. Erdurmus, R. Karadag, A. Gurel, M. Durmus, R. Yigitoglu, Use of melatonin to prevent selenite-induced cataract formation in rat eyes, Curr. Eye Res. 31 (2006) 845–850.
[170] T. Oda, T. Akaike, T. Hamamoto, F. Suzuki, T. Hirano, H. Maeda, Oxygen radicals in influenza-induced pathogenesis and treatment with pyran polymer-conjugated SOD, Science 244 (1989) 974–976.
[171] T. Akaike, M. Ando, T. Oda, T. Doi, S. Ijiri, S. Araki, H. Maeda, Dependence on O2-generation by xanthine oxidase of pathogenesis of influenza virus infection in mice, J. Clin. Invest. 85 (1990) 739–745.
[172] D. Ungheri, C. Pisani, G. Sanson, A. Bertani, G. Schioppacassi, R. Delgado, M. Sironi, P. Ghezzi, Protective effect of n-acetylcysteine in a model of influenza infection in mice, Int. J. Immunopathol. Pharmacol. 13 (2000) 123–128.
[173] H.P. Friedl, G.O. Till, O. Trentz, P.A. Ward, Roles of histamine, complement and xanthine oxidase in thermal injury of skin, Am. J. Pathol. 135 (1989) 203–217.
[174] L.K. Burton, S.E. Velasco, A. Patt, L.S. Terada, J.E. Repine, Xanthine oxidase contributes to lung leak in rats subjected to skin burn, Inflammation 19 (1995) 31–38.
[175] T. Koizumi, H. Tanaka, S. Sakaki, S. Shimazaki, The therapeutic efficacy of edaravone in extensively burned rats, Arch. Surg. 141 (2006) 992–995.
[176] S. Kahl, T.H. Elsasser, Endotoxin challenge increases xanthine oxidase activity in cattle: effect of growth hormone and vitamin E treatment, Domest. Anim. Endocrinol. 26 (2004) 315–328.
[177] C.R. White, V. Darley-Usmar, W.R. Berrington, M. McAdams, J.Z. Gore, J.A. Thompson, D.A. Parks, M.M. Tarpey, B.A. Freeman, Circulating plasma xanthine oxidase contributes to vascular disfunction in hypercholesterolemic rabbits, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 8745–8749.
[178] M.C. Desco, M. Asensi, R. Márquez, J. Martínez-Valls, M. Vento, F.V. Pallardó, J. Sastre, J. Viña, Xanthine oxidase is involved in free radical production in type 1 diabetes: protection by allopurinol, Diabetes 51 (2002) 1118–1124.
[179] S. Matsumoto, I. Koshiishi, T. Inoguchi, H. Nawata, H. Utsumi, Confirmation of superoxide generation via xanthine oxidase in streptozotocin-induced diabetic mice, Free Radic. Res. 37 (2003) 767–772.
[180] L. Chen, Z. Lan, Q. Lin, X. Mi, Y. He, L. Wei, Y. Lin, Y. Zhang, X. Deng, Polydatin ameliorates renal injury by attenuating oxidative stress-related inflammatory responses in fructose-induced urate nephropathic mice, Food Chem. Toxicol. 52 (2013) 28–35.
[181] F.J. Dagher, A. Panossian, S. Saab, The effect of experimental ligation of the superior mesenteric artery on serum xanthine oxidase and transaminase activity, Surgery 62 (1967) 1044–1050.
[182] V.G. Nielsen, A. Weinbroum, S. Tan, P.N. Samuelson, S. Gelman, D.A. Parks, Xanthine oxidoreductase release after descending thoracic aorta occlusion and reperfusion in rabbits, J. Thorac. Cardiovasc. Surg. 107 (1994) 1222–1227.
[183] V.G. Nielsen, A.T. McCammon, S. Tan, K.A. Kirk, P.N. Samuelson, D.A. Parks, Xanthine oxidase inactivation attenuates postocclusion shock after descending thoracic aorta occlusion and reperfusion in rabbits, J. Thorac. Cardiovasc. Surg. 110 (1995) 715–722.
[184] V.G. Nielsen, S. Tan, A. Weinbroum, A.T. McCammon, P.N. Samuelson, S. Gelman, D. A. Parks, Lung injury after hepatoenteric ischemia–reperfusion: role of xanthine oxidase, Am. J. Respir. Crit. Care Med. 154 (1996) 1364–1369.
[185] V.G. Nielsen, S. Tan, M.S. Baird, A.T. McCammon, D.A. Parks, Gastric intramucosal pH and multiple organ injury: impact of ischemia–reperfusion and xanthine oxidase, Crit. Care Med. 24 (1996) 1339–1344.
[186] V.G. Nielsen, S. Tan, M.S. Baird, P.N. Samuelson, A.T. McCammon, D.A. Parks, Xanthine oxidase mediates myocardial injury after hepatoenteric ischemia–reperfusion, Crit. Care Med. 25 (1997) 1044–1050.
[187] L.S. Terada, J.J. Dormish, P.F. Shanley, J.A. Leff, B.O. Anderson, J.E. Repine, Circulating xanthine oxidase mediates lung neutrophil sequestration after intestinal ischemia–reperfusion, Am. J. Physiol. 263 (1992) L394–L401.
[188] V.L. Vega, L. Mardones, M. Maldonado, S. Nicovani, V. Manríquez, J. Roa, P.H. Ward, Xanthine oxidase released from reperfused hind limbs mediate kupffer cell activation, neutrophil sequestration, and hepatic oxidative stress in rats subjected to tourniquet shock, Shock 14 (2000) 565–571.
[189] S. Tan, Y. Yokoyama, E. Dickens, T.G. Cash, B.A. Freeman, D.A. Parks, Xanthine oxidase activity in the circulation of rats following hemorrhagic shock, Free Radic. Biol. Med. 15 (1993) 407–414.
[190] T. Mayumi, C.K. Chan, M.G. Clemens, G.B. Bulkley, Zonal heterogeneity of hepatic injury following shock/resuscitation: relationship of xanthine oxidase activity to localization of neutrophil accumulation and central lobular necrosis, Shock 5 (1996) 324–332.
[191] S. Tan, Y. Yokoyama, Z. Wang, F. Zhou, V. Nielsen, A.D. Murdoch, C. Adams, D.A. Parks, Hypoxia–reoxygenation is as damaging as ischemia–reperfusion in the rat liver, Crit. Care Med. 26 (1998) 1089–1095.
[192] Y. Yokoyama, J.S. Beckman, T.K. Beckman, J.K. Wheat, T.G. Cash, B.A. Freeman, D.A. Parks, Circulating xanthine oxidase: potential mediator of ischemic injury, Am. J. Physiol. 258 (1990) G564–G570.
[193] A. Weinbroum, V.G. Nielsen, S. Tan, S. Gelman, S. Matalon, K.A. Skinner, E. Bradley, D.A. Parks, Liver ischemia–reperfusion increases pulmonary permeability in rat: Role of circulating xanthine oxidase, Am. J. Physiol. 31 (1995) G988–G996.
[194] W. Khoury, M. Namnesnikov, D. Fedorov, S. Abu-Gazala, A.A. Weinbroum, Mannitol attenuates kidney damage induced by xanthine oxidase-associated pancreas ischemia–reperfusion, J. Surg. Res. 160 (2010) 163–168.
[195] A.C. Sisley, T. Desai, J.M. Harig, B.L. Gewertz, Neutrophil depletion attenuates human intestinal reperfusion injury, J. Surg. Res. 57 (1994) 192–196.
[196] X.X. Tian, B.L. Wang, Y.Z. Cao, Y.X. Zhong, Y.Y. Tu, J.B. Xiao, Q.F. He, L.N. Zhai, Comparison of protective effects of Safflor Injection and extract of *Ginkgo biloba* on lung ischemia/reperfusion injury in rabbits, Chin. J. Integr. Med. (Oct 19 2013), http://dx.doi.org/10.1007/s11655-013-1513-8 (Epub ahead of print).
[197] E.G. Wilkins, R.S. Rees, D. Smith, B. Cashmer, J. Punch, G.O. Till, D.J. Smith Jr., Identification of xanthine oxidase activity following reperfusion in human tissue, Ann. Plast. Surg. 31 (1993) 60–65.
[198] M.A. Newaz, N.N. Adeeb, Detection of xanthine oxidase in human plasma, Med. J. Malaysia 53 (1998) 70–75.
[199] M.H. Shamma'a, S. Nasrallah, T. Chaglassian, A.K. Kachadurian, U.A.S. Al-Khalidi, Serum xanthine oxidase: a sensitive test of acute liver injury, Gastroenterology 48 (1965) 226–230.
[200] M.H. Shamma'a, S.M. Nasrallah, U.A.S. AI-Khalidi, Serum xanthine oxidase. An experience with 2000 patients, Am. J. Dig. Dis. 18 (1973) 15–22.
[201] K. Wolko, J. Krawczynski, Diagnostic value of determination of serum xanthine oxidase activity in acute viral hepatitis, Mater. Med. Pol. 6 (1974) 95–98.
[202] S. Giler, O. Sperling, S. Brosh, I. Urca, A. de Vries, Serum xanthine oxidase in jaundice, Clin. Chim. Acta 63 (1975) 37–40.
[203] M.G. Battelli, S. Musiani, M. Valgimigli, L. Gramantieri, F. Tomassoni, L. Bolondi, F. Stirpe, Serum xanthine oxidase in human liver disease, Am. J. Gastroenterol. 96 (2001) 1194–1199.
[204] R. Miesel, M. Zuber, Elevated levels of xanthine oxidase in serum of patients with inflammatory and autoimmune rheumatic diseases, Inflammation 17 (1993) 551–561.
[205] O. Akyol, I. Gokbulut, N. Koksal, H. Akin, H. Ozyurt, Z. Yildirim, The activities of purine catabolizing enzymes in plasma and bronchial washing fluid in patients with lung cancer and pneumonia, Clin. Biochem. 34 (2001) 251–254.
[206] R. Mete, F. Tulubas, M. Oran, A. Yılmaz, B.A. Avci, K. Yildiz, C.B. Turan, A. Gurel, The role of oxidants and reactive nitrogen species in irritable bowel syndrome: a potential etiological explanation, Med. Sci. Monit. 19 (2013) 762–766.
[207] O. Akyol, H. Herken, E. Uz, E. Fadillioğlu, S. Unal, S. Söğüt, H. Ozyurt, H.A. Savaş, The indices of endogenous oxidative and antioxidative processes in plasma from schizophrenic patients. The possible role of oxidant/antioxidant imbalance, Prog. Neuropsychopharmacol. Biol. Psychiatry 26 (2002) 995–1005.
[208] V. Cosić, S. Antić, M. Pesić, O. Jovanović, S. Kundalić, V.B. Djordjević, Monotherapy with metformin: does it improve hypoxia in type 2 diabetic patients? Clin. Chem. Lab. Med. 39 (2001) 818–821.
[209] U.R. Kuppusamy, M. Indran, P. Rokiah, Glycaemic control in relation to xanthine oxidase and antioxidant indices in Malaysian Type 2 diabetes patients, Diabet. Med. 22 (2005) 1343–1346.
[210] D.J. Miric, B.B. Kisic, L.D. Zoric, R.V. Mitic, B.M. Miric, I.M. Dragojevic, Xanthine oxidase and lens oxidative stress markers in diabetic and senile cataract patients, J. Diabetes Complicat. 27 (2013) 171–176.
[211] S. Giler, Y. Eshel, J. Pinkhas, E. Ventura, E. Levy, I. Urca, O. Sperling, A. de Vries, Elevation of serum xanthine oxidase following halothane anesthesia in man, Experientia 33 (1977) 1356–1358.
[212] I. Grattagliano, G. Vendemiale, C. Sabba, P. Buonamico, E. Altomare, Oxidation of circulating proteins in alcoholics: role of acetaldehyde and xanthine oxidase, J. Hepatol. 25 (1996) 28–36.
[213] F.J. Dagher, S. Saab, A. Panossian, Postoperative determinations of serum xanthine oxidase, and serum glutamic and oxalacetic and pyruvic transaminase, Surg. Gynecol. Obstet. 128 (1969) 985–990.

[214] S. Giler, O. Sperling, S. Brosh, I. Urca, A. de Vries, Elevated serum xanthine oxidase activity following biliary tract and gastric surgery, Int. Surg. 61 (1976) 153–155.
[215] B.A. Iwalokun, S.B. Bamiro, A. Ogunledun, Levels and interactions of plasma xanthine oxidase, catalase and liver function parameters in Nigerian children with *Plasmodium falciparum* infection, APMIS 114 (2006) 842–850.
[216] M. Aslan, T.M. Ryan, B. Adler, T.M. Townes, D.A. Parks, J.A. Thompson, A. Tousson, M.T. Gladwin, R.P. Patel, M.M. Tarpey, I. Batinic-Haberle, C.R. White, B.A. Freeman, Oxygen radical inhibition of nitric oxide-dependent vascular function in sickle cell disease, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 15215–15220.
[217] C.M. Grum, R.A. Ragsdale, L.H. Ketai, R.H. Simon, Plasma xanthine oxidase activity in patients with adult respiratory distress syndrome, J. Crit. Care 2 (1987) 22–26.
[218] M.C. Supnet, R. David-Cu, F.J. Walther, Plasma xanthine oxidase activity and lipid hydroperoxide levels in preterm infants, Pediatr. Res. 36 (1994) 283–287.
[219] D. Mitropoulos, G. Deliconstantinos, A. Zervas, V. Villiotou, C. Dimopoulos, J. Stavrides, Nitric oxide synthase and xanthine oxidase activities in the spermatic vein of patients with varicocele: a potential role for nitric oxide and peroxynitrite in sperm dysfunction, J. Urol. 156 (1996) 1952–1958.
[220] H.P. Friedl, D.J. Smith, G.O. Till, P.D. Thomson, D.S. Louis, P.A. Ward, Ischemia–reperfusion in humans — appearance of xanthine oxidase activity, Am. J. Pathol. 136 (1990) 491–495.
[221] H.P. Friedl, G.O. Till, O. Trentz, P.A. Ward, Role of oxygen radicals in tourniquet-related ischemia–reperfusion injury of human patients, Klin. Wochenschr. 69 (1991) 1109–1112.
[222] M. Mathru, D.J. Dries, L. Barnes, P. Tonino, R. Sukhani, M.W. Rooney, Tourniquet-induced exsanguination in patients requiring lower limb surgery. An ischemia–reperfusion model of oxidant and antioxidant metabolism, Anesthesiology 84 (1996) 14–22.
[223] S. Tan, S. Gelman, J.K. Wheat, D.A. Parks, Circulating xanthine oxidase in human ischemia reperfusion, South. Med. J. 88 (1995) 479–482.
[224] A.B. Karabulut, A. Kafkasli, F. Burak, E.M. Gozukara, Maternal and fetal plasma adenosine deaminase, xanthine oxidase and malondialdehyde levels in pre-eclampsia, Cell Biochem. Funct. 23 (2005) 279–283.
[225] A. Yildirim, K. Altinkaynak, H. Aksoy, Y.N. Sahin, F. Akcay, Plasma xanthine oxidase, superoxide dismutase and glutathione peroxidase activities and uric acid levels in severe and mild pre-eclampsia, Cell Biochem. Funct. 22 (2004) 213–217.
[226] E.J. Pesonen, N. Linder, K.O. Raivio, A. Sarnesto, R. Lapatto, K. Hockerstedt, H. Makisalo, S. Andersson, Circulating xanthine oxidase and neutrophil activation during human liver transplantation, Gastroenterology 114 (1998) 1009–1015.
[227] R. Martí, E. Múrio, E. Varela, I. Bilbao, C. Pascual, C. Margarit, R.M. Segura, Xanthine oxidoreductase and preservation injury in human liver transplantation, Transplantation 77 (2004) 1239–1245.
[228] M.G. Battelli, M. Ravaioli, S. Musiani, V. Scicchitano, G.L. Grazi, A. Bolognesi, Increased serum level of xanthine oxidoreductase in liver transplanted patients, J. Biol. Regul. Homeost. Agents 25 (2011) 77–84.
[229] B. Dołęgowska, W. Błogowski, L. Domański, Clinical evidence of the association between serum perioperative changes in xanthine metabolizing enzymes activity and early post-transplant kidney allograft function, J. Am. Coll. Surg. 211 (2010) 587–595.
[230] E. Kwiatkowska, K. Kędzierska, J. Bober, B. Dołęgowska, V. Dziedziejko, E. Gołembiewska, K. Ciechanowski, M. Wiśniewska, Urinary hepatocyte growth factor indicates ischemia/reperfusion injury after kidney transplantation, Pol. Arch. Med. Wewn. 120 (2010) 437–442.
[231] E.D. Jarasch, G. Bruder, H.W. Heid, Significance of xanthine oxidase in capillary endothelial cells, Acta Physiol. Scand. Suppl. 548 (1986) 39–46.
[232] A. Kooij, H.J. Schiller, M. Schijns, C.J.F. Van Noorden, W.M. Frederiks, Conversion of xanthine dehydrogenase into xanthine oxidase in rat liver and plasma at the onset of reperfusion after ischemia, Hepatology 19 (1994) 1488–1495.
[233] T. Adachi, T. Fukushima, Y. Usami, K. Hirano, Binding of human xanthine oxidase to sulphated glycosaminoglycans on the endothelial-cell surface, Biochem. J. 289 (1993) 523–527.
[234] M. Houston, A. Estevez, P. Chumley, M. Aslan, S. Marklund, D.A. Parks, B.A. Freeman, Binding of xanthine oxidase to vascular endothelium. Kinetic characterization and oxidative impairment of nitric oxide-dependent signaling, J. Biol. Chem. 274 (1999) 4985–4994.
[235] R.S. Poggetti, F.A. Moore, E.E. Moore, K. Koeike, A. Banerjee, Simultaneous liver and lung injury following gut ischemia is mediated by xanthine oxidase, J. Trauma 32 (1992) 723–727.
[236] K. Koike, F.A. Moore, E.E. Moore, R.A. Read, V.S. Carl, A. Banerjee, Gut ischemia mediates lung injury by a xanthine oxidase-dependent neutrophil mechanism, J. Surg. Res. 54 (1993) 469–473.
[237] R.N. Axon, M.S. Baird, J.D. Lang, A.E. Brix, V.G. Nielsen, PentaLyte decreases lung injury after aortic occlusion–reperfusion, Am. J. Respir. Crit. Care Med. 157 (1998) 1982–1990.
[238] D.L. Carden, D.N. Granger, Pathophysiology of ischaemia–reperfusion injury, J. Pathol. 190 (2000) 255–266.
[239] A.N. Shafik, Febuxostat improves the local and remote organ changes induced by intestinal ischemia/reperfusion in rats, Dig. Dis. Sci. 58 (2013) 650–659.
[240] G. Allan, D. Cambridge, L. Lee-Tsang-Tan, C.W. Van Way, M.V. Whiting, The protective action of allopurinol in an experimental model of haemorrhagic shock and reperfusion, Br. J. Pharmacol. 89 (1986) 149–155.
[241] P.H. Chan, J.W. Schmidley, R.A. Fishman, S.M. Longar, Brain injury, edema, and vascular permeability changes induced by oxygen-derived free radicals, Neurology 34 (1984) 315–320.
[242] N. Tapuria, Y. Kumar, M.M. Habib, M. Abu Amara, A.M. Seifalian, B.R. Davidson, Remote ischemic preconditioning: a novel protective method from ischemia reperfusion injury—a review, J. Surg. Res. 150 (2008) 304–330.
[243] C.X. Santos, N. Anilkumar, M. Zhang, A.C. Brewer, A.M. Shah, Redox signaling in cardiac myocytes, Free Radic. Biol. Med. 50 (2011) 777–793.
[244] D.N. Granger, Ischemia–reperfusion: mechanisms of microvascular dysfunction and the influence of risk factors for cardiovascular disease, Microcirculation 6 (1999) 167–178.
[245] J.B. Seal, B.L. Gewertz, Vascular dysfunction in ischemia–reperfusion injury, Ann. Vasc. Surg. 19 (2005) 572–584.
[246] A. Meneshian, G.B. Bulkley, The physiology of endothelial xanthine oxidase: from urate catabolism to reperfusion injury to inflammatory signal transduction, Microcirculation 9 (2002) 161–175.
[247] R.M. Wright, L.A. Ginger, N. Kosila, N.D. Elkins, B. Essary, J.L. McManaman, J.E. Repine, Mononuclear phagocyte xanthine oxidoreductase contributes to cytokine-induced acute lung injury, Am. J. Respir. Cell Mol. Biol. 30 (2004) 479–490.
[248] S. Gibbings, N.D. Elkins, H. Fitzgerald, J. Tiao, M.E. Weyman, G. Shibao, M.A. Fini, R.M. Wright, Xanthine oxidoreductase promotes the inflammatory state of mononuclear phagocytes through effects on chemokine expression, peroxisome proliferator-activated receptor-{gamma} sumoylation, and HIF-1{alpha}, J. Biol. Chem. 286 (2011) 961–975.
[249] S.A. Nicholas, V.V. Bubnov, I.M. Yasinska, V.V. Sumbayev, Involvement of xanthine oxidase and hypoxia-inducible factor 1 in Toll-like receptor 7/8-mediated activation of caspase 1 and interleukin-1beta, Cell. Mol. Life Sci. 68 (2011) 151–158.
[250] J.W. de Jong, R.G. Schoemaker, R. de Jonge, P. Bernocchi, E. Keijzer, R. Harrison, H.S. Sharma, C. Ceconi, Enhanced expression and activity of xanthine oxidoreductase in the failing heart, J. Mol. Cell. Cardiol. 32 (2000) 2083–2089.
[251] U.E. Ekelund, R.W. Harrison, O. Shokek, R.N. Thakkar, R.S. Tunin, H. Senzaki, D.A. Kass, E. Marbán, J.M. Hare, Intravenous allopurinol decreases myocardial oxygen consumption and increases mechanical efficiency in dogs with pacing-induced heart failure, Circ. Res. 85 (1999) 437–445.
[252] W.F. Saavedra, N. Paolocci, M.E. St John, M.W. Skaf, G.C. Stewart, J.S. Xie, R.W. Harrison, J. Zeichner, D. Mudrick, E. Marbán, D.A. Kass, J.M. Hare, Imbalance between xanthine oxidase and nitric oxide synthase signaling pathways underlies mechanoenergetic uncoupling in the failing heart, Circ. Res. 90 (2002) 297–304.
[253] L.C. Amado, A.P. Saliaris, S.V. Raju, S. Lehrke, M. St John, J. Xie, G. Stewart, T. Fitton, K.M. Minhas, J. Brawn, J.M. Hare, Xanthine oxidase inhibition ameliorates cardiovascular dysfunction in dogs with pacing-induced heart failure, J. Mol. Cell. Cardiol. 39 (2005) 531–536.
[254] V. Mellin, M. Isabelle, A. Oudot, C. Vergely-Vandriesse, C. Monteil, B. Di Meglio, J.P. Henry, B. Dautreaux, L. Rochette, C. Thuillez, P. Mulder, Transient reduction in myocardial free oxygen radical levels is involved in the improved cardiac function and structure after long-term allopurinol treatment initiated in established chronic heart failure, Eur. Heart J. 26 (2005) 1544–1550.
[255] H. Kögler, H. Fraser, S. McCune, R. Altschuld, E. Marbán, Disproportionate enhancement of myocardial contractility by the xanthine oxidase inhibitor oxypurinol in failing rat myocardium, Cardiovasc. Res. 59 (2003) 582–592.
[256] A.V. Naumova, V.P. Chacko, R. Ouwerkerk, L. Stull, E. Marbán, R.G. Weiss, Xanthine oxidase inhibitors improve energetics and function after infarction in failing mouse hearts, Am. J. Physiol. Heart Circ. Physiol. 290 (2006) H837–H843.
[257] O.S. Ali, H.M. Abdelgawad, M.S. Mohammed, R.R. El-Awady, Ischemic heart diseases in Egypt: role of xanthine oxidase system and ischemia-modified albumin, Heart Vessels (Oct 4 2013), http://dx.doi.org/10.1007/s00380-013-0413-3 (Epub ahead of print).
[258] W. Doehner, N. Schoene, M. Rauchhaus, F. Leyva-Leon, D.V. Pavitt, D.A. Reaveley, G. Schuler, A.J. Coats, S.D. Anker, R. Hambrecht, Effects of xanthine oxidase inhibition with allopurinol on endothelial function and peripheral blood flow in hyperuricemic patients with chronic heart failure: results from 2 placebo-controlled studies, Circulation 105 (2002) 2619–2624.
[259] C.A. Farquharson, R. Butler, A. Hill, J.J. Belch, A.D. Struthers, Allopurinol improves endothelial dysfunction in chronic heart failure, Circulation 106 (2002) 221–226.
[260] S. Baldus, R. Köster, P. Chumley, T. Heitzer, V. Rudolph, M.A. Ostad, A. Warnholtz, H.J. Staude, F. Thuneke, K. Koss, J. Berger, T. Meinertz, B.A. Freeman, T. Münzel, Oxypurinol improves coronary and peripheral endothelial function in patients with coronary artery disease, Free Radic. Biol. Med. 39 (2005) 1184–1190.
[261] H.E. Cingolani, J.A. Plastino, E.M. Escudero, B. Mangal, J. Brown, N.G. Pérez, The effect of xanthine oxidase inhibition upon ejection fraction in heart failure patients: La Plata Study, J. Card. Fail. 12 (2006) 491–498.
[262] F. Khan, J. George, K. Wong, S. McSwiggan, A.D. Struthers, J.J. Belch, Allopurinol treatment reduces arterial wave reflection in stroke survivors, Cardiovasc. Ther. 26 (2008) 247–252.
[263] S.W. Muir, C. Harrow, J. Dawson, K.R. Lees, C.J. Weir, N. Sattar, M.R. Walters, Allopurinol use yields potentially beneficial effects on inflammatory indices in those with recent ischemic stroke: a randomized, double-blind, placebo-controlled trial, Stroke 39 (2008) 3303–3307.
[264] J.M. Hare, B. Mangal, J. Brown, C. Fisher Jr., R. Freudenberger, W.S. Colucci, D.L. Mann, P. Liu, M.M. Givertz, R.P. Schwarz, OPT-CHF Investigators, Impact of oxypurinol in patients with symptomatic heart failure. Results of the OPT-CHF study, J. Am. Coll. Cardiol. 51 (2008) 2301–2309.
[265] U. Landmesser, S. Spiekermann, C. Preuss, S. Sorrentino, D. Fischer, C. Manes, M. Mueller, H. Drexler, Angiotensin II induces endothelial xanthine oxidase activation: role for endothelial dysfunction in patients with coronary disease, Arterioscler. Thromb. Vasc. Biol. 27 (2007) 943–948.
[266] D.R. Gonzalez, A.V. Treuer, J. Castellanos, R.A. Dulce, J.M. Hare, Impaired S-nitrosylation of the ryanodine receptor caused by xanthine oxidase activity contributes to calcium leak in heart failure, J. Biol. Chem. 285 (2010) 28938–28945.

[267] D.B. Sawyer, D.A. Siwik, L. Xiao, D.R. Pimentel, K. Singh, W.S. Colucci, Role of oxidative stress in myocardial hypertrophy and failure, J. Mol. Cell. Cardiol. 34 (2002) 379–388.
[268] M. Pinelli, M. Bindi, F. Moroni, M. Castiglioni, Relationship between serum uric acid levels and urinary albumin excretion in patients with heart failure, Acta Cardiol. 63 (2008) 191–195.
[269] Y.Y. Sautin, R.J. Johnson, Uric acid: the oxidant–antioxidant paradox, Nucleosides Nucleotides Nucleic Acids 27 (2008) 608–619.
[270] F. Viazzi, G. Leoncini, E. Ratto, R. Pontremoli, Serum uric acid as a risk factor for cardiovascular and renal disease: an old controversy revived, J. Clin. Hypertens. (Greenwich) 8 (2006) 510–518.
[271] P. Strazzullo, J.G. Puig, Uric acid and oxidative stress: relative impact on cardiovascular risk? Nutr. Metab. Cardiovasc. Dis. 17 (2007) 409–414.
[272] I. Suzuki, T. Yamauchi, M. Onuma, S. Nozaki, Allopurinol, an inhibitor of uric acid synthesis—can it be used for the treatment of metabolic syndrome and related disorders? Drugs Today (Barc.) 45 (2009) 363–378.
[273] J.M. Hare, R.J. Johnson, Uric acid predicts clinical outcomes in heart failure: insights regarding the role of xanthine oxidase and uric acid in disease pathophysiology, Circulation 107 (2003) 1951–1952.
[274] T. Neogi, J. George, S. Rekhraj, A.D. Struthers, H. Choi, R.A. Terkeltaub, Are either or both hyperuricemia and xanthine oxidase directly toxic to the vasculature? A critical appraisal, Arthritis Rheum. 64 (2012) 327–338.
[275] A.F. Cicero, M. Rosticci, A. Parini, C. Baronio, S. D'Addato, C. Borghi, Serum uric acid is inversely proportional to estimated stroke volume and cardiac output in a large sample of pharmacologically untreated subjects: data from the Brisighella Heart Study, Intern. Emerg. Med. (Nov 9 2013), http://dx.doi.org/10.1007/s11739-013-1016-9 (Epub ahead of print).
[276] A.G. Ioachimescu, D.M. Brennan, B.M. Hoar, S.L. Hazen, B.J. Hoogwerf, Serum uric acid is an independent predictor of all-cause mortality in patients at high risk of cardiovascular disease: a preventive cardiology information system (PreCIS) database cohort study, Arthritis Rheum. 58 (2008) 623–630.
[277] J. Dawson, M. Walters, Uric acid and xanthine oxidase: future therapeutic targets in the prevention of cardiovascular disease? Br. J. Clin. Pharmacol. 62 (2006) 633–644.
[278] A. Harzand, L. Tamariz, J.M. Hare, Uric acid, heart failure survival, and the impact of xanthine oxidase inhibition, Congest. Heart Fail. 18 (2012) 179–182.
[279] D.I. Feig, D.H. Kang, R.J. Johnson, Uric acid and cardiovascular risk, N. Engl. J. Med. 359 (2008) 1811–1821.
[280] M. Mazzali, M. Kanbay, M.S. Segal, M. Shafiu, D. Jalal, D.I. Feig, R.J. Johnson, Uric acid and hypertension: cause or effect? Curr. Rheumatol. Rep. 12 (2010) 108–117.
[281] M. Mazzali, J. Hughes, Y.G. Kim, J.A. Jefferson, D.H. Kang, K.L. Gordon, H.Y. Lan, S. Kivlighn, R.J. Johnson, Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism, Hypertension 38 (2001) 1101–1106.
[282] L.G. Sánchez-Lozada, E. Tapia, V. Soto, C. Avila-Casado, M. Franco, L. Zhao, R.J. Johnson, Treatment with the xanthine oxidase inhibitor febuxostat lowers uric acid and alleviates systemic and glomerular hypertension in experimental hyperuricaemia, Nephrol. Dial. Transplant. 23 (2008) 1179–1185.
[283] F. Stirpe, E. Della Corte, E. Bonetti, A. Abbondanza, A. Abbati, F. De Stefano, Fructose-induced hyperuricaemia, Lancet 2 (1970) 1310–1311.
[284] T. Nakagawa, H. Hu, S. Zharikov, K.R. Tuttle, R.A. Short, O. Glushakova, X. Ouyang, D. I. Feig, E.R. Block, J. Herrera-Acosta, J.M. Patel, R.J. Johnson, A causal role for uric acid in fructose-induced metabolic syndrome, Am. J. Physiol. Renal. Physiol. 290 (2006) F625–F631.
[285] R.J. Johnson, M.S. Segal, Y. Sautin, T. Nakagawa, D.I. Feig, D.H. Kang, M.S. Gersch, S. Benner, L.G. Sánchez-Lozada, Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease, Am. J. Clin. Nutr. 86 (2007) 899–906.
[286] K. Nakazono, N. Watanabe, K. Matsuno, J. Sasaki, T. Sato, M. Inoue, Does superoxide underlie the pathogenesis of hypertension? Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 10045–10048.
[287] M.A. Newaz, N.N. Adeeb, N. Muslim, T.A. Razak, N.N. Htut, Uric acid, xanthine oxidase and other risk factors of hypertension in normotensive subjects, Clin. Exp. Hypertens. 18 (1996) 1035–1050.
[288] D.I. Feig, B. Soletsky, R.J. Johnson, Effect of allopurinol on blood pressure of adolescents with newly diagnosed essential hypertension: a randomized trial, JAMA 300 (2008) 924–932.
[289] J. George, A.D. Struthers, The role of urate and xanthine oxidase inhibitors in cardiovascular disease, Cardiovasc. Ther. 26 (2008) 59–64.
[290] A.R. Weseler, A. Bast, Oxidative stress and vascular function: implications for pharmacologic treatments, Curr. Hypertens. Rep. 12 (2010) 154–161.
[291] M.A. Newaz, Z. Yousefipour, A. Oyekan, Oxidative stress-associated vascular aging is xanthine oxidase-dependent but not NAD(P)H oxidase-dependent, J. Cardiovasc. Pharmacol. 48 (2006) 88–94.
[292] A. Boueiz, M. Damarla, P.M. Hassoun, Xanthine oxidoreductase in respiratory and cardiovascular disorders, Am. J. Physiol. Lung Cell. Mol. Physiol. 294 (2008) L830–L840.
[293] S. Martinez-Hervas, J.T. Real, C. Ivorra, A. Priego, F.J. Chaves, F.V. Pallardo, J.R. Viña, J. Redon, R. Carmena, J.F. Ascaso, Increased plasma xanthine oxidase activity is related to nuclear factor kappa beta activation and inflammatory markers in familial combined hyperlipidemia, Nutr. Metab. Cardiovasc. Dis. 20 (2010) 734–739.
[294] O. Yiginer, F. Ozcelik, T. Inanc, M. Aparci, N. Ozmen, B.Y. Cingozbay, E. Kardesoglu, S. Suleymanoglu, G. Sener, B.S. Cebeci, Allopurinol improves endothelial function and reduces oxidant-inflammatory enzyme of myeloperoxidase in metabolic syndrome, Clin. Res. Cardiol. 97 (2008) 334–340.
[295] P. Higgins, J. Dawson, K.R. Lees, K. McArthur, T.J. Quinn, M.R. Walters, Xanthine oxidase inhibition for the treatment of cardiovascular disease: a systematic review and meta-analysis, Cardiovasc. Ther. 30 (2012) 217–226.
[296] H. Cai, D.G. Harrison, Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress, Circ. Res. 87 (2000) 840–844.
[297] J. Sabán-Ruiz, A. Alonso-Pacho, M. Fabregate-Fuente, C. de la Puerta González-Quevedo, Xanthine oxidase inhibitor febuxostat as a novel agent postulated to act against vascular inflammation, Antiinflamm. Antiallergy Agents Med. Chem. 12 (2013) 94–99.
[298] X. Xu, X. Hu, Z. Lu, P. Zhang, L. Zhao, J.L. Wessale, R.J. Bache, Y. Chen, Xanthine oxidase inhibition with febuxostat attenuates systolic overload-induced left ventricular hypertrophy and dysfunction in mice, J. Card. Fail. 14 (2008) 746–753.
[299] L. Zhao, B.M. Roche, J.L. Wessale, A. Kijtawornrat, J.L. Lolly, D. Shemanski, R.L. Hamlin, Chronic xanthine oxidase inhibition following myocardial infarction in rabbits: effects of early versus delayed treatment, Life Sci. 82 (2008) 495–502.
[300] D.I. Feig, Serum uric acid and the risk of hypertension and chronic kidney disease, Curr. Opin. Rheumatol. 26 (2014) 176–185.
[301] J.E. Baker, J. Su, X. Fu, A. Hsu, G.J. Gross, J.S. Tweddell, N. Hogg, Nitrite confers protection against myocardial infarction: role of xanthine oxidoreductase, NADPH oxidase and K(ATP) channels, J. Mol. Cell. Cardiol. 43 (2007) 437–444.
[302] S.M. Ghosh, V. Kapil, I. Fuentes-Calvo, K.J. Bubb, V. Pearl, A.B. Milsom, R. Khambata, S. Maleki-Toyserkani, M. Yousuf, N. Benjamin, A.J. Webb, M.J. Caulfield, A.J. Hobbs, A. Ahluwalia, Enhanced vasodilator activity of nitrite in hypertension: critical role for erythrocytic xanthine oxidoreductase and translational potential, Hypertension 61 (2013) 1091–1102.