Chapter

The Good and the Bad: The Bifunctional Enzyme Xanthine Oxidoreductase in the Production of Reactive Oxygen Species

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Abstract

Xanthine oxidoreductase (XOR) is a molybdoflavin enzyme which occurs in two forms; the reduced form known as xanthine dehydrogenase (XDH, EC 1.17.1.4) and the oxidised form known as xanthine oxidase (XO, EC 1.17.3.2). In humans, it is a 293 kDa homodimer which catalyses consecutive hydroxylation steps of purine degradation. The oxidised form of the enzyme produces hydrogen peroxide and superoxide ($O_2^{\bullet -}$), both of which are reactive oxygen species (ROS) that can interact with several biomolecules producing adverse reactions. XOR can also produce nitric oxide, a cardiovascular protective molecule. Overproduction of nitric oxide results in the formation of the highly reactive peroxynitrite radical. XOR-produced ROS may provide protection against infection, while at the same time can also lead to inflammation, oncogenesis, brain injury and stroke. XOR is also involved in tumour lysis syndrome in chemotherapy patients as well in ischaemia-reperfusion injury, increasing the levels of ROS in the body. Consequently, the presence of XOR in blood can be used as a biomarker for a number of conditions including oxidative stress and cardiovascular disease.

Keywords: xanthine oxidoreductase, reactive oxygen species, superoxide, cardiovascular disease, oxidative stress

1. Introduction

Xanthine Oxidoreductase (XOR) is ubiquitous amongst organisms being found in both prokaryotes and eukaryotes. It is a complex flavoenzyme and one of a few that require a molybdenum cofactor (Moco), in the form of molybdopterin (MPT). As well as a requirement for flavin adenine dinucleotide (FAD) it also contains two iron–sulphur ([2Fe-2S]) clusters. XOR exists in two forms: most organisms have only the dehydrogenase, XDH (EC 1.17.1.4), while mammals also produce the oxidase, XO (EC 1.17.3.2). The XDH to XO conversion has been studied [1, 2]. It has been demonstrated that conversion could be accomplished in two ways; either through

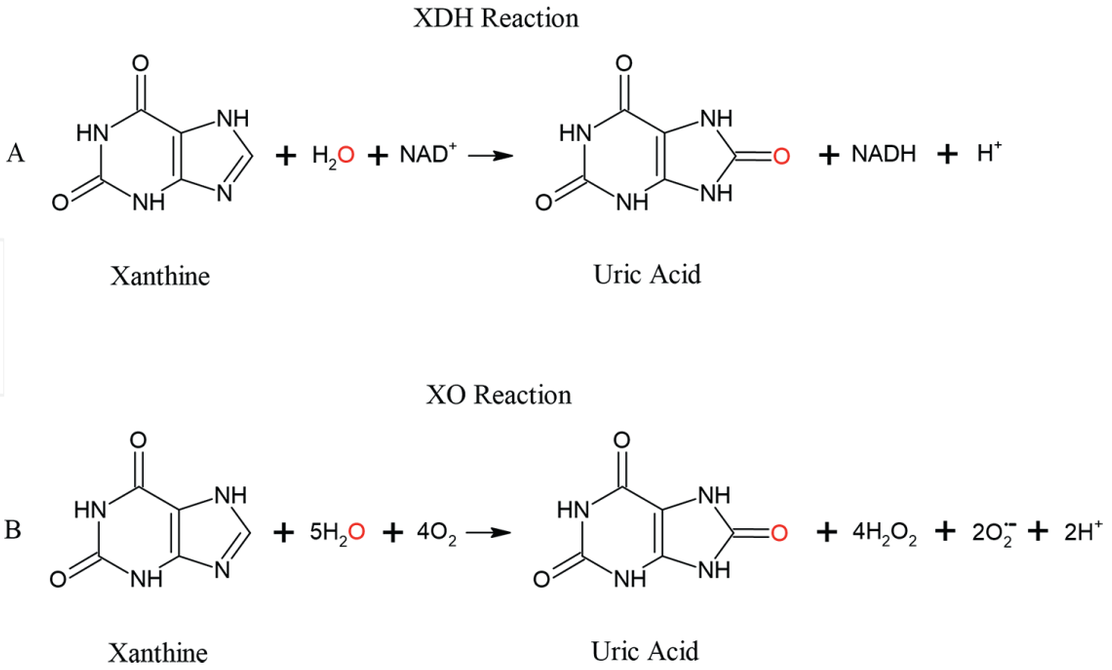

Figure 1.
Xanthine oxidoreductase (XOR) catalysed reactions. A. Xanthine dehydrogenase (XDH) reaction with NAD+ as electron acceptor. B. Xanthine oxidase (XO) reaction with molecular oxygen as electron acceptor. Note that the majority of XOR enzymes are found in the XDH form while mammalian enzymes may be converted from XDH to XO (see text for details).

disulphide formation of specific cysteine residues or by limited proteolysis. The major reactions catalysed by the dehydrogenase (XDH), that of oxidising hypoxanthine to xanthine and then xanthine to uric acid (**Figure 1A**), are important last steps in the degradation of purines (and therefore in the degradation of DNA and RNA). The oxidase (XO) on the other hand, can generate reactive oxygen species (ROS) which are involved in the inflammatory defensive response (**Figure 1B**) [3].

The enzyme also has nitrite reductase activity and is therefore important in the production of nitric oxide and involved in the physiological control of blood pressure. In this review we discuss the structure, catalytic mechanism, and tissue localisation of human XOR and its role in health and disease.

2. Mammalian xanthine oxidoreductase

Mammalian XOR exists as a homodimer with a molecular weight of about 290 kDa (**Figure 2**). XOR can be found in both XO and XDH forms and has a wide distribution in mammalian tissues, with the highest amounts observed in the intestines and liver. XOR is present in milk and has in fact, been detected in mammary gland epithelial and capillary endothelial cells. Intracellularly, XDH is the predominant species whilst XO is present in the gastrointestinal lumen and body fluids. Inactive forms of XOR do occur in mammalian tissues and usually lack either molybdenum or sulphur in the Moco cofactor. It was estimated that up to 60% of XOR found in bovine milk may be in an inactive state [4]. Demolybdo XOR, lacks the molybdenum (Mo) and possibly the whole MPT cofactor, while desulfo XOR, has a molybdenum-bound sulphur atom replaced by oxygen in the Moco. Due to the presence of these inactive forms, immunodetection of XOR may not correlate well with level of XOR activity. There

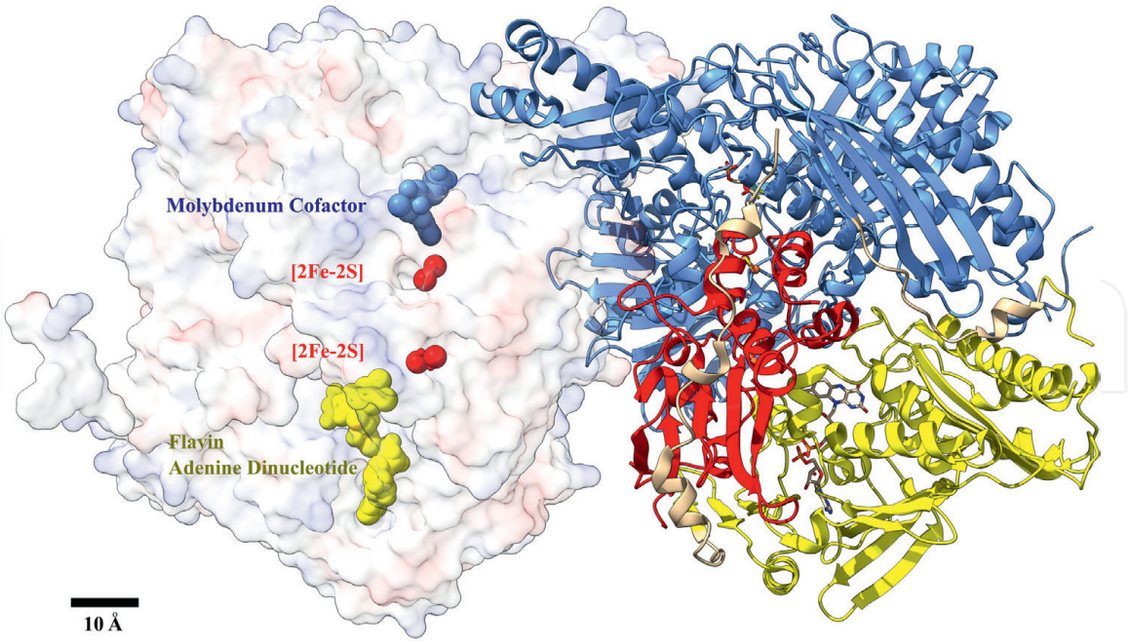

Figure 2.
*Crystal structure of bovine xanthine oxidoreductase, (PDB 3UNC). The two subunits of the biologically active dimeric enzyme are shown as different representations. On the right-hand side is a cartoon of one monomer; blue represents the Moco domain (including the C terminus), yellow represents the FAD domain and red represent the iron–Sulphur domain (and includes the N-terminus). Cream represents the link portions and cofactors are also shown in stick form. On the left hand side the monomer is shown as a surface coloured by electrostatic potential with cofactors as spheres coloured as their domains in the cartoon and labelled accordingly. Note that the cofactors in each monomer are orientated similarly from top to bottom, which is also the direction of the flow of electrons during the catalytic reaction (***Figure 3****).*

is considerable homology between XOR and aldehyde oxidases, exemplified by the observed cross-reactivity of XOR antibodies, further complicating the analysis by immunodetection [5, 6].

3. Human xanthine oxidoreductase

Human XOR activity has been found to be low in organs other than the liver and the intestines [5, 6]. XOR was shown to be a source of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in vasculature and digestive tract. In an inflammatory response, it has been shown that XOR protects against infection through the generation of ROS and RNS, and so was designated as an innate immunity agent [7]. Additionally, it is deduced that XOR provides antimicrobial defence in the gastrointestinal tract where it can concentrate on the epithelial cells' surface [8]. Milk XOR has bactericidal characteristics and provides immunity in the new-born gut, where other immune responses have not yet formed [9]. In the liver, XOR may catalyse the oxidation of numerous metabolites such as purines, pyrimidines, heterocyclic compounds, and aldehydes, as well as other xenobiotics such as anti-cancer drugs. As a result, XOR contributes to liver detoxification [10].

At normal physiological conditions, XOR is present in very low amounts in serum [11]. Due to the presence of serum proteases, circulating XOR is virtually all in XO form [12]. During certain disease states, the levels of blood XO increase. For example, during viral hepatitis, the level of blood XO is 1000 times higher than the controls [13]. Other disease states were blood XO was found to be elevated include

artherosclerosis, mixed connective tissue disease and scleroderma [14, 15]. Moreover, the level of circulating XO has been reported to also increase during ischaemia-reperfusion injury induced by liver transplantation or aortic cross-clamp procedures amongst others [16, 17]. Circulating XO can also bind to glycoslaminoglycans on the vascular endothelial cells' surface and, if concentrated enough, can cause oxidative damage to organs that are far from the original site of XO release/damage and/or have low content of XOR during normal physiological conditions [18].

Purification of human XOR from liver tissue and breast milk, and (recombinantly) from bacteria has been described [19–21]. The activity of pure human liver and milk XO was found to be just 5% that of bovine milk, which is consistent with the molybdenum content measurement, where human milk XOR only has 4% molybdenum content, while bovine milk XOR has between 70 and 60% molybdenum content [4, 20]. Human XOR activity was observed to be the highest in the liver. However, as compared to other mammals, namely *Bos taurus*, *Ovis aries* and *Capra aegagrus,* the specific activity of human XOR is low [4, 22, 23].

4. Structure and catalysis

Due to the evolutionary loss of uricase in humans and higher primates, XOR serves as the ultimate catalyst in purine degradation, catalysing the last two hydroxylation reactions by first converting hypoxanthine to xanthine, and then xanthine to uric acid [24].

The first XDH and XO crystal structures were elucidated by Enroth and coworkers using protein isolated from bovine milk [25]. The crystal structure of bovine XOR shows that it is organised as a homodimer with three redox-centered domains per monomer. Each subunit consists of a polypeptide chain of 1332 amino acids of 146.8 kDa and contains two iron sulphur clusters (each [2Fe-2S]), one Moco and one flavin adenine dinucleotide (FAD) coenzyme (**Figure 2**).

The 20 kDa N-terminal domain that houses the two [2Fe-2S] is connected via a linker peptide to a central 40 kDa FAD domain. This is, in turn, connected by another linker to the larger 85 kDa C-terminal molybdopterin domain. The molybdopterin lies close to the interfaces of both the Fe-S and the FAD domains. The two hydroxylation reactions that convert hypoxanthine to xanthine, and xanthine to urate both occur at the Moco active site of each monomer.

During catalysis, electrons flow sequentially through the redox centers of the three domains in a nearly linear manner commencing at the Moco domain where the oxidative half-reaction takes place, to the two [2Fe-2S] domains, and finally, the FAD domain, where the reductive half-reaction takes place. Here NAD^+ is reduced to NADH (**Figure 3**). As the two Mo centers in the homodimer are 52 Å apart, no electron transfer is possible between the two monomers (**Figure 2**) [25].

The active site Glu-1261 (bovine numbering; sequence accession number P80457) initiates the reaction by abstraction of a proton from a hydroxide bound to Mo at the Moco site (**Figure 4**). This results in a deprotonated ion, $Mo\text{-}O^-$, that is nucleophilic and attacks carbon-8 of the xanthine substrate. This causes a hydride shift from the substrate onto the Moco sulphur, changing the valency of the Mo from (VI) to (IV). The subsequent reactions depend on the reaction conditions and substrate. If electron transfer from the Mo center to the sulphur precedes the product displacement, Path A (**Figure 4**), occurs. This path occurs at high pH and substrate concentrations, and with slow substrates, such as 2-hydroxy-6-methylpurine [26]. Under these conditions,

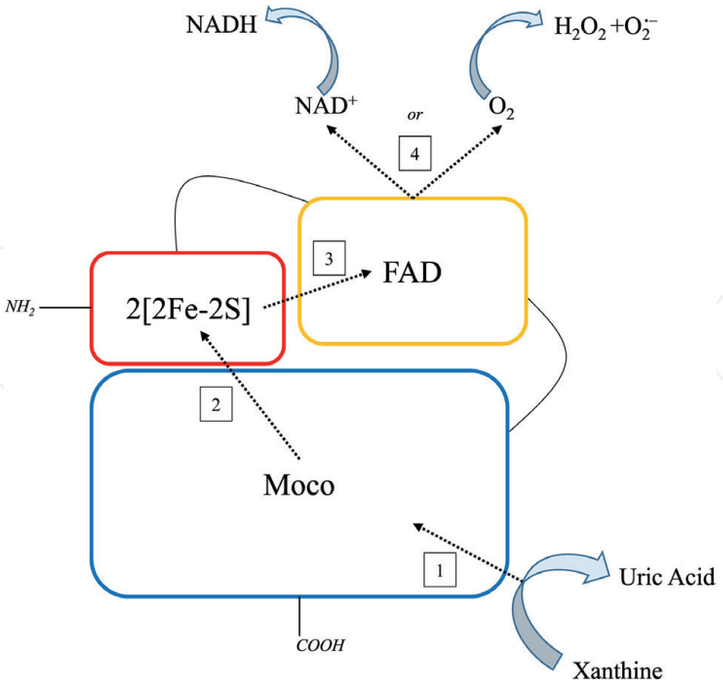

Figure 3.
Electron transfer in XOR. Arrows represent electron transfer. Xanthine donates its electrons at the Moco site (1) and oxidises to form uric acid in the process. The electrons propagate through the 2[2Fe-2S] clusters (2) to the FAD (3) and are then ultimately accepted by either NAD+ (XDH reaction) or oxygen (XO reaction), depending on whether the enzyme is reduced or oxidised respectively (4). Superoxide production occurs when oxygen is the electron acceptor.

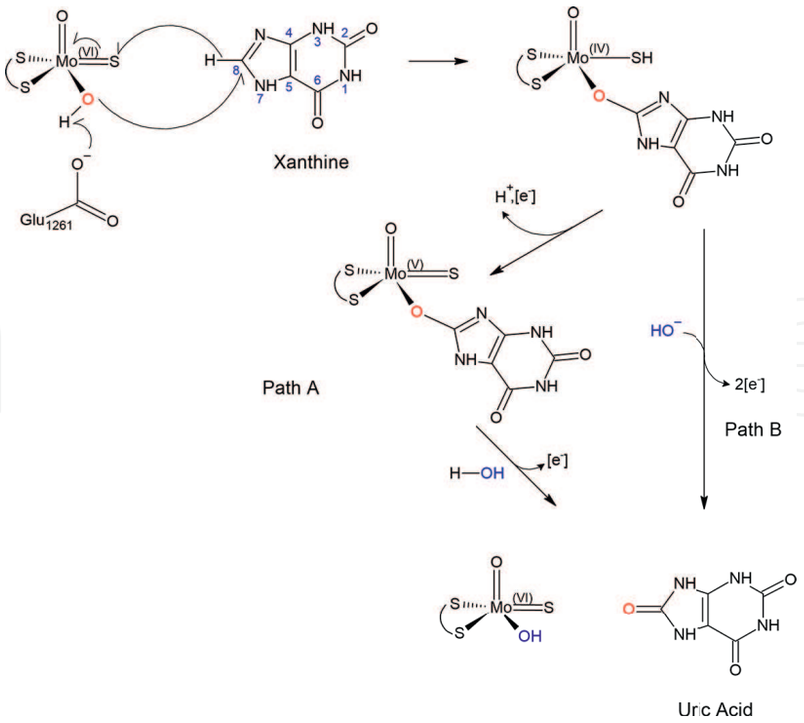

Figure 4.
Reaction mechanism of XOR with xanthine as a substrate. Glu-1261 abstracts a hydrogen from the hydroxyl group coordinated with Mo, resulting in a cascade of reactions, ending with the first intermediate (top right). This intermediate, can go through one of two alternative paths. Which pathway takes place is dependent on the reaction conditions; Path A occurs at high pH or high substrate concentrations, and slow substrates, whereas path B occurs at a pH lower than 8.3.

a Mo(V) species will form which displaces the product and forms the Mo(VI) species via hydrolysis. Under normal physiological conditions, however, the Mo(V) intermediate is skipped and product dissociation occurs first (Path B, **Figure 4**) [26, 27].

The XDH and XO forms of the mammalian XOR are mechanistically distinct as XDH preferentially uses NAD^+ as the final electron acceptor at the FAD site, forming NADH, while oxygen is the final electron acceptor in XO which produces hydrogen peroxide and superoxide radical (**Figure 3**). These XO-generated ROS may serve a physiological purpose but may also have pathological consequences. In mammals XDH can be converted to XO either by the reversible oxidation of cysteine residues to form a disulfide bond or by irreversible limited proteolysis [28, 29]. The formation of XO has three effects: hindrance of the entry of NAD^+ to the FAD active site, the formation of an access channel to this active site and an increase in the redox potential that supports the generation of superoxide [30].

As XO is the principle source of XOR-produced ROS, this conversion process is of particular interest. The mechanism of XDH-XO conversion was elucidated by comparing *Bos taurus* XOR, *Rattus norvegicus* XOR (both of which can be found in both XDH and XO forms) and *Gallus gallus* XOR (can be found only as the XDH form). On comparing the *R. norvegicus* XOR with the *G. gallus* XOR, Nishino et al. showed that two pairs of cysteine residues were responsible for formation of disulphide bridges during this conversion: Cys535 with Cys992 and Cys1316 with Cys1324 (*R. norvegicus* numbering) [31].

In the bovine XOR crystal structure, Cys992 is positioned on the molecular surface, while Cys535 is on a linker peptide (Lys532 - Ser589) between the Moco and FAD. Cys535-Cys992 disulphide bridge has a far-reaching conformational effect on the Gln422 - Lys432 C-terminal loop that would otherwise structurally contribute the FAD active site in XOR. The displaced loop partly covers the FAD active site in XO, thus blocking NAD^+ from entering and acting as the electron acceptor. Furthermore, this disturbance alters the electrostatic environment surrounding the FAD, making the semiquinone less stable and more reactive to oxygen. As a result, oxygen becomes the primary electron acceptor in XO [25]. NAD^+ reactivity in XO is further decreased due to the oxidation and disulfide bond formation at Cys1316 and Cys1324. Upon oxidation, the XOR C-terminus is obstructed from interacting with the NAD^+ binding cavity. This interaction was shown to be critical for NAD^+ binding in the bovine structure [31]. Similarly, irreversible proteolysis near Leu 215 and Leu 569 causes considerable structural disturbance of the Gln422 - Lys432 C-terminal loop that interferes with the reaction at the FAD (**Figure 5**).

Apart from its canonical activity, XOR can also act as a nitrite reductase by reducing NO_2^- to $NO^\bullet$ at the Moco site using either xanthine or NADH as the electron donors [32, 33]. Li et al. noted that the site of electron donation differs depending on the electron donor involved in the reaction [34]. While NADH donates its electrons to the FAD site, xanthine donates its electrons directly to the Moco site. Under normal physiological conditions, the preference for xanthine as an electron acceptor to produce uric acid inhibits nitric oxide ($NO^\bullet$) formation. As such, it is suggested that NADH acts as the primary electron donor for $NO^\bullet$ production, indicating that XDH, not XO, is the preferred catalyst under hypoxic conditions [35]. The presence of oxygen results in lower production of $NO^\bullet$ due to oxidation of the Moco site, while NO is formed under hypoxic conditions. Additionally, $NO^\bullet$ formation is an acid catalysed reaction and only occurs under acidic pH conditions such as those observed during inflammation or intracellular acidosis in ischaemic conditions where pH falls to 6.0 or below [36].

5. XOR-generated ROS/RNS

Although chronic and elevated levels of ROS/RNS are culpable of extensive cellular and molecular damage and identified as primary suspects in pathogenicity, when within threshold levels, reactive species do play vital roles in cellular signalling, vasodilation, neutrophil burst and neurotransmission for example [37, 38]. There is, therefore, a fine line that separates the benefits exerted by these reactive species from the harm they may cause that is in turn influenced by the activity of circulating XO.

Expression of XOR in arterial endothelial cells increases in response to hypoxia and inflammatory cytokines stimuli [39]. Once secreted, the circulating XO can bind to glycosaminoglycans (GAGs) on the surface of endothelial cells, leading to localised amplification of XO activity [40, 41]. XOR is converted to XO especially during inflammation and ischaemia-reperfusion injury. Ischaemia is a condition characterised by limited oxygen supply, while reperfusion refers to the restoration of blood flow and oxygen to tissues. During ischaemia, a rise in calcium levels activates calpain protease, which irreversibly converts XDH to XO through limited proteolysis [42]. Furthermore, under these conditions, levels of hypoxanthine also increase due to the release of ATP into the extracellular space [43]. Although XO is not the only molecule that causes vascular damage due to oxidative stress, it is of particular interest because it is a known druggable target.

Levels of ROS also continue to rise during the reperfusion stage as XDH is oxidatively converted to XO. The level of oxygen tension influences the type of ROS that forms, with superoxide being the predominant ROS species at higher oxygen tension while more hydrogen peroxide is produced as hypoxia worsens. Increases in hydrogen peroxide have far-reaching physiological effects since it is a significant regulator of signalling pathways [44, 45].

XDH too may contribute to ROS levels. Although oxygen is not a preferred substrate of XDH, it may still be reduced by XDH under conditions of ischaemia/hypoxia when NAD^+ levels are characteristically low due to a decrease in mitochondrial oxidation of NADH (**Figure 6**) [40].

Superoxide may cause further damage by reacting with hydrogen peroxide or nitric oxide. Even though H_2O_2 is not a very potent ROS, in the presence of iron or copper catalysts it may react with $O_2^{\bullet-}$ to produce the more reactive hydroxyl radicals ($OH^{\bullet}$) via the Fenton reaction [46]. Superoxide may also react with nitric oxide ($NO^{\bullet}$), which produces peroxynitrite ($ONOO^-$). The latter is a very strong oxidising agent that is capable of damaging biomolecules, including aconitase, Hsp90, and mitochondrial membrane components [47–49].

Polyunsaturated fatty acids are highly susceptible to lipid peroxidation by ROS that attack polyunsaturated lipids to produce highly reactive lipid peroxy radicals and 4-hydroxyononenal [50]. These reactive species can then react with DNA and membrane proteins, leading to changes in the fluidity and permeability of the lipid bilayer [51]. ROS have been linked to neurodegenerative disorders and acute neurological diseases, including strokes and epilepsy [52, 53]. This may be due to the production of $OH^{\bullet}$ radicals via the Fenton reaction, as the brain has a high concentration of iron and copper. Research suggests that XO contributes to brain injury and stroke [53].

Reactive nitrogen species (RNS) are a group of nitrogen-based compounds that function as highly reactive free radicals. This includes nitrogen dioxide, dinitrogen trioxide, and peroxynitrite ($ONOO^-$). The $NO^{\bullet}$ and $O_2^{\bullet-}$ that may both be generated by XOR, can react with each other to produce $ONOO^-$ [32, 54]. This leads to the rapid drop in the available amount of $NO^{\bullet}$. Since $NO^{\bullet}$ is a molecule that protects

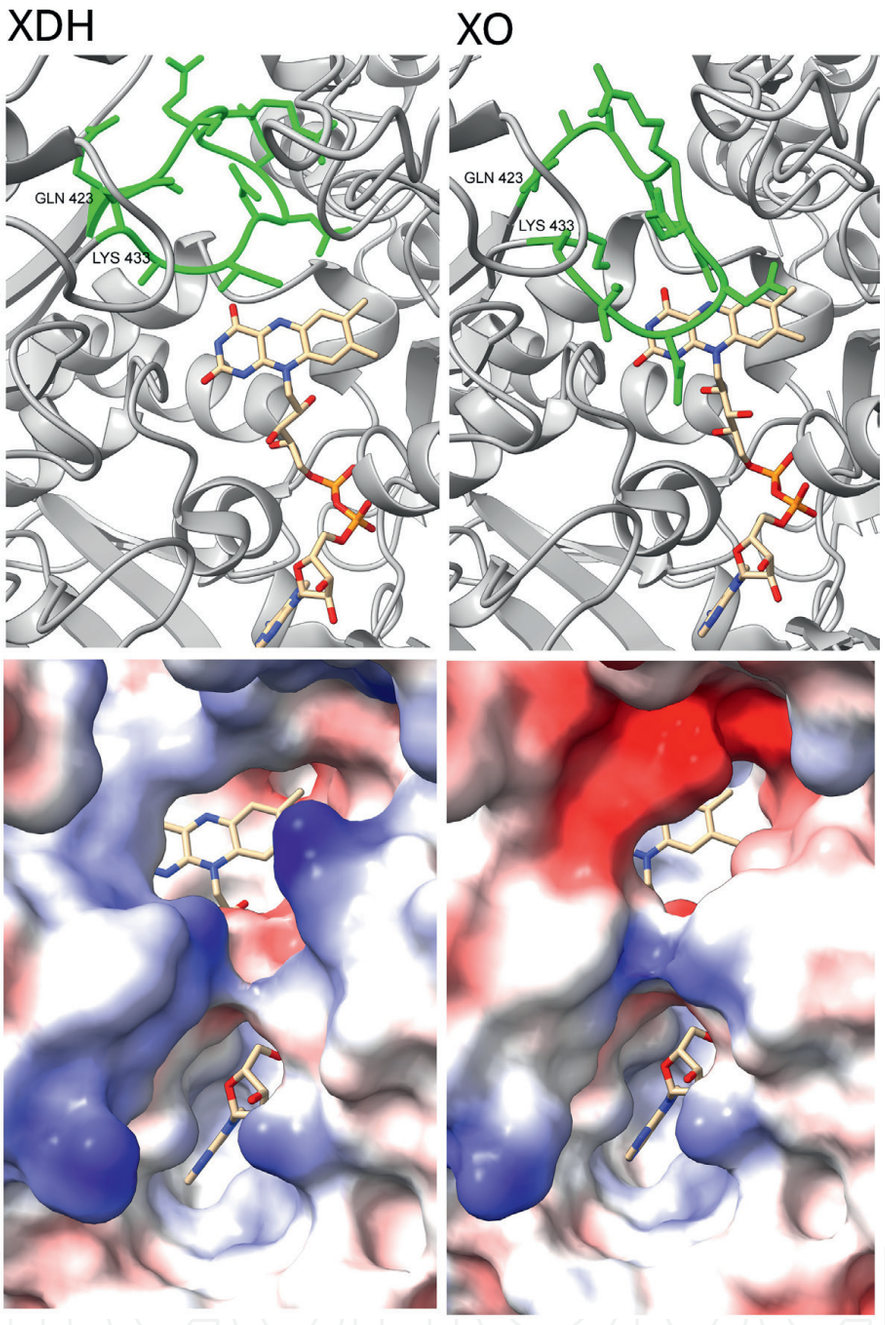

Figure 5.
The electrostatic environment around the XOR FAD domain in XDH (left panels) and XO (right panels). Top: The Gln423-Lys433 loop (green) with respect to the FAD where the loop covers the FAD's quinone moiety in XO (right panel). Bottom: Electrostatic surface around the FAD where blue and red regions represent electropositive and electronegative regions respectively. XDH contains more electropositive areas surrounding the FAD and larger active site pores, allowing both oxygen and NAD^+ to enter the active site. XO contains more electronegative areas around FAD and narrower pores, preventing the NAD^+ approach.

the cardiovascular system, its deficiency may contribute to various cardiovascular diseases such as atherosclerosis, hypertension, and coronary artery disease [55].

The production of $ONOO^-$ is linked to the location where $O_2^{\bullet-}$ is produced. This is because $O_2^{\bullet-}$ has a shorter half-life and is less able to diffuse than $NO^{\bullet}$. Although $ONOO^-$ has a very brief half-life of approximately 10 ms under normal pH conditions, it can still permeate cell membranes and interact with molecules in neighbouring cells [56]. In fact, the impact of $ONOO^-$ can be felt up to a distance of 20 μm from its origin [49].

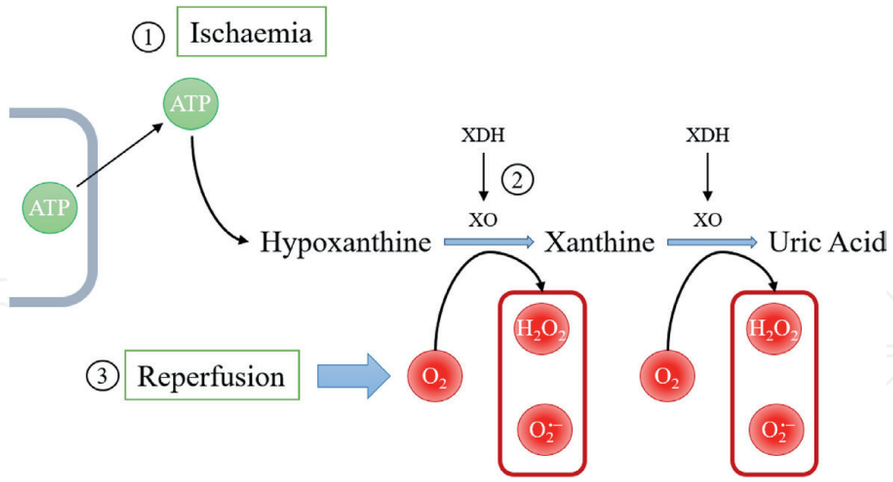

Figure 6.
Ischaemia-reperfusion effects on XOR action. 1. During ischaemia ATP is released into the extracellular space where it is catabolised to adenosine by membrane bound enzymes. Adenosine is further catabolised to hypoxanthine. 2. XDH is converted to XO due to limited proteolysis during ischaemia or oxidation during reperfusion. 3. Oxygen supply is back during reperfusion which can act as an electron acceptor producing reactive oxygen species marked in red.

The highly reactive and oxidative $ONOO^-$ anion may damage cellular structures such as mitochondria by inactivating manganese superoxide dismutase [57]. $ONOO^-$ also oxidises cysteine and nitrates tyrosine residues in membrane channels, including calcium pumps, which impairs cellular ionic balance [58]. Additionally, $ONOO^-$ induces DNA double-strand breaks that can cause cell death and reduces the amount of antioxidants like cysteine, which consequently leads to an increase in free-radical damage [59, 60].

6. Uric acid

Impaired kidney urate excretion is a common cause of hyperuricemia as are excessive alcohol consumption, high protein diet and chemotherapy. The underlying inflammatory state that is maintained by chronic hyperuricemia (6.8 mg/dl) exacerbates the symptoms of metabolic syndrome and is associated with type 2 diabetes, hypertension and kidney inflammatory disease [61–63]. This urate-induced inflammatory response is also a risk factor for cardiovascular disease including stroke and heart failure.

The association of low, but not high, urate levels with neurological disorders such as multiple sclerosis, Parkinson's and Huntington is curious as in these pathologies, hyperuricemia was in fact, deemed responsible of slower progression of the disease [64, 65]. It is hypothesised that the antioxidant behaviour of urate as a scavenger of superoxide radicals is neuroprotective [66, 67].

7. XOR in cardiovascular disease and hypertension

Evidence shows that elevated levels of XOR and its associated oxidative stress are risk factors of cardiovascular disease, causing endothelial damage, hypertension and affecting vascular tone. In particular, the protein damage due to the nitration of tyrosine residues by peroxynitrite has been implicated in the pathophysiology of

cardiovascular disease [68]. Whether hyperuricaemia contributed to cardiovascular disease, especially hypertension, has been a controversial topic. Evidence suggests that the accumulation of intracellular urate may be associated with hypertension due to an increase in oxidative stress and inflammation [69]. while extracellular urate may cause calcification of blood vessels [69, 70]. Recent studies by Yoshida et al. and Furuhashi et al. conclude that serum levels of XOR are positively and independently associated with hypertension via the oxidative stress it forms [71, 72]. A number of polymorphisms in the XDH gene that increase enzyme activity such as G172R, A932T, N1109T have been linked to hypertension and related morbidities [73].

8. XOR and cancer

Although the level of XOR expression varies in different cancer types, dysregulated XOR expression and alterations in activity have been observed in various cancers and appear to be closely associated with clinical outcomes [74, 75]. Due to the biochemical complexity of XOR, it is not easy to predict or determine its involvement in cancer development and progression nor whether XOR inhibitors may be beneficial in prevention. The activity of XOR is greatly influenced by its microenvironment, its isoform state, cofactor availability, its broad substrate specificity and its multiple catalytic properties as a xanthine dehydrogenase, a xanthine oxidase, a NADH oxidase and nitrite/nitrate reductase. There is also the issue of which and how much product is formed as some may have pro- while others may have anti-tumorigenic potential. The final products of XDH are uric acid and NADH, although superoxide and hydrogen peroxide may be formed when oxygen is limited. XO generates uric acid, superoxide, hydrogen peroxide and when in the presence of nitrites/nitrates, nitric oxide. To complicate matters further, not only are these ROS capable of directly causing cellular damage, but both also serve as signalling molecules that affect tissue physiology [76–78].

The irreversible degradation of purines by XOR limits the formation of nucleotides by the salvage pathway which in turn limits cellular proliferation. The expression of XOR responds to various signals that include oxygen tension and inflammatory cytokines [79]. In breast, ovarian, gastric, lung and colorectal cancer tissue, significant down-regulation of XOR expression was observed in advanced, aggressive cancer stages. The loss of XOR expression is suggested to be an independent predictor for poor patient outcomes [80–84]. In fact, the situation is different during early stages of oncogenic transformation.

Xu et al. report that alternol, a natural compound, can effectively trigger a significant response of ROS and cell death through activating XO. This activation occurs when alternol interacts with the catalytic molybdenum-binding domain of XOR, without interfering with the FAD cofactor. According to computational analysis, alternol interacts with the catalytic domain of the XOR protein and enhances its oxidative activity, leading to an increase in XOR activity and proteolytic processing in prostate cancer cells, but not in benign cells. This might provide novel insights on how to treat cancer without harming other tissues [85].

9. XOR inhibitors

XOR inhibitors (XOI) that reduce elevated serum urate levels in the treatment of gout, also curb oxidative stress and increase adenosine triphosphate levels. These

drugs are prescribed when the concentration of urate in the bloodstream is 6.8 mg/dl or more [86]. Allopurinol, a purine analogue, has been on the market since 1956 and is the most prescribed XOI in clinical settings and is administered for the treatment of gout and the prevention of tumour lysis syndrome as a consequence of cancer chemotherapy. The rapid increase in uric acid levels characteristic of tumour lysis syndrome may precipitate death. Allopurinol is effective and relatively inexpensive, being a non-specific competitive and suicide inhibitor of XOR. XOR transforms allopurinol into oxypurinol, which binds to the Moco site's Mo(IV) thereby inhibiting catalysis of the hypoxanthine/xanthine substrates [87]. Allopurinol may cause various side effects such as nephropathy, hepatitis, Stevens-Johnson syndrome, and allopurinol hypersensitivity syndrome. A second XOI is febuxostat. This is a non-purine specific inhibitor that binds tightly to the Moco site of both forms of XOR and is more effective than allopurinol at lowering serum urate. The common side effects of febuxostat include diarrhoea, nausea, and elevated levels of liver enzymes. The main disadvantage of febuxostat is its cost, and therefore, it is considered a second choice after allopurinol.

The third XOI is topiroxostat. This a non-purine analogue that exhibits mechanistic properties of both allopurinol and febuxostat. It initially binds in a competitive manner instead of the substrate to the XOR active site after which it binds covalently with the molybdenum and blocks the Moco site. The dissociation of topiroxostat from the Moco site has a prolonged half-life of 20 hours [88, 89]. Although allopurinol and febuxostat have been approved for use in the United States, European Union, and Japan, topiroxostat is currently only approved for use in Japan.

10. Conclusion

Despite being an evolutionally ancient and highly conserved enzyme that has been studied since the early 1900s, xanthine oxidoreductase does not fail to surprise [90]. What has clearly emerged over these years is that XOR is a biomolecule with many options. As an enzyme, XOR is multifunctional protein with a broad substrate specificity. This not only permits it to catalyse the final two consecutive hydroxylation steps in the degradation pathway of purines but also to detoxify many other endogenous and exogenous molecules. The XOR protein itself can exist in two forms, XDH which may be converted post-translationally, in a reversible or irreversible manner, to XO in response to the microenvironment, oxygen tension and various other signals. This makes it versatile and capable of adapting rapidly to physiological signals. Even expression of its gene is tightly regulated by physiological stimuli such as inflammatory cytokines. Although both isoforms conduct the same reactions at the Moco site to produce uric acid, XO is sufficiently structurally distinct at the FAD domain to only permit the binding of oxygen as the final electron acceptor at this active site. This results in the generation of superoxide and hydrogen peroxide reactive species rather than NADH. These ROS in turn, exert diverse physiological effects that include amongst others anti-microbial activity, wound healing, proliferation, cellular ageing. The versatility of XOR catalysis includes a nitrite reductase activity that results in the formation of nitric oxide that is an essential signalling molecule in vascular physiology.

The complicated enzymatic profile of XOR does benefit many physiological systems but when dysregulated may result in pathological outcomes. The levels of ROS may damage endothelial cells, may form highly oxidative peroxynitrite and hydroxyl

radicals, and may disrupt cellular pathways, cause inflammation and all its ensuing downstream effects. Changes in expression and activity are influenced by external factors including diet, alcohol and drugs while clinically relevant polymorphisms have been associated with hypertension.

The close relationship between XOR activity and certain pathophysiologies including cancer, metabolic syndrome and hypertension highlights the potential of XOR as a biomarker and as a therapeutic target.

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