Pharmacological Reports 67 (2015) 669–674

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Review article

A new paradigm for XOR-catalyzed reactive species generation in the endothelium

Eric E. Kelley

University of Pittsburgh, School of Medicine, Department of Anesthesiology and Vascular Medicine Institute, Pittsburgh, USA

ARTICLE INFO

Article history:
Received 19 February 2015
Received in revised form 6 May 2015
Accepted 8 May 2015
Available online 23 May 2015

Keywords:
Nitrite
Xanthine oxidoreductase
Nitric oxide
Inflammation
Hypoxia

ABSTRACT

A plethora of vascular pathology is associated with inflammation, hypoxia and elevated rates of reactive species generation. A critical source of these reactive species is the purine catabolizing enzyme xanthine oxidoreductase (XOR) as numerous reports over the past 30 years have demonstrated XOR inhibition to be salutary. Despite this long standing association between increased vascular XOR activity and negative clinical outcomes, recent reports reveal a new paradigm whereby the enzymatic activity of XOR mediates beneficial outcomes by catalyzing the one electron reduction of nitrite (NO_2^-) to nitric oxide ($^{\bullet}NO$) when NO_2^- and/or nitrate (NO_3^-) levels are enhanced either *via* dietary or pharmacologic means. These observations seemingly countervail numerous reports of improved outcomes in similar models upon XOR inhibition in the absence of NO_2^- treatment affirming the need for a more clear understanding of the mechanisms underpinning the product identity of XOR. To establish the micro-environmental conditions requisite for *in vivo* XOR-catalyzed oxidant and $^{\bullet}NO$ production, this review assesses the impact of pH, O_2 tension, enzyme–endothelial interactions, substrate concentrations and catalytic differences between xanthine oxidase (XO) and xanthine dehydrogenase (XDH). As such, it reveals critical information necessary to distinguish if pursuit of NO_2^- supplementation will afford greater benefit than inhibition strategies and thus enhance the efficacy of current approaches to treat vascular pathology.

Contents

Introduction

In primates, the molybdopterin-flavin enzyme xanthine oxidoreductase (XOR) catalyzes the final steps in purine catabolism; oxidation of hypoxanthine to xanthine and xanthine to uric acid. Although XOR is reported to be expressed in numerous human tissues including the lung, kidney and myocardium, the greatest XOR specific activity is localized in the splanchnic system [1]. Several inflammatory cytokines (TNF-α, IL-1β, IFN-γ) as well as hypoxia are noted to induce XOR expression. In particular, vascular endothelial cells respond to hypoxia, cytokine stimulation

E-mail address: ekelley@pitt.edu.

http://dx.doi.org/10.1016/j.pharep.2015.05.004
1734-1140/

and shear stress by upregulating XOR and exporting the enzyme to the circulation [1,2]. Structurally, XOR is a 295 kD homodimer with each subunit composed of four redox centers: a molybdopterin cofactor (Mo-co), a flavin adenine dinucleotide (FAD) and two iron-sulfur centers (Fe/S). The Mo-co is comprised of a pterin derivative and one Mo atom which is pentacoordinated with a dithiolene, two oxygen atoms and a sulfur atom. The Mo-co is the site of hypoxanthine and xanthine oxidation whereas NAD^+ and O_2 reduction occur at the FAD. The two Fe/S clusters provide the conduit for electron flux between the Mo-co and the FAD. Distinguishable by their specific electron paramagnetic resonance (EPR) spectra, these Fe/S centers are both of the ferredoxin type but are not identical [3–5].

Xanthine oxidoreductase is transcribed as xanthine dehydrogenase (XDH) where its substrate-derived electrons reduce NAD^+ to NADH, Fig. 1. However, during hypoxia/inflammation, reversible oxidation of critical cysteine residues (535 and 992) and/or limited proteolysis converts XDH to xanthine oxidase (XO) [6]. In the oxidase form, affinity for NAD^+ at the FAD is greatly diminished while affinity for oxygen is enhanced resulting in univalent and divalent electron transfer to O_2 to generate $O_2^{\bullet-}$ and hydrogen peroxide (H_2O_2), respectively, Fig. 1 [7]. Therefore, when situated at critical sites in the tissue and vasculature, XO can serve as an abundant source of ROS mediating alterations in vascular function by reducing $^{\bullet}NO$ bioavailability *via* direct reaction with $O_2^{\bullet-}$ ($^{\bullet}NO + O_2^{\bullet-} \rightarrow ONOO^-$) or indirectly, by mediating redox-dependent cell signaling reactions [8–10].

Oxidant formation

In the literature XO is rarely recognized as a direct source of H_2O_2 and mainly referred to as a source of $O_2^{\bullet-}$ with H_2O_2 formation resulting from reaction of $O_2^{\bullet-}$ with superoxide dismutase (SOD) or spontaneous dismutation. However, XO is actually a H_2O_2-producing enzyme that also produces some $O_2^{\bullet-}$ under physiological conditions. For example, achievement of 100% $O_2^{\bullet-}$ production from XO requires 100% O_2 and pH 10 whereas at pH 7.0 and 21% O_2, XO generates ~25% $O_2^{\bullet-}$ and ~75% H_2O_2 [11]. At normal pH and under hypoxia, XO-catalyzed H_2O_2 increases approaching 90–95% of total electron flux though the enzyme [12]. Thus, under conditions such as ischemia and/or hypoxia, where both O_2 tension and pH are reduced, H_2O_2 formation is favored suggesting that XOR may participate in the numerous signaling cascades where H_2O_2 has been implicated [13,14]. While the post-translational conversion of XDH to XO has become synonymous with the conversion from a "good" housekeeping enzyme to a "bad" ROS-producing enzyme, it is critical to recognize that XDH can also reduce O_2 and generate ROS. For example, ischemia/hypoxia either localized, regional or systemic mediates O_2-dependent alterations in cellular respiration leading to decreased mitochondrial NADH oxidation and thus significantly decreases NAD^+ concentration [15]. Although NAD^+ is the preferred electron acceptor for XDH, when levels of this substrate are low XDH will utilize O_2 and thus care should be taken not to exclusively associate XDH with the form of XOR that does not produce ROS [16]. In the aggregate, XO is primarily a source of H_2O_2 that also produces $O_2^{\bullet-}$ while, under certain circumstances, both forms of the enzyme are capable of producing oxidants.

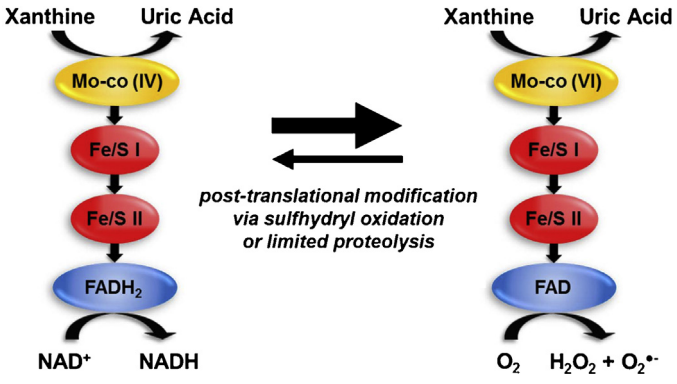

Fig. 1. Xanthine oxidoreductase reactions. For XDH, xanthine is oxidized to uric acid at the Mo-co and electrons transferred *via* 2 Fe/S centers to the FAD where NAD^+ is reduced to NADH (*left*). For XO, xanthine is oxidized to uric acid at the Mo-co and electrons are transferred to the FAD where O_2 is reduced to $O_2^{\bullet-}$ and H_2O_2 (*right*). Conversion from XDH to XO is mediated by post-translational modification.

XOR-endothelium interaction

Intravenous administration of heparin in the clinic and in animal models of vascular disease results in elevated XO activity in the plasma suggesting XO is bound to the vascular endothelium [17–19]. During ischemic/hypoxic conditions XDH released into the circulation is rapidly (<1 min) converted to XO and subsequently binds to negatively charged glycosaminoglycans (GAGs) on the surface of endothelial cells [18,20]. Although XO exhibits a net negative charge at physiological pH, pockets of cationic amino acid motifs on the surface of the protein confer high affinity for GAGs (K_d = 6 nM) [18,21–23]. Sequestration of XO by vascular endothelial GAGs amplifies local XO concentration and significantly alters XO kinetic properties. For example, when compared to XO free in solution, GAG-immobilized XO demonstrates an increased K_m for xanthine (6.5 *vs.* 21.2 μM) and an increased K_i for allo/oxypurinol (85 *vs.* 451 nM) [23]. In addition to affecting kinetic properties at the Mo-co, binding of XO to GAGs confers alterations to the FAD resulting in reduction of $O_2^{\bullet-}$ production by 34% and thus elevation of H_2O_2 formation [23]. Combined, XO-GAG interaction results in: (1) diminished affinity for hypoxanthine/xanthine, (2) resistance to inhibition by the pyrazalopyrimidine-based inhibitors allo/oxypurinol and (3) diminished $O_2^{\bullet-}$ production and thus enhanced H_2O_2 generation. This vascular milieu where XO is sequestered on the surface of the endothelium is prime for prolonged enhancement of oxidant formation that is partially resistant to inhibition by the most commonly prescribed clinical agents.

Nitrite reductase activity

For over 40 years, dogma dictates that elevation of XO activity during hypoxia/ischemia/inflammation equates to increased XO-derived ROS generation and ultimately to poor clinical outcomes. However, recent reports have proposed a paradigm shift by demonstrating XO-mediated formation of salutary $^{\bullet}NO$ under similar pathologic conditions. Indeed, under anoxic conditions and acidic pH, XO demonstrates a nitrite reductase activity by catalyzing the reduction of NO_2^- to $^{\bullet}NO$ in the presence of either xanthine or NADH as reducing substrates (sources of electrons) [24–27]. The Mo-co has been identified as the site of NO_2^- reduction where xanthine oxidation directly reduces the cofactor; alternatively, NADH can indirectly provide reducing equivalents *via* electron donation at the FAD with subsequent retrograde flow to the Mo-co, Fig. 2. This catalytic activity has also been demonstrated is tissue homogenates in the presence of xanthine or aldehyde [28]. In addition to *in vitro*/*ex vivo* biochemical studies, diminution or ablation of NO_2^--mediated beneficial effects upon co-treatment with allo/oxypurinol has been observed *in vivo* suggesting XOR involvement as a NO_2^- reductase. For example, XOR inhibition has diminished protective effects mediated by NO_2^- therapy both clinically and in animal models of intimal hyperplasia following vessel injury [29], acute lung injury and