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Involvement of Reactive Oxygen Species in Hypertension: Its Roles, Production and Therapeutic Strategies

Hongzhuan Yin[1,2], Long Liao[2,3] and Jun Fang[2,4,5*]

[1]Department of General Surgery, Sheng Jing Hospital, China Medical University, Shenyang City, Liaoning Province, 110004, China.
[2] Institute of DDS, Sojo University, Ikeda 4-22-1, Nishi-Ku, Kumamoto 860-0082, Japan.
[3]Department of Applied Microbial Technology, Faculty of Biotechnology & Life Science, Sojo University, Japan.
[4]Laboratory of Microbiology & Oncology, Faculty of Pharmaceutical Sciences, Sojo University, Japan.
[5]School of Public Health, Anhui Medical University, 81th Meishan Road, Hefei City, Anhui Province, 230032, China.

Authors' contributions

This work was carried out in collaboration between all authors. Authors HY and LL wrote the manuscript and prepared the figures. Author JF designed the study and edit the manuscript. All authors read and approved the final manuscript.

Mini-review Article

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ABSTRACT

Hypertension is a major risk factor to human health. Many factors are known to involved in the pathogenesis and progression of hypertension, among which overproduction of reactive oxygen species (ROS) is closely associated with it in part by impairing endothelial function. In our laboratory, we found that ROS exert an important biological effect on the regulation of normal physiological responses of the cardiovascular system and the pathogenesis of hypertension. Namely, superoxide and hydrogen peroxide are over-produced under various pathological states which subsequently reduce the bioavailability of endothelium-derived nitric oxide, the vital molecule to maintain vasorelaxation. Understanding the roles of ROS in hypertension is thus important to develop new therapeutic strategies for the control of hypertension. The present review addresses the putative function of ROS in the pathogenesis of hypertension and focuses

**Corresponding author: Email: fangjun@ph.sojo-u.ac.jp;*

on the therapeutical potentials of the inhibitors of Xanthine oxidase that is a main source of ROS in diseased inflammatory conditions including hypertension.

Keywords: Reactive oxygen species; hypertension; Xanthine oxidase; NADPH oxidase.

1. INTRODUCTION

Hypertension is a major risk factor for renal failure, cardiovascular disease and stroke [1,2]. Although the exact etiology of hypertension still remains largely unknown, it is clear that hypertension is a multi factorial, complicate polygenic disorder with many interacting mechanisms contributing to its pathophysiology and involving many organ systems, such as the heart, kidney, brain, vessels and even the immune system [3,4]. A common pathological phenomenon in the processes of hypertension is the increased oxidative stress due to excess reactive oxygen species (ROS) production, reduced nitric oxide (NO) levels and its bioavailability and decreased antioxidant capacity in the cardiovascular system [5,6], which may impair endothelial function of vascular systems that partly contributes to the pathogenesis of hypertension [7-9].

ROS such as superoxide anion ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) are potentially injurious metabolic cellular by-product. It is now known to play important physiological actions at physiological low or moderate levels, including the induction of host defense genes [10], stimulation of transcription factors [11-13] and activation of ion transporters [14]. However, ROS is a double-edged sword, at high concentrations, it react readily with proteins, lipids, carbohydrates and nucleic acids, often inducing irreversible functional alterations or even complete destruction. When ROS were initially integrated into biomedical concepts it was thought that they would cause exclusively toxic effects and are associated with pathologies of many diseases.

In view of the roles of ROS in hypertension, the first literature was published in 1960s [15]. And in the past decade, more and more attentions were paid in this field, the behaviors, sources of ROS and related mechanisms in hypertension were extensively clarified. Rajagopalan et al. showed increased vascular ROS production in Angiotension II (Ang II) – mediated hypertension in rats probably via non-phagocytic NADPH oxidase activation [16]. ROS-generating enzymes deficient mice showed lower blood pressure versus wild-type counterparts and Ang II infusion fails to induce hypertension in these mice [17,18]. These findings are thus greatly helpful to develop new therapeutics for hypertension by focusing on the production and/or elimination of ROS. In this review, we summarized the implications of ROS in hypertension, especially focusing on a therapeutic strategy by modulating ROS production.

2. THE SOURCE OF ROSVASCULAR SYSTEMS

Major sources of ROS include cellular respiration and metabolic processes, though ROS may also be generated by radiation [19]. During the process of cellular respiration, oxygen is reduced by the successive transfer of single electrons and the intermediates with odd electrons can escape the chain. Metabolic processes also generate ROS; for instance, the peroxisome catabolizes bio-molecules using enzymes that remove hydrogen in an oxidative reaction, thereby creating H_2O_2. Moreover, some ROS-generating enzymes are known for example, NADPH oxidase (NOX) that is one of the major sources of ROS in the body and its

primary function is to produce ROS as an important host defense system [20,21]. Other ROS generating enzymes include Xanthine oxidase (XO), Nitric oxide synthase (NOS), p450 cytochromes and some organelles (mitochondria, peroxisomes) can also produce ROS as metabolic byproducts [22].

2.1 NOX

NOX is a family of membrane-bound proteins that function to transfer electrons across membranes, where the final electron receptor is oxygen and $O_2^{\cdot-}$ is generated. To date, seven isoforms of NOX were identified and these enzymes are known to participate in cellular differentiation, growth, proliferation, apoptosis, cytoskeletal regulation, migration and contraction, mostly via the redox-dependent signaling processes [23]. Though NOX plays important roles in host defense as described above [20,21], it is also known to be involved in many diseases.

Regarding cardiovascular diseases, it is clear that vascular cells simultaneously express multiple NOX enzymes and changes in expression of NOX are associated with the pathogenesis of cardiovascular diseases [24-27]. Namely, in vascular smooth muscle cells from large arteries, NOX1 is required for migration, hypertrophy and proliferation [24]; NOX4 mediates differentiation [25] and NOX1 and 2 are implicated in hypertension [26,27]. The implication of NOX in hypertension was also verified in genetic studies from mice, rats, as well as humans: NOX1-deficient mice have decreased basal blood pressure and they are less responsive to Ang II; spontaneously hypertensive rats have a polymorphism in the promoter of the $p22^{phox}$ gene, which leads to an over expression of this NOX subunit and a subsequent increase in ROS [28]; humans homozygous for a polymorphism in the gene encoding $p22^{phox}$ showed reduced oxidative stress in the vascular system and probably related to the reduced blood pressure [28]. All these findings suggested the potential of NOX as a candidate target molecule for hypertension.

2.2 XO

XO is an iron-containing metalloflavoprotein that catalyzes the oxidation of hypoxanthine and xanthine, to uric acid, in which molecular oxygen (O_2) is used as an electron acceptor and ROS including $O_2^{\cdot-}$and H_2O_2 are generated [29]. XO is widely distributed in various tissues, particularly in the intestine, lung and liver [30]. In normal conditions, most XO is present as Xanthine dehydrogenase, with very low $O_2^{\cdot-}$generating activity [31]. However, the expression and activity are significantly increased in the diseased conditions, such as ischemia and infection/inflammation, resulting in the progression of diseases due to the excessive generation of ROS [30-32].

In vascular systems, XO/Xanthine system is one of the major sources of $\bullet O_2^-$generation [33-35]. Because of cationic charge of XO, it has high affinity to the luminal surface of blood vessels [35]. Therefore it is reasonable that the NO-mediated endothelial functions such as vasorelaxation could be regulated by XO in vascular systems, in which $O_2^{\cdot-}$rapidly reacts with NO thus decreasing the bioavailability of NO [36,37]. Based on this view point, we have demonstrated that inhibition of XO potentiated NO-induced vasodilation, both in vitro and in vivo experiments, suggesting the potential of XO inhibitors as antihypertensive agent [38,39], which is discussed in details below. A new therapeutic strategy may thus be developed for hypertension as well as other vascular diseases by modulating the activity of XO and thus ROS generation. Diagrammatic illustration of the association of XO/ROS in hypertension is summarized in Fig. 1.

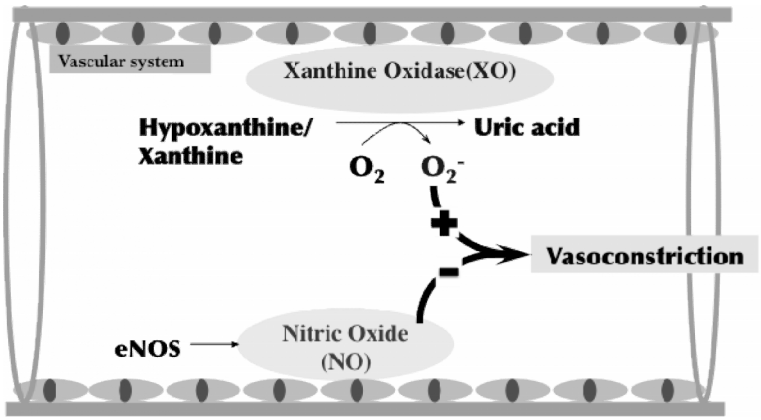

Fig. 1. A mechanism of XO-mediated vasoconstriction via generating $O_2^{\cdot-}$ and decreasing the bioavailability of NO.

Under pathological conditions such as injury of endothelial cells and inflammation, the expression and activity of XO is highly up regulated, resulting in the burst of $O_2^{\cdot-}$ generation which will reacts with NO rapidly. Consequently, the decrease of circulation NO induces vasoconstriction that may result in hypertension.

3. ROLES OF ROS IN HYPERTENSION AND THE MECHANISMS INVOLVED

With regard of the mechanisms ROS involved in hypertension, $O_2^{\cdot-}$ is known the major effector, which induces vascular dysfunction in hypertension by reacting with NO as described above [35-37]. This notion was also supported by in vivo studies, e.g., Banday et al. showed that high-salt intake, along with L-buthionine sulfoximine treatment, reduced the NO levels and endothelial NO synthase activity thus causing vascular dysfunction in rats [40]. Similarly, it was reported that Ang II induced ROS production thus triggering vascular contractions in mesenteric arteries of Wistar rats, which were reversed by atorvastatin treatment possibly via inhibition of NOX1-induced ROS [41]. Lavi et al further described the association of local oxidative stress with reduced NO bioavailability and the consequent coronary endothelial dysfunction in human [42].

According to this knowledge, the importance of effective antioxidant systems was thus realized in vascular diseases such as hypertension. In superoxide disumutase (SOD3) knockout animals, Ang II infusion increased $O_2^{\cdot-}$ generation and reduced endothelium-dependent vasodilatation in small mesenteric arterioles [43]. Similar results were described by Qin et al showing that reduced SOD3 activity caused by mutation of copper transporter Menkes ATPase led to impairment of ACh-induced endothelium-dependent vasorelaxation [44]. Moreover, deletion of glutathione peroxidase 1, an enzyme that metabolizes H_2O_2 to water, augmented Ang II–induced vasoconstriction [45]. In contrast, over expression of human thioredoxin 2, a mitochondrial-specific antioxidant enzyme, attenuated the generation of $O_2^{\cdot-}$, H_2O_2 induced by Ang II and significantly reduced NOX2 expression [46].

Though large numbers of studies suggested that ROS induced by Ang II is tightly associated with hypertension, recent studies indicated that Mineralocorticoid receptor (MR)-induced ROS was implicated in hypertension as well. Savoia et al. showed that MR antagonist eplerenone significantly reduced arterial wall stiffness, medial collagen: Elastin ratio and circulating inflammatory mediators in hypertensive patients [47]. Similarly, MR antagonist spironolactone protected Ren2 rats from vascular apoptosis and injury via NOX-dependent

mechanisms [48]. Along this line, in endothelial cells, aldosterone treatment increased ROS generation by trans locating p47phox to the membrane from the cytosol; eplerenoneor knockdown of p47phox reversed the reduced NO levels and the reduction in endothelial NO synthase Ser 1177 phosphorylation [49]. Likewise, pretreatment of eplerenone ameliorated aortic endothelial dysfunction during myocardial infarction in rats, in part because of normalization of NO bioavailability by reducing p22phox expression and aortic ROS generation and restoration of endothelial NO synthase phosphorylation [50].

One of the critical pathological changes of hypertension is hypertrophy or thickening of the heart muscle. Similar to the central nervous, renal and vascular systems, many ROS sources participate in cardiac hypertrophy and remodeling and various antioxidant systems play important roles in reducing hypertrophic conditions [51]. For example, glutathione peroxidase 1 prevented cardiac hypertrophy in Ang II–dependent hypertension; glutathione peroxidase 1 knockout mice exhibited accelerated cardiac hypertrophy and dysfunction [52]. Carbon monoxide, one of the end products of an important antioxidative enzyme heme oxygenase 1, inhibits Ang II–induced left ventricular hypertrophy by reducing the expression of p47phox, p67phox and ROS generation [53]. Interestingly, it was also reported that inhibition of mitochondrial ROS by MitoQ treatment reduced hypertrophy in stroke-prone SHRs [54].

Cross-talk between Ang II and MR is also observed in cardiac hypertrophy and remodeling. In transgenic mice with conditional, cardiomyocyte-restricted over expression of human MR, Ang II infusion caused a greater increase in left ventricle mass/body weight than in wild-type mice [55]. These effects are associated with increased expression of hypertrophic markers (collagen and fibronectin) and NOX2 [55]. Similarly, in uremic rats, MR antagonist spironolactone attenuated left ventricular hypertrophy, cardiac $O_2^{\cdot-}$ production and NOX2, NOX4 and p47phox expressions, which indicated a direct role for MR in cardiac hypertrophy.

4. THERAPEUTIC STRATEGY FOR HYPERTENSION BY MODULATING ROS

Because of the vital roles of ROS in the pathogenesis of various diseases including hypertension, modulating ROS is thus considered a reasonable therapeutic strategy for hypertension as well as other ROS-related diseases. To control ROS, there are commonly two approaches; one is to elucidate ROS by use of antioxidants such as Vitamin C, phenol compounds, flavoid etc and antioxidative enzymes such as superoxide dismutase (SOD) and catalase. Actually, many antioxidants are used in healthy supplements and cosmetics, and SOD or polymer modified SOD were challenged in many ROS related diseases, for example pyran-SOD showed remarkable therapeutic effect in influenza virus infection in mice [56] and lecithinized SOD showed great therapeutic potential to pulmonary emphysema that is a well-known disease related to ROS, which is now under clinical development [57].

Another approach is to target the ROS generating systems, i.e. NOX and XO as discussed above, thus decreasing the generation of ROS from the source of ROS. In this review, we will discuss this approach in details, especially focusing on the major ROS generating enzymes in disease condition, i.e., XO based on our experimental findings.

4.1 NOX Inhibitors

Although multiple sources of ROS contribute to disease development, NOX family appears particularly important and thus NOX could be good drug targets. NOX isoforms are cell and

tissue-specific, having different modes of activation and serving distinct roles. When NOX inhibitors were developed as therapeutics, the possible "on-target" side effects were noted with concerns. For example, inhibition of NOX2 was thought to be undesirable because it might lead to immunodeficiency because of the decreased ROS that serves as important unspecific protection against infections. In the light of recent knowledge, however, these on-target effects are unlikely to be a major concern. In the case of NOX2, partly inhibition of its activity will not induce dire consequences, in fact, very small amounts of ROS generation (~0.1% of normal) is enough to fulfill substantial protection against severe infection [58]. Also, no obvious signs of disease were found in NOX1 and NOX4 knockout mice. These findings suggested that pharmacological inhibition of these NOX should be well tolerated.

The first generation NOX inhibitors were mostly non-specific inhibitors which showed many toxic side effects. For example, the widely-used compound diphenyliodonium (DPI) not only inhibits all NOX enzymes but also other electron transporters as well as the mitochondrial respiratory chain. Thus, new generations of NOX inhibitors with high specificity are developed recently, with less side effects compared to the first generation of NOX inhibitors.

4.2 XO Inhibitors

Compared to NOX, XO may be a more ideal target for ROS related diseases including hypertension, because unlike NOX which constitutively expresses in many normal tissues, XO commonly shows very low expression and activity in normal tissues [30-32,56,59]. But in diseased conditions, such as infections, ischemia-reperfusion injury, inflammation, the expression and activity of XO are significantly up regulated [30-32,56,59]. Inhibition of XO may thus exhibit specific disease-targeted effect, while showing less side effects.

Among various XO inhibitors, allopurinolis well-known and is used in clinic for treating hyperuricemia and its complications, including chronic gout [60], though it was not designed as a ROS blocker. In our laboratory, we first reported the importance of XO-induced ROS in the pathogenesis of virus infection [32,56] and later hypertension [38] and ischemia-reperfusion injury [59]. Accordingly, we developed a potent XO inhibitor 4-amino-6-hydroxypyrazolo[3,4-d]pyrimidine (AHPP), which showed much higher XO inhibitory activity than other XO inhibitors, e.g., 20 times higher than alloxanthine, 3 times higher than allopurinol [38]. More important AHPP exhibited XO inhibition in a dose-dependent manner, whereas allopurinol or alloxanthine might become the substrate of XO to produce ROS at high concentrations [38]. By using AHPP, we found that the AHPP was beneficial for potentiating bioactivity of NO in a dose-dependent fashion, i.e., augmenting NO-mediated relaxation of aortic rings from both rabbits and spontaneously hypertensive rats (SHR) [38] (Fig. 2A). Systemic administration of AHPP significantly reduced the blood pressure of SHR to 70% (Fig. 2B), whereas only transient and moderate decrease in blood pressure was found for allopurinol. These findings strongly suggested the potential of AHPP as the XO inhibitor for the treatment of hypertension.

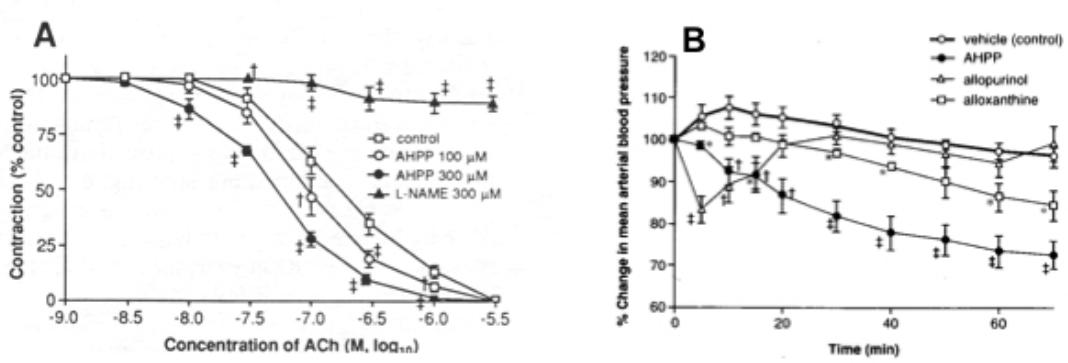

Fig. 2. Effects of AHPP on acetylcholine-induced relaxation of the aorta rings of SHR (A) and on the blood pressure of SHR after intravenous administration (B).

*The relaxation of vascular rings was achieved by acetylcholine (ACh) with or without AHPP (A). In (B), AHPP was injected via the jugular vein, after which mean arterial blood pressure was continuously measured. Data are means ± SE (n=4-8). * + ++ the value with each XO inhibitor is significantly different from the control value without inhibitors: * P<0.05; + P<0.025; ++ P<0.01. Data are from [38] with permission.*

However, AHPP is almost insoluble in physiological solutions, which hamper its in vivo application. Therefore, we further prepared a water soluble polymeric conjugate of AHPP, by using a styrene maleic acid copolymer (SMA, SMA-AHPP) (Fig. 3A) [39]. Compared to native AHPP, the water solubility of SMA-AHPP was greatly improved, with comparable XO inhibitory activity (Ki of 0.25 μM vs 0.17 μM of free AHPP) [39]. In addition, because of the albumin-binding property of SMA, SMA-AHPP will bind to albumin in circulation after systemic administration, thus behaving as a macromolecule [59], which will thus show various beneficial characteristics including prolonged in vivo half-time, AUC (area under concentration/time curve), decreased kidney clearance and selective accumulation in solid tumors and inflammatory tissues because of their unique pathophysiological and anatomical characteristics, a phenomenon coined as EPR effect [61]. Thus, conjugation of SMA-AHPP not only solved the problem of water solubility but it will give advantage in the pharmacokinetic merits of macromolecular drugs after binding with albumin in vivo, consequently resulting in a significant and constant antihypertensive effect in SHR rats, i.e., one injection of SMA-AHPP induced an about 30% decrease of blood pressure and the effect continued to at least 24 h (Fig. 3B). SMA-AHPP is thus considered a candidate drug as the therapeutic for hypertension, as well as other ROS related diseases including inflammation, ischemia-reperfusion injury and microbial infection, which warrants further investigations.

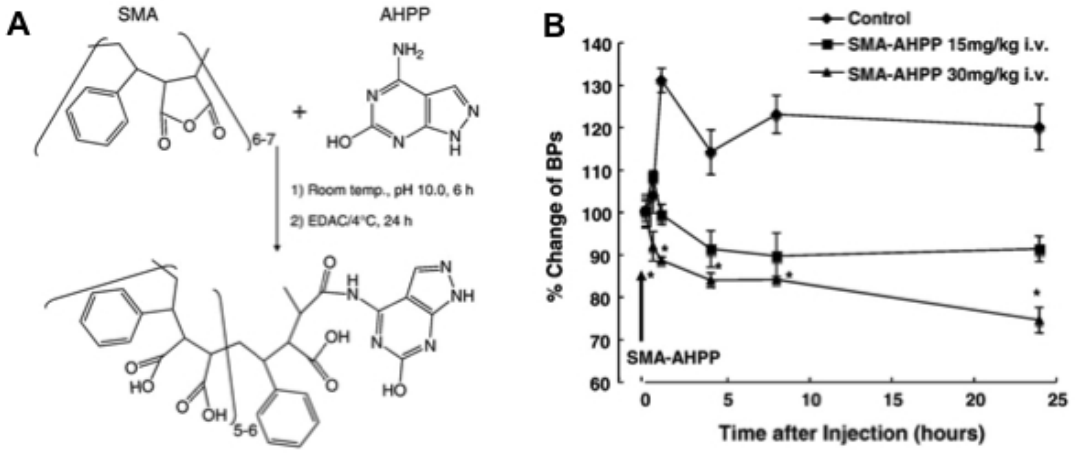

Fig. 3. Schematic representation for the formation of SMA–AHPP conjugate (A) and its in vivo antihypertensive effect after intravenous injection in SHR rats (B).

*The synthesis of SMA-AHPP was carried out by the coupling reaction of SMA with AHPP in the presence of a water soluble carbodiimide (EDAC). One molecule of AHPP is conjugated to each SMA chain containing 6–7 styrene maleic acid repeating units to yield a loading of 10–15% w/w of AHPP. Noted that one injection of SMA-AHPP resulted in a 30% decrease of blood pressure and it continued for at least 24 h (B). Data are expressed as means ± SE (n = 3–4). * P < 0.01 between each treatment group (15 mg/kg or 30 mg/kg i.v.) and the untreated control group. # P<0.05 between SMA–AHPP 100 mg/kg oral group and control group. Data are from [39] with permission.*

5. CONCLUSIONS AND PERSPECTIVE

Upon understanding the roles and sources of ROS in hypertension and many other diseases, new therapeutics for such diseases through modulating ROS is anticipated. As the major sources of ROS, NOX and XO may become the target molecules and development of their inhibitors for clinical use may thus be of importance for drug discovery. We believe this will be an effective strategy for such ROS related diseases including hypertension and promise the development of new anti-ROS and antihypertensive drugs.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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