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Role of oxidative stress in angiotensin-II mediated contraction of human conduit arteries in patients with cardiovascular disease

Valentina O. Püntmann [b,*], Monira B. Hussain [a,c], Manuel Mayr [b], Qingbo Xu [b], Donald R.J. Singer [c]

[a] Department of Pharmacology and Clinical Pharmacology, St. George's, University of London, London, SW17 0RE, UK
[b] Department of Cardiac and Vascular Sciences, St. George's, University of London, London, SW17 0RE, UK
[c] Clinical Pharmacology Section, Division of Clinical Sciences, Warwick Medical School (LWMS), University of Warwick Coventry CV4 7AL, UK

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Abstract

Background: Angiotensin II is a powerful vasoconstrictor involved in the development of high blood pressure and in the regulation of cardiovascular growth. Recent reports have suggested that in addition to the classical pathways involved in transducing responses to receptor activation, formation of reactive oxygen species by angiotensin II may also be involved. We investigated the importance of oxidative stress in angiotensin II induced contraction in human conduit arteries from patients with cardiovascular disease.
Methods and results: Isometric contraction studies using human radial arteries entailed probes modulating the redox-dependent reactions to define the oxidative pathways involved in angiotensin II contraction. In situ oxidative fluorescence was employed to detect immediate superoxide tissue production in radial and internal mammary arteries. Treatment with TEMPOL, human superoxide dismutase, diphenyleneiodonium, oxypurinol, NG-monomethyl L-arginine considerably decreased contractile response to angiotensin II in radial arteries. Similarly, angiotensin II-stimulated arterial superoxide production was reduced in the presence of the above inhibitors. On the contrary, used as controls, norepinephrine vasoconstriction was not associated with increase of superoxide and neither ciprofloxacin nor aminophylline altered basal or angiotensin II induced superoxide generation.
Conclusions: Our findings provide evidence for the role of oxidative pathways in contractile response of human conduit arteries to angiotensin II. Angiotensin II induced superoxide anion production may be mediated by multiple inter-dependent rate-limiting enzymes in both types of artery. Our studies may have important implication for future therapeutic approaches involving inhibition of angiotensin II mediated superoxide generation in hypertension and prevention of cardiovascular disease.
Condensed abstract: We studied the role of oxidant species in contraction responses to angiotensin II in human conduit arteries. Treating radial artery segments with the anti-oxidants with a range of inhibitors, affecting the redox dependent pathways, markedly reduced contraction to angiotensin II. In parallel experiments, oxidative fluorescence was assessed and compared in human radial and internal mammary artery. Angiotensin II induced superoxide anion production may be mediated by multiple inter-dependent rate-limiting enzymes in both types of artery.

Keywords: Angiotensin II; Coronary artery disease; Reactive oxygen species; Superoxide dismutase; Vasoconstriction

1. Introduction

Angiotensin II (Ang-II) is a powerful vasoconstrictor produced by renal and extra-renal synthetic pathways and involved in the development of high blood pressure and in the regulation of cardiovascular growth (Touyz and Schiffrin, 2000). Ang-II is an important modulator of vascular smooth muscle cell (VSMC) biology and many effects of Ang-II are mediated by the generation of reactive oxygen species (ROS) (Touyz and Schiffrin, 2000). Vascular superoxide interacts with nitric oxide (NO) to reduce NO bioavailability and generate the potent oxidative peroxynitrite radical, playing important roles in vascular pathophysiology (Cai and Harrison, 2000; White et al., 1994). In addition to NO scavenging, Ang-II evoked oxidative stress has been implicated in development of potentially proatherogenic actions on VSMC proliferation, inflammatory cell recruitment, and redox-sensitive gene expression (Nickenig and Harrison, 2002; Keidar et al.,

* Corresponding author. Tel.: +44 208 725 5912; fax: +44 208 725 3328.
E-mail address: vgiacome@sgul.ac.uk (V.O. Püntmann).

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1995). However, evidence from animal models suggests that increased ROS production may also contribute to Ang-II-mediated vasoconstriction and hypertension (Kawazoe et al., 2000; Wang et al., 2002).

In human blood vessels, the membrane associated NAD(P)H oxidase was thought to be the principal source of basal and Ang-II induced superoxide and functionally related to cardiovascular risk factors and systemic endothelial dysfunction (Griendling et al., 1994; Berry et al., 2000; West et al., 2001; Huraux et al., 1999). However, the relative non-selectivity of diphenyleneiodonium (DPI) to block NAD(P)H oxidase in above studies implies that many other flavine oxidase dependent enzyme systems may also be important in mediating vascular oxidant stress, including xanthine oxidase and nitric oxide synthase (NOS).

Moreover, the pteridine cofactor tetrahydrobiopterin (BH4) has emerged as a critical determinant of NOS (NOS) activity: when BH4 availability is limiting, NOS no longer produces NO but instead generates superoxide. This phenomenon has been referred to as uncoupling of NOS from producing NO, resulting in superoxide production (Berry et al., 2000; Schmalfuss et al., 1999; Guzik et al., 2002; Griendling et al., 2000).

The vascular signalling of ROS in Ang-II-mediated vasoconstriction of human conduit arteries remains poorly understood. Human internal mammary (IMA) and radial artery (RA) serve as conduits for a coronary artery bypass graft (CABG) that preserve functional patency longer than saphenous vein (SV). Much is known about the biology of human IMA and SV, (Schmalfuss et al., 1999) however, the precise differences in oxidative character of IMA and RA are rather unclear. In this study, we investigated whether oxidant stress does contribute to Ang-II-induced contractions of human conduit arteries by performing functional study of segments of RA and direct visualisation of oxidative status in RA and IMA tissue from patients undergoing routine coronary revascularization.

2. Methods

2.1. Subjects and methods

Twenty patients undergoing routine revascularization surgery for coronary artery disease were invited to participate in the study (16 men, mean age 61±3(SEM)years, BP 142±9/79±3 mmHg, cholesterol 4.5±0.5 mmol/L, glucose 5.8±1.4 mmol/L). All had hypertension and hypercholesterolemia, 16 were smokers and 11 had diabetes mellitus. As expected, patients were on a wide range of cardiovascular drug treatments including statins, nitrates, β-blockers and calcium channel antagonists. For patients on treatment with aspirin or angiotensin converting enzyme (ACE) inhibitors, these drugs were stopped at least 3 days before surgery as a part of standard hospital protocol. None of the patients was treated with AT_1-receptor blockers. The St. George's Hospital Research Ethics Committee approved the study. Written informed consent was obtained from all subjects.

2.2. Vasomotor studies

Vasoconstriction responses to Ang-II were determined as previously described (Harrison et al., 2001; Hamilton et al., 1997). In brief, development of isometric tension of isolated RA was measured in parallel rings from twenty patients. After viability test (KCl, 124 mM) and test for receptor-dependent endothelial responsiveness, vessels were pre-treated for 30 min with DPI (100 μmol/L), xanthine oxidase inhibitor (oxypurinol: 100 μmol/L), NO synthase inhibitor NG-monomethyl L-arginine (L-NMMA: 100 μmol/L), the cell-permeable superoxide dismutase (SOD) mimetic TEMPOL (1 mmol/L), human SOD (50 U/ml), or vehicle (DMSO for DPI, oxypurinol and TEMPOL; saline for L-NMMA and SOD). A single cumulative concentration–response-curve (CRC) to Ang-II (0.1–100 nmol/L was obtained for each vessel ring as contractions to Ang-II are subject to tachyphylaxis (Mulvany and Halpern, 1977). To test the specificity of Ang-II responses, vessels were routinely primed with KCL in the presence of each inhibitor. Using the above single curve design, the effect of TEMPOL (1 mM) was tested for norepinephrine (NE) contractions (0.1–100 nmol/L).

2.3. Oxidative fluorescence microscopy

In situ ROS production was evaluated in parallel vascular cryosections (30 μm) of IMA ($n=10$) and RA ($n=8$) using oxidative fluorescent dye dihydroethidium (DHE) (5 μmol/L) with or without DPI (100 μmol/L), oxypurinol (100 μmol/L), L-NMMA (100 μmol/L), exogenous human SOD (500 U/L), TEMPOL (100 μmol/L). Ciprofloxacin (0.5 nM), a DNA gyraze inhibitor, and aminophylline (50 nM), cAMP inhibitor, served as negative controls. Generation of ROS was evaluated after 40 min treatment with Ang-II (1 μmol). To assess the effects of a positive control of vasoconstriction, cryosections of RA and IMA were incubated with norepinephrine (1

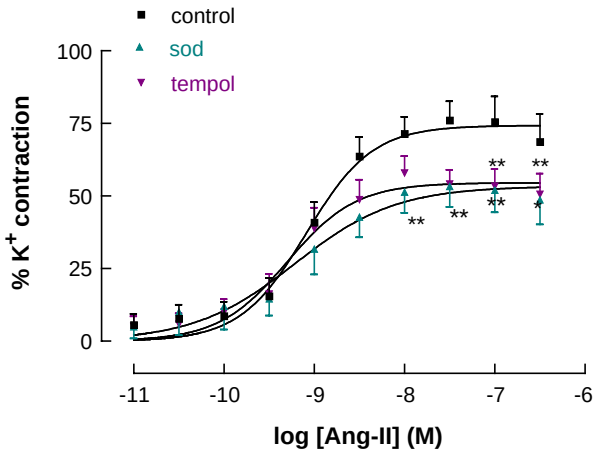

Fig. 1. Effects of the superoxide anion scavenger TEMPOL (1 mmol/l) and exogenous human superoxide dismutase (SOD: 50 U/ml) on angiotensin-II induced contraction in human radial artery ($n=10$, $F=31.1$ and 56.1, respectively, $P<0.0001$). $^{**}P<0.01$ for post-hoc testing for a given concentration of constrictor vs. constrictor and inhibitor.