ORIGINAL ARTICLE

Modulation of antioxidative response in the therapy of hypertension and other cardiovascular diseases

Ima Dovinova [1], Roman Gardlik [2], Roland Palffy [2,4], Frantisek Kristek [1], Sona Cacanyiova [1], Zuzana Vantova [3], Helena Paulikova [3]

[1] Institute of Normal and Pathological Physiology, Centre of Excellence for Cardiovascular Research, Slovak Academy of Sciences;
[2] Institute of Pathophysiology, Comenius University, Faculty of Medicine;
[3] Department of Biochemistry and Microbiology, Slovak Technical University, Faculty of Chemical and Food Technology;
[4] Institute of Molecular Biomedicine, Comenius University, Faculty of Medicine; Slovak Republic

Correspondence to: RNDr. Ima Dovinová, Ph.D., Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Sienkiewiczova 1, 813 71 Bratislava, Slovak Republic.
PHONE: +421-252 926 271, FAX: +421-252 968 516,
E-MAIL: ima.dovinova@savba.sk

Submitted: 2009-07-15 Accepted: 2009-09-11 Published online: 2009-11-15

Key words: **hypertension; reactive oxygen species; nitric oxide; antioxidants; gene therapy**

Neuroendocrinol Lett 2009; **30**(Suppl 1): 32–35 **PMID:** 20027141 NEL300709A04

Abstract

OBJECTIVES: This paper reviews and compares major approaches and strategies to modulation of antioxidative response in the therapy of hypertension and cardiovascular diseases.
DESIGN: There are two major strategies of modulation of antioxidative response in hypertension and cardiovascular diseases: (i) modulation of NO levels by NOS stimulation, increase of NO bioavailability, administration of NO, and NOS gene incorporation; (ii) scavenging of superoxide and suppression of oxidative stress by activation of antioxidant gene expression or by suppression of selected genes by RNA silencing. These strategies are accomplished by several concepts, including (1) delivery of external agents, (2) antioxidant gene therapy and RNA silencing, and (3) combined therapies and approaches.
CONCLUSION: Combined therapies and approches often achieve multiplicative effects and are the most promising attitude in antioxidant-oriented therapy of hypertension and cardiovascular diseases.

INTRODUCTION

Blood pressure in the cardiovascular system depends on a delicate balance between vasoconstrictory and vasodilatory systems. Among them, the equilibrium between the production and metabolism of reactive oxygen species (ROS) plays an important role. Overproduction of ROS due to increased activity of NADPH oxidase, cyclooxygenases, xanthine oxidases, or the mitochondrial electron chain can evoke alterations in other systems involved in the regulation of blood pressure. Increased ROS levels can also eliminate vasodilatory actions of other substances, such as nitric oxide (NO). Activation of major antioxidant enzymes and/or the glutathione system is one of the compensatory mechanisms against ROS increase. The NO/ROS balance was proved in several types of hypertension (Torok 2008; Kopincova et al.. 2008). A strong association between blood pressure and oxidative stress was found in the pathophysiology of essential hypertension (Rodrigo *et al.* 2008).

Abbreviations:
SHR – spontaneously hypertensive rat
NO – nitric oxide
ROS – reactive oxygen species
NOS – nitric oxid synthase
SOD – superoxide dismutase
GPx – glutathione peroxidase
CAT – catalase
RVLM – rostral ventrolateral medulla
cDNA – complementary DNA
NADPH oxidase – nicotinamide adenine dinucleotide phosphate-oxidase

Modulation of blood pressure through changes in oxidative stress, in the glutathione system and in the antioxidant defense system can lead to improvement in endothelial functions due to increased eNOS and NO bioavailability, and to regression of cardiovascular pathology. Delivery of external antioxidants, antihypertensive drugs or NO donors can stimulate antioxidative response. Antioxidant response can also be directly evoked via antioxidant gene transfer or stimulated by physical exercise (Kung *et al.* 2008; Leung *et al.* 2008)

The aim of the present study is to compare some approaches of antioxidative response modulation in experimental and human hypertension.

We review the following approaches currently used in antioxidant-oriented therapy: (1) Delivery of external agents (antioxidants, NO donors), (2) Stimulation of antioxidant enzymes / Antioxidant gene therapy / RNA silencing, (3) Combined therapies and approaches.

1. Delivery of External Agents (Antioxidants, NO donors)

Over the past decade, the role of ROS in the cardiovascular system has been the subject of intense research. Major effects of redox-dependent signaling pathway throught ROS include regulation of kinases and phosphatases, regulation of metalloproteinase, ion channels and /or cell differentiation, growth and death. They can play an important role in pathological changes associated with hypertension. The most relevant sources of ROS with respect to cardiovascular diseases and hypertension appear to be NADPH oxidase, xanthine oxidase, uncoupled endothelial NO synthase (Paravicini & Touyz, 2006). Moreover, several studies point out at alterations in expression and activity of main antioxidant enzymes (SOD, catalase and glutathione peroxidase) in SHR or in SHR with pre-disposition towards infarction (Csonka *et al.* 2000, Umemoto *et al.* 2004).

Various studies on hypertension models indicate that antioxidant therapy by natural compounds such as provinol or melatonin, or by synthetic antioxidants such as N-acetylcysteine or apocynin leads to beneficial effects on hypertension and on blood vessel damage (Kojsova *et al.* 2006, Pechanová *et al.* 2009). *Tempol* is one of the most extensively studied antioxidants. Intravenous or oral administration of tempol to hypertensive rodents caused rapid and reversible dose-dependent reduction in blood pressure, accompanied either with effects on vasodilation, increased nitric oxide activity, reduced sympathetic nervous system activity at central and peripheral sites, or with correction of salt sensitivity, endothelial dysfunction and/or oxidative stress (Wilcox & Pearlman, 2008). *Vitamins C and E* administered to patients with essential hypertension decreased systolic blood pressure and increased erythrocyte and serum antioxidant capacity compared to control (Rodrigo *et al.* 2008). The molecular mechanisms underlying the *in vivo* antioxidant effects of vitamin C is mediated by the ability of vitamin C to protect tetrahydrobiopterin from oxidation and thereby to increase the enzymatic activity of eNOS. The effect of α-tocopherol depends on tissue saturation with vitamin C, and both vitamins may act synergically, behaving not only as ROS scavengers but also providing optimal conditions for endothelial NO formation and inducing downregulation of NADPH oxidase (Rodrigo *et al.* 2008). Similarly, other antioxidants such as *N-acetyl cystein* and *melatonin* changed NO/ROS balance, blood pressure, and parameters of cardiovascular system in hypertensive rats (Kojsova *et al.* 2006, Krskova *et al.* 2007).

The hypertension treatment strategies can include, along with application of antioxidants, also specific therapies aimed at improving endothelial dysfunction and decreasing the vascular tone. Administration of an NO donor (pentaerythrityl tetranitrate) to SHR resulted in a decrease of cardiac hypertrophy and MDA level, and in an increase of antioxidant enzyme activities of SOD and GPx, without any effect on blood pressure (Dovinova *et al.* 2009). Thus, increased level of NO in SHR had a beneficial effect on antioxidant properties. It has been demonstrated that the NO donor protects endothelium via the antioxidant defense protein heme oxygenase 1 (Grosser & Schroder, 2004). Also, a combined treatment with L-arginine (NO donor) and losartan (AT II receptor antagonist) had an antihypertensive effect in SHR and a beneficial effect on renal function, mediated in part by increased SOD activity (Miloradovic et al. 2008).

2. Stimulation of Antioxidant Enzymes, Gene Therapy and RNA Silencing

Studies on hypertension models indicate that application of some antihypertensives induced increase in expression of antioxidant enzymes, and that the therapy with antihypertensives has, along with the main effect on hypertension, an important side effect of a decrease of blood vessel damage (Umemoto *et al.* 2004).

In recent years, *antioxidant gene therapy* based on incorporation of cDNA of antioxidant genes for the SOD, catalase and GPx has been used in experimental treatment of cancer and in the therapy of cardiovascular system (Li *et al.* 2001). The most important therapeutic effects, as regards decreased risk of myocardial infarc-

tion and reduction of arterial pressure in SHR, were found after a direct gene transfer of the cDNA encoding membrane-bound extracellular SOD - EcSOD (Chu *et al.* 2003). Downregulation of antioxidant gene expression and enzyme activity may underlie the augmented levels of superoxide and hydrogen peroxide in the rostral ventrolateral medulla (RVLM), leading to oxidative stress and hypertension in the SHR. A causative relationship between biochemical correlates of oxidative stress and neurological hypertension was established after a gene transfer by microinjection of adenovirus encoding SOD1 and SOD2 or CAT into brain (RVLM), which promoted a long-term reduction of blood pressure in SHR (Chan *et al.* 2006). A novel approach, yet not published in literature, is the systemic administration of live attenuated bacterial vectors and expression systems for delivery of antioxidant genes/enzymes. This method may feature several advantages over viral vectors (such as repetitive administration, longer duration of the effect) (Palffy *et al.* 2006). However, this has yet to be proved in further *in vivo* studies.

The rationale behind using RNA interference (RNAi) for treatment of cardiovascular diseases, in particular hypertension, has been proved in a number of experimental studies. Knock-down of several molecular targets has been investigated to ameliorate hypertension in animal models. In SHR, silencing of matrix metalloproteinase-7 by systemic RNAi resulted in attenuation of hypertension and stopped development of cardiac hypertrophy (Wang *et al.* 2009). Similar results were obtained by knocking-down the angiotensin-converting enzyme (He *et al.* 2009) and *β1*-adrenergic receptor (Arnold *et al.* 2007) in SHR. More importantly, it was found that depletion of NADPH oxidase subunits with small interfering RNAs inhibits ROS production and, thus, has the potential to reduce blood pressure. Silencing of p22phox component of NADPH oxidase *in vivo* by RNAi resulted in reduced ROS and mean atrial pressure in angiotensin II-induced hypertension in rats (Modlinger *et al.* 2006). These results make RNAi-based antioxidative approach a reasonable strategy for the treatment of hypertension.

3. Combined Therapies and Approaches

It is known that over-expression of a single antihypertensive gene can often lead to its enhanced interaction with another endogenous compound, resulting in insufficient reduction of blood pressure. Therefore, simultaneous activation of multiple genes may represent a useful solution. Co-transduction of AdeNOS and AdSOD2 to RVLM of SHR elicited a significantly greater decrease in arterial pressure and heart rate than those promoted by the individual transgenes, and prevented the AdeNOS-induced rebound hypertension. The interplay between NO and superoxide in RVLM via formation of peroxynitrite contributes to unsustained hypotensive effects of NO after overexpression of eNOS in SHR. Also, the mitochondria-derived superoxide mediates the rebound hypertension induced by eNOS transgene in RVLM of SHR (Kung *et al.* 2008). Because the superoxide anion level is increased and synthesis or activity of mitochondrial manganese superoxide dismutase (SOD2) is reduced in RVLM during hypertension, the interaction between NO and superoxide in RVLM is probably using the mitochondrial respiratory enzyme complexes as the cellular target (Kung *et al.* 2008).

Many studies suggest that cardiovascular diseases are largely associated with physical inactivity. Human and animal data confirm an important beneficial role of physical exercise in the prevention and treatment of cardiovascular diseases. The effects of exercise on endothelial function include regulation of endothelial genes mediating oxidative metabolism, vascular smooth muscle function, antioxidant systems (by elevation of Mn-SOD, Cu/Zn-SOD and CAT), increases in glutathione peroxidase activity, and heat shock protein expression in myocardium. The benefits of exercise include improved blood flow, reduced blood pressure, increased activity of antioxidant enzymes, reduced oxidative stress and restored vascular endothelial dysfunction (Leung *et al.* 2008). In this way, regulated physical exercise is associated with stimulation of main antioxidant enzymes, increase of reduced glutathione, and increase of eNOS expression and activity, thus acting as an efficient antioxidant therapy (Kojda & Hambrecht, 2005).

Conclusion

The work reviewed in this paper is an important indication of the potential of antioxidant therapy in the treatment of hypertension and cardiovascular diseases. Of the candidate therapies, the combined approaches seem to be the most efficient.

Delivery of antioxidant substances and dietary therapies induce multiple effects and usually require continuous and long-term administration of a narrow range of doses to produce an antihypertensive effect. More often than not, these approaches cannot produce critical antioxidant levels in target cells.

Expression of antioxidant genes specifically directed by gene delivery systems has shown remarkable results. Some major obstacles of gene therapy of hypertension include the inflammatory response to the adenoviral vector, short duration of the effect of gene transfer, impossibility of repetitive treatment with the adenoviral vector. Applying other viral or nonviral vectors, such as bacterial systems, using RNA silencing to knock down selected genes, together with repeatable administration may represent an advantageous therapeutic option in terms of enhanced duration and potency of antihypertensive effect.

Acknowledgements

This research was supported by the VEGA of Slovak Republic No 2/0066/08.

REFERENCES

1 Arnold AS, Tang YL, Qian K, Shen L, Valencia V, Phillips MI, *et al.* (2007). Specific beta1-adrenergic receptor silencing with small interfering RNA lowers high blood pressure and improves cardiac function in myocardial ischemia. J Hypertens. **25**: 197–205.
2 Csonka C, Pataki T, Kovacs P, Muller SL, Schroeter ML, Tosaki A, *et al.* (2000). Effects of oxidative stress on the expression of antioxidative defense enzymes in spontaneously hypertensive rat hearts. Free Rad Biol Med. **29**: 612–619.
3 Dovinova I, Cacanyiova S, Faberova V, Kristek F (2009). The effect of an NO donor, pentaerythrityl tetranitrate, on biochemical, functional, and morphological attributes of cardiovascular system of spontaneously hypertensive rats. Gen Physiol Biophys. **28**: 86–93.
4 Grosser N, Schröder H (2004). Therapy with NO donors-antiatherogenic and antioxidant actions. Herz. **29**: 116–122. German.
5 He J, Bian Y, Gao F, Li M, Qiu L, Wu W, *et al.* (2009). RNA interference targeting the ACE gene reduced blood pressure and improved myocardial remodelling in SHRs. Clin Sci (Lond). **116**:249–255.
6 Chan SHH, Tai MH, Li ChY, Chan YH (2006). Reduction in molecular synthesis or enzyme activity of superoxide dismutases and catalase contributes to oxidative stress and neurogenic hypertension in spontaneously hypertensive rats. Free Rad Biol Med. **40:** 2028–2039.
7 Chu Y, Iida S, Lund DD, Weiss RM, DiBona GF, Watanabe Y, *et al.* (2003). Gene transfer of extracellular superoxide dismutase reduces arterial pressure in spontaneously hypertensive rats.Circ Res. **92**:461–468.
8 Kojda G, Hambrecht R (2005). Molecular mechanisms of vascular adaptations to exercise. Cardiovasc Res. **67**:187–197.
9 Kojsova S, Jendekova L, Zicha J, Kunes J, Andriantsitohaina R, Pechánová O (2006). The effect of different antioxidants on nitric oxide production in hypertensive rats. Physiol Res. **55**:S3-16.
10 Kopincova J, Puzserova A, Bernatova I (2008). Chronic low-dose L-NAME treatment effect on cardiovascular system of borderline hypertensive rats. Neuroendocrinol Lett. **29**: 784–789.
11 Krskova L, Vrabcova M, Zeman M (2007). Effect of melatonin on exploration and anxiety in normotensive and hypertensive rats with high activity of renin-angiogensin system. Neuroendocrinol Lett. **28**: 295–301.
12 Kung LC, Chan SH, Wu KL, Ou CC, Tai MH, Chan JY (2008). Mitochondrial respiratory enzyme complexes in RVLM as cellular targets of nitric oxide and superoxide interaction in the antagonism of antihypertensive action of eNOS transgene. Mol Pharmacol. **74**: 1319–32.
13 Leung FP, Yung LM, Laher I; Yao X, Chen ZY, Huang Y (2008). Exercise, vascular wall and cardiovascular diseases. Sports Medicine **38**: 1009–1024.
14 Li Q, Bolli R, Qui Y, Tang XL, Yiru G, French BA (2001). Gene therapy with extracellular superoxide dismutase protects conscious rabbits against myocardial infarction. Circulation. **103**: 1893–1898.
15 Miloradovic Z, Jovovic D, Mihailovic N, Milanovic JG, Milanovic S (2008). Effect of long-term losartan and L-arginine treatment on haemodynamics and SOD activity in SHR. Can J Physiol Pharmacol. **86**: 210–214.
16 Modlinger P, Chabrashvili T, Gill PS, Mendonca M, Harrison DG, Griendling KK, *et al.* (2006). RNA silencing in vivo reveals role of p22phox in rat angiotensin slow pressor response. Hypertension. **47**: 238–44.
17 Palffy R, Gardlik R, Hodosy J, Behuliak M, Resko P, Radvansky J, Celec P (2006). Bacteria in gene therapy: bactofection versus alternative gene therapy. Gene Therapy **13**: 101-105.
18 Paravicini TM, Touyz RM (2006). Redox signaling in hypertension. Cardiovasc Res. **71**: 247–258.
19 Pechanová O, Jendekova L, Vrankova S (2009). Effect of chronic apocynin treatment on nitric oxide and reactive oxygen species production in borderline and spontaneous hypertension. Pharmacol Rep. **61**: 116–122.
20 Rodrigo R, Prat H, Passalacqua W, Araya J, Bachler JP (2008). Decrease in oxidative stress through supplementation of vitamins C and E is associated with a reduction in blood pressure in patients with essential hypertension. Clin Sci (Lond). **114**: 625–634.
21 Torok J (2008). Participation of Nitric Oxide in Different Models of Experimental Hypertension. Physiol Res. **57**: 813–825.
22 Umemoto S, Tanaka M, Kawahara S, Kubo M, Umeji K., Hashimoto R, *et al.* (2004). Calcium Antagonist Reduces Oxidative Stress By Upregulating Cu/Zn Superoxide Dismutase In Stroke-Prone SHR. Hypertens Res. **27**: 877–885.
23 Wang X, Chow FL, Oka T, Hao L, Lopez-Campistrous A, Kelly S, et al. (2009). Matrix metalloproteinase-7 and ADAM-12 define a signaling axis in agonist-induced hypertension and cardiac hypertrophy. Circulation. **119**: 2480–2489.
24 Wilcox CS, Pearlman A (2008). Chemistry and antihypertensive effects of tempol and other nitroxides. Pharmacol Rev. **60**: 418–469.