Post N Med 2016; XXIX(12B): 52-56

*Anna Gajos-Draus

New concepts of the pro-atherogenetic mechanism of vascular oxidative stress. Protective and harmful roles of NADPH oxidase**

Nowa koncepcja promiażdżycowego mechanizmu naczyniowego stresu oksydacyjnego. Protekcyjna i szkodliwa rola oksydazy NADPH

Department of Clinical Physiology, Centre of Postgraduate Medical Education, Warsaw
Head of Department: Michał Mączewski, MD, PhD

Key words

NADPH oxidative, Nox4, ROS, vascular oxidative stress, CVD

Słowa kluczowe

oksydaza NADPH, Nox4, ROS, naczyniowy stres oksydacyjny, CVD

S u m m a r y

Atherosclerosis and other forms of cardiovascular disease (CVD) remain the leading cause of morbidity and mortality in contemporary societies. Vascular oxidative stress, defined as increased vascular production of reactive oxygen species leading to endothelial dysfunction and cellular damage arising from disturbed ROS-mediated redox-signalling reactions, are likely common underlying mechanisms of CVD. Nevertheless, using antioxidants to prevent CVD has been demonstrated to be ineffective in clinical trials, which most probably reflects an incomplete understanding of the oxidative stress.

A major source of the vascular ROS and a mediator of CVD is the NADPH oxidase family of enzymes. Four NADPH homologues, Nox1, Nox2, Nox4, and Nox5, differing in various features and biological functions are expressed in the cardiovascular system. Until recently, the prevailing view was that the cardiovascular risk factors mediate the vascular oxidative stress, endothelial dysfunction, and progression of CVD by increasing vascular activity and/or expression of Nox1, Nox2, and Nox5. Paradoxically, the recent evidence suggests that Nox4-mediated cellular signalling plays a vasculo-protective and an antiatherogenic role, and that induction of Nox2 is associated with reduction of Nox4. Collectively, these data support the view that the mechanism of the vascular oxidative stress and endothelial dysfunction associating risk factors of CVD, in fact, encompasses two interrelated processes: the increase of the harmful Nox1/2/5 and the decrease of the protecting Nox4. This, in turn, implicates that treatment of CVD should include either selective inhibition of Nox1/2/5 or selective activation of Nox4.

S t r e s z c z e n i e

Choroba sercowo-naczyniowa (CVD) jest najczęstszą przyczyną zgonów w krajach cywilizacji zachodniej. Wspólnym elementem patomechanizmu różnych postaci CVD jest zwiększona naczyniowa produkcja reaktywnych form tlenu (ROS, ang. Reactive Oxygen Species) skutkująca naczyniowym stresem oksydacyjnym. Stan ten prowadzi do zakłócenia homeostazy redox na rzecz ROS, co skutkuje inaktywacją tlenku azotu (NO) i dysfunkcją śródbłonka. Do tej pory wykorzystanie antyoksydantów w leczeniu CVD okazało się być nieskuteczne, co wskazuje, że mechanizm stresu oksydacyjnego jest wciąż słabo poznany.

Głównym źródłem ROS w naczyniach jest enzym oksydaza NADPH (Nox), którego jedyną znaną funkcją jest produkcja ROS. Nox ma kilka izoform różniących się mechanizmem aktywacji, komórkową lokalizacją i rodzajem produkowanego ROS. W układzie krążenia u ludzi obecne są izoformy Nox1, Nox2, Nox4 i Nox5, a u myszy i szczurów – tylko Nox1, Nox2 i Nox4. Wykazano, że naczyniowemu stresowi oksydacyjnemu u ludzi i zwierząt towarzyszy zwykle wzrost aktywności enzymatycznej oraz ekspresji Nox1/2/5. Ostatnio wykazano, że farmakologiczna indukcja lub zwiększona ekspresja Nox4 ma protekcyjne działanie naczyniowe, a eliminacja genu Nox4 skutkuje niekorzystnymi efektami śródbłonkowymi. Wykazano również, że wzrost ekspresji Nox2 jest związany ze zmniejszeniem ekspresji Nox4. Dane te wskazują, że mechanizm naczyniowego stresu oksydacyjnego oraz zaburzenia czynności śródbłonka w rzeczywistości obejmuje dwa powiązane ze sobą procesy: zwiększone szkodliwe działanie Nox1/2/5 oraz zmniejszone protekcyjne działanie Nox4. Dowodzi to tego, że leczenie CVD powinno polegać albo na selektywnym hamowaniu Nox1/2/5, albo na selektywnej aktywacji Nox4.

Conflict of interest
Konflikt interesów

None
Brak konfliktu interesów

Address/adres:

*Anna Gajos-Draus
Department of Clinical Physiology
Centre of Postgraduate Medical Education
Marymoncka 99/103, 01-813 Warszawa
tel. +48 (22) 569-38-40
anna.gajos@cmkp.edu.pl

**This work was supported by grant No: 2015/17/N/NZ5/00328 (Preludium 9) from the National Science Centre, Poland.

CONTEMPORARY CONCEPT OF THE VASCULAR OXIDATIVE STRESS

Oxygen free radicals, such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), and other reactive oxygen species (ROS) have long been recognised as essentially biologically noxious. Accordingly, oxidative stress has been traditionally defined as ROS-induced structural damage to proteins, phospholipids, nucleic acids and other vitally important cellular constituents (1, 2)

However, it has been recognised that ROS are signalling molecules, which via redox-mediated modification of specific cellular proteins, play important regulatory roles in physiological cellular processes. In this context, it has been demonstrated that ROS can activate or inactivate various members of cellular signalling pathways, including some phosphatases, kinases, and transcription factors. Consequently, the term "oxidative stress" has been recently redefined as cellular damage arising from disturbed ROS-mediated redox-signalling reactions (3). As discussed in detail below, the implications of this new concept are twofold. Firstly, while some ROS may be harmful, the others may be protective for the cells. Secondly, increased, as well as decreased production of some ROS may be harmful.

In the context of CVD, it is believed that cardiovascular risk factors (e.g. hypercholesterolemia, hypertension, diabetes, smoking, ageing, and others) acting through factors such as angiotensin II and endothelin-1, mediate the production of excess vascular O_2^-. Superoxide acting: (a) per se or as a by-product of its dismutation, H_2O_2; (b) via O_2^- induced inactivation of nitric oxide (NO, seen in clinical studies as endothelial dysfunction), and/or (c) via peroxynitrite ($ONOO^-$, the reaction product of NO and O_2^-) is thought to affect cellular signalling pathways, which ultimately mediate atherogenic vascular inflammation and remodelling (4, 5). Superoxide is a highly reactive, short-lived, and poor membrane-penetrating species predominantly active at a site of its generation. H_2O_2 has a longer half-life and is freely diffusible and, therefore, is more likely to exert more distant effects (3;6). Actually, in healthy vascular system, cellular signalling is dominated by endothelial NO that induces an anti-atherosclerotic phenotype of the endothelium and the vascular wall. CVD risk factors are associated with ROS-mediated decreased NO bioavailability (seen as endothelial dysfunction) (oxidative stress) and adverse signalling by $ONOO^-$ (nitrosative stress) (1, 7). In accord with the above hypothesis: (i) vascular increase of redox-regulated Nuclear factor κB (NF-κB) (encoding numerous pro-inflammatory genes) and a parallel reduction of redox-regulated Nuclear factor (erythroid-derived 2)-like-2 factor (Nrf2) (encoding genes for antioxidant and anti-inflammatory enzymes), associate endothelial dysfunction and diseased vascular phenotype accompanying cardiovascular risk factors (8, 9) and (ii) markers of endothelial dysfunction correlate with classical cardiovascular risk factor profile and predict the occurrence of myocardial infarction and stroke in humans (10, 11). However, treatment with antioxidants to prevent CVD has been demonstrated to be effective only in small clinical studies and in experimental models of CVD (12, 13). However, the data from large clinical trials have shown antioxidants to be ineffective, which most probably reflect the incomplete understanding of the oxidative stress (14-16).

NADPH OXIDASES AND THE VASCULAR OXIDATIVE STRESS

The NADPH oxidase family of enzymes (Noxs) is the major source of the vascular ROS. Noxs produce ROS as their primary function, and therefore are thought to play essential roles in tightly regulated cellular redox-signalling pathways, including those underlying vascular inflammation and atherogenesis. The effects of the Nox undergo further reinforcement because Noxs-derived ROS activates ROS production by other cellular sources, including mitochondria, xanthine oxidase, and other sources, which are known to produce ROS only as a by-product of their normal functions. Collectively, Noxs appear to play a role of "the maestro" of vascular oxidative stress (17, 18).

Of the seven known Nox homologes, only four (Nox1, Nox2, Nox4 and Nox5) have been identified as important sources of ROS in the cardiovascular system. These four Noxes appear to differ in their mechanism of the activation, type of the ROS produced, cellular localisation, and the role they play in the cellular regulations and pathology (tab. 1).

Tab. 1. Characterisation of cell/tissue distribution, sub-cellular localisation and regulation of Nox isoforms in the cardiovascular disease (19), modified

Isoforms of Nox	Cell/tissue distribution	Subcellular localisation	Regulators
NOX1	Vessels, colon,prostate,uterus, muscle	Plasma membrane, Caveolae Endosomes Perinuclear	p22phox, NOX01,NOXA1, Rac1
NOX2	Phagocytes, kidney, vessels, heart	Plasma membrane Perinuclear Nuclear pore	p22phox, p47hox, p67phox, Rac1/2
NOX4	Kidney, vessels, bone	ER* Perinuclear Nucleus Mitochondria Focal adhesions Stress fibres Cytoplasm	p22phox
NOX5	Lymph nodes, testes, heart	Plasma membrane Intracellular	Calcium

*ER – endoplasmic reticulum

First, the activity and the expression of Nox1 and Nox5 are regulated by the number of agonists, including angiotensin II and endothelin-1. The activity of Nox5 is activated by intracellular calcium ions. Nox4 is unique among Noxs because it is constitutively active, i.e., its activity is regulated on the transcriptional level only (20-22).

Second, Nox1/2/5 generate O_2^-, which may affect signalling pathways either per se or via H_2O_2, by decreasing bioavailability of vascular NO or by $ONOO^-$. In contrast, Nox4 generates predominantly H_2O_2, which is incapable of inactivating NO and producing $ONOO^-$ and which exerts predominantly H_2O_2-specific cellular signalling (23).

Third, Nox1/2/5 are plasmalemma bound enzymes releasing O_2^- intra- and extracellularly, while Nox4 is bound to the intracellular membranes (predominantly the membrane of nucleus and endoplasmic reticulum (fig. 1).

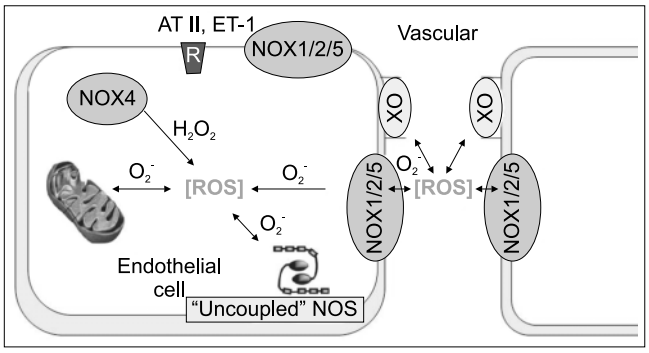

Fig. 1. Spatial organisation of vascular Nox enzymes
Nox1, Nox2, and Nox5 are localised in different cellular compartments such as within cells or at the plasma membrane. They release O_2^- inside vesicles or extracellularly after activation of receptor (R) by ligand (L) and may cause their cytosolic effect after crossing membrane. Nox4 is always intracellular and constitutively produces a higher proportion of membrane-permeable H_2O_2 than other oxidases. Nox 1/2/5 derived ROS activates ROS production by other cellular sources, including mitochondria, xanthine oxidase, and other sources, which are known to produce ROS only as a by-product of their normal functions. Collectively, Noxs appear to play a role of "the maestro" of the vascular oxidative stress
XO – xanthine oxidase; ATII – Angiotensin; ET1 – Endothelin; ROS – reactive oxygen species; NOS- Nitric Oxide Synthase

Fourth, several arguments support the view that it is the increase of Nox1, Nox2, and perhaps of Nox5, which mediates the vascular oxidative stress, endothelial dysfunction, and progression of CVD (19, 24, 25). Thus: (a) vascular activity and/or expression of Nox1 and Nox2 is increased in animal models of the oxidative stress (Nox5 is not found in rodents) (24, 26, 27), and increased vascular expression of Nox1, Nox2, and Nox5 was noted in humans with coronary artery disease or diabetes (28, 29); (b) Nox1 and Nox2 knockout mice do not develop endothelial dysfunction in response to angiotensin II (30); (c) knockout studies in atherosclerosis–prone, ApoE deficient (ApoE-/-) mice, identified important roles of Nox1 and Nox2 in atherogenesis (31-33), and (d) human carriers of hereditary deficiency of Nox2 demonstrated reduced isoprostanes generation, greater flow-mediated vasorelaxation, increased NO availability, and reduced intima-media thickness, comparing to healthy subjects (34). In contrast, to harmful Nox1/2/5, Nox4 appears to be a protective enzyme (see below).

PURPORTED PROTECTIVE AND ANTI-OXIDATIVE ROLE OF NOX4

The knowledge of Nox4 is still in its infancy, however thanks to latest research there are the following significant arguments for the engagement of Nox4 in a protective function in cardiovascular research.

First, studies involving Nox4 gene manipulations (i.e., gene knockout vs. its forced overexpression) strongly implied a protecting, rather than deleterious, role of Nox4, and that the protection is mediated by H_2O_2. H_2O_2 is continuously produced and this enables to maintain a basal level of antioxidant and protect cells against oxidative stress. A significant quantity of data showed that low concentrations of H_2O_2 have positive effects in the vascular system and signalling of H_2O_2 plays a key role in vascular function and homeostasis (35).

It has been reported in this context that Nox4: (a) is vasculoprotective (via increased angiogenesis, induction of endothelial NO synthase (eNOS), and increased vascular NO availability) (20, 36-38); (b) prevents load-induced myocardial injury (partially via increased angiogenesis) (39, 40); (c) mediates protection against atherosclerosis (21, 41, 42), and (d) either prevents (43) or increases (44) atherogenic changes in vascular smooth muscle cells (VSMCs) in Apoe-/- mice.

Moreover, the data showed that H_2O_2 derived from Nox4, activates and induce expression eNOS and also production of NO by several mechanisms such as: (a) activation of various signal transduction pathway such as PI3K/Akt (45, 46) and Erk 1/2 (46) (b) laminar shear stress, which promotes the formation of signalling level of H_2O_2 that in turn activate p38 MAPK (47) and SHP2 sulfenylation – FAK (Focal adhesion kinase) – mediated eNOS activation (48).

Secondly, in contrast to Nox1/2/5, only fragmentary and inconsistent data is available concerning Nox4 expression in CVD and its preclinical models. In humans, Nox4 was found to be upregulated in atherosclerotic coronary arteries (49) and downregulated in atherosclerotic plaques (21) and in myocardium of patients with aortic valve stenosis (50). In rats, Nox4 was upregulated in stenotic carotid arteries (51), but unchanged in aortas of diabetic animals (52). In $ApoE^{-/-}$ mice, vascular Nox4 was upregulated at 10 week and downregulated at 20 week of streptozotocin-induced diabetes (21).

Thirdly, empirical evidence suggests that Nox1/2/5 and Nox4 are oppositely regulated by agonists and each other. Thus, angiotensin II simultaneously upregulated Nox1 and downregulated Nox4 in VSMCs (53), and Nox1 was upregulated in VSMCs of Nox4-deficient $ApoE^{-/-}$ mice (43). In $ApoE^{-/-}$ mice with Nox4 deleted or over expressed vascular Nox2 gene and protein levels

inversely correlated with Nox4 levels (21). Conversely, in mice with elimination of Nox2, upregulation of Nox4 was noted (54). Moreover, Nox4 or Nox2 elimination upregulated the mRNA and protein expression of the other in human pulmonary artery endothelial cells (54). Collectively, the data imply the existence of the regulatory cross-talk between Nox1/2 and Nox4.

Fourthly, this cross-talk seems to involve the transcription factor Nrf2, which is known to undergo H_2O_2-mediated activation. In this context, Nox4 has been identified as an upstream activator of Nrf2 in transgenic mice models of inflammatory vascular injury and of load-induced myocardial injury (20, 40, 55). Alternatively, Nrf2 has been identified as an upstream activator of Nox4 expression and H_2O_2 production, and an inhibitor of Nox2 expression and O_2^- production in brain hippocampal tissue (56). Altogether, the data suggest an instrumental role of Nrf2 in the purported cross-talk between, at least, Nox2 and Nox4 (fig. 2).

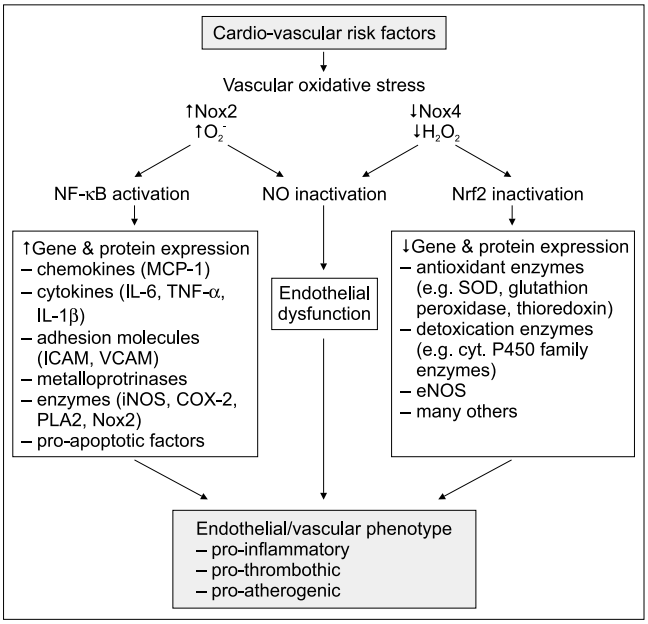

Fig. 2. The probable sequence through which cardiovascular risk factors lead to development pro-atherosclerotic endothelial phenotype.
The cardiovascular disease risk factors such as diabetes, hypertension, hyperlipidemia and others increase the activity and expression of NADPH oxidase isoforms Nox1/2/5 and decrease Nox4 in endothelial cells. This causes more production of reactive oxygen species (ROS) and causes activation of pro-atherosclerotic vascular phenotype due to decreased bioavailability of NO by maintaining the NFkB signalling pathway (4; 23). Moreover the data suggest an instrumental role of Nrf2 in the purported cross-talk between Nox2 and Nox4 (20; 36; 55; 56; 62; 63).

NOX4 AS A POTENTIAL THERAPEUTIC TARGET IN CVD

Vascular oxidative stress has a critical role in the pathogenesis of cardio-vascular disease (CVD). The data cited above suggest that the mechanism of the vascular oxidative stress and endothelial dysfunction associating risk factors of CVD, in fact, encompasses two interrelated processes: the upregulation of the harmful Nox1/2/5 and the downregulation of the protecting Nox4.

This, in turn, implies that treatment of CVD should involve interventions intended either for selective inhibition of Nox1/2/5 or at selective activation of Nox4.

However, use of antioxidants to prevent CVD proved to be ineffective in clinical trials, which most probably confirms the incomplete understanding of the oxidative stress. The antioxidants tested in large clinical trials are known to scavenge both O_2^- and H_2O_2, which partially explains their inability to prevent CVD. Moreover, the evidence indicates that ROS such as O_2^- and H_2O_2 may function as second messengers in physiological cellular signalling and various forms of ROS may have different signalling properties.

Subsequently, several inhibitors of Noxs have been developed and they are already tested in a number of preclinical studies. However, the problem is that usually these inhibitors are not sufficiently selective (they block all Noxs together). As discussed above, to be clinically efficient, the inhibitor should selectively block Nox1/2/5, and should not affect Nox4. An alternative therapeutic option would be a direct or an indirect (e.g., via the activation of Nrf2) activation of Nox4. Actually, flavonoids, which were shown to exert various favourable effects in CVD, were demonstrated to activate Nrf2 (57-61) (fig. 2).

Better understanding of Nox4 regulation and its involvement in signalling pathways gives hope to control the development of CVD. Therefore, all Nox4 inhibitors, which are currently being used for clinical trial, should be in control. Based on these recent studies a new paradigm is emerging that Nox4 may be vascular protective and that upregulation of this Nox isoform may have potential therapeutic benefit in preventing vascular disease.

BIBLIOGRAPHY

1. Cai H, Harrison DG: Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res 2000; 87: 840-844.
2. Li JM, Shah AM: Endothelial cell superoxide generation: regulation and relevance for cardiovascular pathophysiology. Am J Physiol 2004; 287: R1014-R1030.
3. Brandes RP, Weissmann N, Schröder K: Redox-mediated signal transduction by cardiovascular Nox NADPH oxidases. J Mol Cell Cardiol 2014; 73: 70-79.
4. Truong TH, Carroll KS: Redox regulation of protein kinases. Crit Rev Biochem Mol Biol 2013; 48, 4: 332-356.
5. Droge W: Free radicals in the physiological control of cell function. Physiol Rev 2002; 82: 47-95.
6. Brandes RP, Kreuzer J: Vascular NADPH oxidases: molecular mechanisms of activation. Cardiovasc Res 2005; 65: 16-27.
7. Beresewicz A, Gajos-Draus A: Enjoy your heart-beets. The role of dietary inorganic nitrate in cardiovascular health. Kardiol Pol 2016; 74, 5: 403-410.
8. Tebay LE, Robertson H, Durant ST et al.: Mechanisms of activation of the transcription factor Nrf2 by redox stressors, nutrient cues, and energy status and the pathways through which it attenuates degenerative disease. Free Radical Biology and Medicine 2015; 88: 108-146.
9. Gimbrone MA, García-Cardeña G: Endothelial Cell Dysfunction and the Pathobiology of Atherosclerosis. Circ Res 2016; 118: 620-636.

10. Giannotti G, Landmesser U: Endothelial dysfunction as an early sign of atherosclerosis. Herz 2007; 32: 568-572.
11. Kleinbongard P, Dejam A, Lauer T et al.: Plasma nitrite concentrations reflect the degree of endothelial dysfunction in humans. Free Radic Biol Med 2006; 40: 295-302.
12. Rodrigo R, Prat H, Passalacqua W et al.: Decrease in oxidative stress through supplementation of vitamins C and E is associated with a reduction in blood pressure in patients with essential hypertension. Clinical Science 2008; 14; 114: 625.
13. Plantinga Y, Ghiadoni L, Magagna A et al.: Supplementation With Vitamins C and E Improves Arterial Stiffness and Endothelial Function in Essential Hypertensive Patients. Am J Hypertens 2007; 20: 392-397.
14. Czernichow S, Bertrais S, Blacher J et al.: Effect of supplementation with antioxidants upon long-term risk of hypertension in the SU.VI.MAX study: association with plasma antioxidant levels. Journal of Hypertension 2005; 23, 11: 2013-2018.
15. Greig L, Maxwell S: Anti-oxidants – a protective role in cardiovascular disease? Expert Opinion on Pharmacotherapy 2001; 2: 1737-1750.
16. Vivekananthan DP, Penn MS, Sapp SK et al.: Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. Lancet 2003; 361: 2017-2023.
17. Wojtera E, Konior A, Fedoryszak-Kuska N et al.: Obligatory role of intraluminal $O_2^{\cdot}$ in acute endothelin-1 and angiotensin II signaling to mediate endothelial dysfunction and MAPK activation in guinea-pig hearts. Int J Mol Sci 2014; 15: 19417-19443.
18. Dikalov S: Cross talk between mitochondria and NADPH oxidases. Free Radic Biol Med 2011; 51: 1289-1301.
19. Lassegue B, Griendling KK: NADPH oxidases: functions and pathologies in the vasculature. Arterioscler Thromb Vasc Biol 2010; 30: 653-661.
20. Schröder K, Zhang M, Benkhoff S et al.: Nox4 is a protective reactive oxygen species generating vascular NADPH oxidase. Circ Res 2012; 110: 1217-1225.
21. Gray SP, Di Marco E, Kennedy K et al.: Reactive Oxygen Species Can Provide Atheroprotection via NOX4-Dependent Inhibition of Inflammation and Vascular Remodeling. Arteriosclerosis, Thrombosis, and Vascular Biology 2016; 36: 295-307.
22. Konior A, Schramm A, Czesnikiewicz-Guzik M et al.: NADPH Oxidases in Vascular Pathology. Antioxid Redox Signal 2014; 20: 2794-2814.
23. Takac I, Schröder K, Zhang L et al.: The E-loop is involved in hydrogen peroxide formation by the NADPH oxidase Nox4. J Biol Chem 2011; 286: 13304-13313.
24. Brandes RP: Vascular Functions of NADPH Oxidases. Hypertension 2010; 56: 17-21.
25. Drummond GR, Sobey CG: Endothelial NADPH oxidases: which NOX to target in vascular disease? Trends in Endocrinology & Metabolism 2014; 25: 452-463.
26. Polotow TG, Vardaris CV, Mihaliuc AR et al.: Astaxanthin Supplementation Delays Physical Exhaustion and Prevents Redox Imbalances in Plasma and Soleus Muscles of Wistar Rats. Nutrients 2014; 6: 5819-5838.
27. Konior A, Klemenska E, Brudek M et al.: Seasonal superoxide overproduction and endothelial activation in guinea-pig heart; seasonal oxidative stress in rats and humans. J Mol Cell Cardiol 2011; 50: 686-694.
28. Guzik TJ, Chen W, Gongora MC et al.: Calcium-dependent NOX5 nicotinamide adenine dinucleotide phosphate oxidase contributes to vascular oxidative stress in human coronary artery disease. J Am Coll Cardiol 2008; 52: 1803-1809.
29. Guzik TJ, Griendling KK: NADPH oxidases: molecular understanding finally reaching the clinical level? Antioxid Redox Signal 2009; 11: 2365-2370.
30. Matsuno K, Yamada H, Iwata K et al.: Nox1 is involved in angiotensin II-mediated hypertension: a study in Nox1-deficient mice. Circulation 2005; 112: 2677.
31. Barry-Lane PA, Patterson C, van der Merwe M et al.: p47phox is required for atherosclerotic lesion progression in ApoE -/-. mice. J Clin Invest 2001; 108: 1513-1522.
32. Judkins CP, Diep H, Broughton BR et al.: Direct evidence of a role for Nox2 in superoxide production, reduced nitric oxide bioavailability, and early atherosclerotic plaque formation in ApoE-/- mice. Am J Physiol 2010; 298: H24-H32.
33. Gray SP, Di Marco E, Okabe J et al.: NADPH Oxidase 1 Plays a Key Role in diabetes mellitus-accelerated atherosclerosis. Circulation 2013; 127: 1888.
34. Carnevale R, Iuliano L, Nocella C et al.: Relationship between platelet and urinary 8-Iso-PGF2α levels in subjects with different degrees of NOX2 regulation. J Am Heart Assoc 2013; 2: e000198.
35. Cai H: Hydrogen peroxide regulation of endothelial function: Origins, mechanisms, and consequences. Cardiovascular Research 2005 Oct 1; 68: 26.
36. Craige SM, Chen K, Pei Y et al.: NADPH oxidase 4 promotes endothelial angiogenesis through endothelial nitric oxide synthase activation. Circulation 2011; 124: 731-40.
37. Ray R, Murdoch CE, Wang M et al.: Endothelial Nox4 NADPH oxidase enhances vasodilatation and reduces blood pressure *in vivo*. Arterioscler Thromb Vasc Biol 2011; 31: 1368-1376.
38. Peshavariya HM, Liu GS, Chang CW et al.: Prostacyclin signaling boosts NADPH oxidase 4 in the endothelium promoting cytoprotection and angiogenesis. Antioxid Redox Signal 2014; 20: 2710-2725.
39. Zhang M, Brewer AC, Schroder K et al.: NADPH oxidase-4 mediates protection against chronic load-induced stress in mouse hearts by enhancing angiogenesis. Proc Natl Acad Sci U S A 2010; 107: 18121-18126.
40. Smyrnias I, Zhang X, Zhang M et al.: Nicotinamide adenine dinucleotide phosphate oxidase-4-dependent upregulation of nuclear factor erythroid-derived 2-like 2 protects the heart during chronic pressure overload. Hypertension 2015 Mar 1; 65: 547-553.
41. Schürmann C, Rezende F, Kruse C et al.: The NADPH oxidase Nox4 has anti-atherosclerotic functions. European Heart Journal 2015 Dec 21; 36: 3447.
42. Craige SM, Kant S, Reif M et al.: Endothelial NADPH oxidase 4 protects ApoE-/- mice from atherosclerotic lesions. Free Radical Biology and Medicine 2015; 89: 1-7.
43. Di Marco E, Gray SP, Kennedy K et al.: NOX4-derived reactive oxygen species limit fibrosis and inhibit proliferation of vascular smooth muscle cells in diabetic atherosclerosis. Free Radical Biology and Medicine 2016; 97: 556-567.
44. Tong X, Khandelwal AR, Wu X et al.: Pro-atherogenic role of smooth muscle Nox4-based NADPH oxidase. Journal of Molecular and Cellular Cardiology 2016; 92: 30-40.
45. Thomas SR, Chen K, Keaney JF: Hydrogen Peroxide Activates Endothelial Nitric-oxide Synthase through Coordinated Phosphorylation and Dephosphorylation via a Phosphoinositide 3-Kinase-dependent Signaling Pathway. J Biol Chem 2002; 277: 6017-6024.
46. Cai H, Li Z, Davis ME et al.: Akt-Dependent Phosphorylation of Serine 1179 and Mitogen-Activated Protein Kinase Kinase/Extracellular Signal-Regulated Kinase 1/2 Cooperatively Mediate Activation of the Endothelial Nitric-Oxide Synthase by Hydrogen Peroxide. Molecular Pharmacology 2003; 63: 325-331.
47. Bretón-Romero R1, González de Orduña C, Romero N et al.: Critical role of hydrogen peroxide signaling in the sequential activation of p38 MAPK and eNOS in laminar shear stress. Free Radical Biology and Medicine 2012; 52: 1093-1100.
48. Sánchez-Gómez FJ, Calvo E, Bretón-Romero R et al.: NOX4-dependent Hydrogen peroxide promotes shear stress-induced SHP2 sulfenylation and eNOS activation. Free Radical Biology and Medicine 2015; 89: 419-430.
49. Sorescu D, Griendling KK: Reactive oxygen species, mitochondria, and NAD(P)H oxidases in the development and progression of heart failure. Congest Heart Fail 2002; 8: 132-140.
50. Moreno MU1, Gallego I, López B et al.: Decreased Nox4 levels in the myocardium of patients with aortic valve stenosis. Clinical Science 2013; 125: 291.
51. Szöcs K, Lassègue B, Sorescu D et al.: Upregulation of Nox-based NAD(P)H oxidases in restenosis after carotid injury. Arterioscler Thromb Vasc Biol 2002; 22: 21-27.
52. Wendt MC, Daiber A, Kleschyov AL et al.: Differential effects of diabetes on the expression of the gp91phox homologues nox1 and nox4. Free Radic Biol Med 2005; 39: 381-391.
53. Lassègue B, Sorescu D, Szöcs K et al.: Novel gp91(phox) homologues in vascular smooth muscle cells: nox1 mediates angiotensin II-induced superoxide formation and redox-sensitive signaling pathways. Circ Res 2001; 88: 888-894.
54. Pendyala S, Usatyuk PV, Gorshkova IA et al.: Regulation of NADPH oxidase in vascular endothelium: the role of phospholipases, protein kinases, and cytoskeletal proteins. Antioxid Redox Signal 2009; 11: 841-860.
55. Brewer AC, Murray TV, Arno M et al.: Nox4 regulates Nrf2 and glutathione redox in cardiomyocytes *in vivo*. Free Radic Biol Med 2011; 51: 205-215.
56. Kovac S, Angelova PR, Holmström KM et al.: Nrf2 regulates ROS production by mitochondria and NADPH oxidase. Biochim Biophys Acta 2015 Apr 22; 1850: 794-801.
57. Bondonno CP, Croft KD, Ward N et al.: Dietary flavonoids and nitrate: effects on nitric oxide and vascular function. Nutrition Reviews 2015; 73: 216.
58. Howden R: Nrf2 and Cardiovascular Defense. Oxid Med Cell Longev 2013; 2013: 104308.
59. Zhou S, Jin J, Bai T et al.: Potential drugs which activate nuclear factor E2-related factor 2 signaling to prevent diabetic cardiovascular complications: A focus on fumaric acid esters. Life Sciences 2015; 134: 56-62.
60. Hur W, Gray NS: Small molecule modulators of antioxidant response pathway. Current Opinion in Chemical Biology 2011; 15: 162-173.
61. Mann GE, Bonacasa B, Ishii T et al.: Targeting the redox sensitive Nrf2-Keap1 defense pathway in cardiovascular disease: protection afforded by dietary isoflavones. Current Opinion in Pharmacology 2009; 9: 139-145.
62. Touati S, Montezano AC, Meziri F et al. Exercise training protects against atherosclerotic risk factors through vascular NADPH oxidase, extracellular signal-regulated kinase 1/2 and stress-activated protein kinase/c-Jun N-terminal kinase downregulation in obese rats. Clin Exp Pharmacol Physiol 2015; 42, 2: 179-185
63. Omar SA, Webb AJ: Nitrite reduction and cardiovascular protection. J Mol Cell Cardiol 2014; 73: 57-69.

received/otrzymano: 10.11.2016
accepted/zaakceptowano: 29.11.2016