Biased activity of soluble guanylyl cyclase: the Janus face of thymoquinone

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Received 8 June 2017; accepted 13 June 2017

Abstract The natural compound thymoquinone, extracted from Nigella sativa (black cumin), is widely used in humans for its anti-oxidative properties. Thymoquinone is known for its acute endothelium-independent vasodilator effects in isolated rat aortae and pulmonary arteries, depending in part on activation of adenosine triphosphate-sensitive potassium channels and inhibition of voltage-dependent calcium channels. The compound also improves endothelial dysfunction in mesenteric arteries of ageing rodents and in aortae of rabbits treated with pyrogallol, by inhibiting oxidative stress. Serendipitously, thymoquinone was found to augment contractions in isolated arteries with endothelium of both rats and pigs. The endothelium-dependent augmentation it causes counterintuitively depends on biased activation of soluble guanylyl cyclase (sGC) producing inosine 3’,5’-cyclic monophosphate (cyclic IMP) rather than guanosine 3’,5’-cyclic monophosphate. This phenomenon shows a striking mechanistic similarity to the hypoxic augmentation previously observed in porcine coronary arteries. The cyclic IMP preferentially produced under thymoquinone exposure causes an increased contractility of arterial smooth muscle by interfering with calcium homeostasis. This brief review summarizes the vascular pharmacology of thymoquinone, focussing in particular on how the compound causes endothelium-dependent contractions by biasing the activity of sGC.

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Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.
1. Introduction

Quinones are a class of organic substances derived from aromatic structures and include both endogenous (ubiquinone) and xenobiotic (for example, thymoquinone and 1,4-benzoquinone) compounds. Ubiquinone (coenzyme Q10) is the ubiquitous component of the mitochondrial electron transport chain, which, upon activation, generates energy from adenosine triphosphate (ATP). Of xenobiotic quinones, thymoquinone (2-methyl-5-isopropyl-1,4-benzoquinone; Fig. 1) is one of the most thoroughly studied for its pharmacological properties. It is the most active component of Nigella sativa, commonly called black cumin, the essential oil of the seeds of which are used for treatment or prevention of a range of diseases and conditions including hypertension, diabetes, inflammation, infection, asthma, diarrhoea and dyslipidaemia. Thymoquinone has well-documented anti-oxidant, anti-inflammatory, nephro-, hepat-, neuro-protective and anticancer properties. It is, in general, safe to use with limited adverse effects; in mice, the oral median lethal dose (LD₅₀) is estimated to be 105 mg/kg, while in patients with cancer, a dose of 2600 mg/day is well-tolerated.

This brief review discusses the vascular properties of thymoquinone, whether administered acutely or chronically, as determined by experiments on isolated blood vessels.

2. Vasodilator effects

The in vitro vasodilator effect of thymoquinone upon acute exposure has been demonstrated in contracted isolated rat pulmonary arteries (Fig. 2A), rat aortae (Fig. 2B), rat mesenteric arteries and porcine coronary arteries. The relaxations caused by thymoquinone are observed in the absence of endothelium. In the rat pulmonary artery they are due both to activation of ATP-sensitive potassium channels and to competitive blockade of the adrenergic, serotonin- and endothelin-pathways (Fig. 3A), while in the aorta of the same species they are caused partially by blockade of voltage-dependent calcium influx (Fig. 3B).

Upon chronic treatment with the compound, endothelial function improved in mesenteric arteries of ageing rats. This effect can, at least partially, be explained by an inhibition of oxidative stress and stabilization of the angiotensin system, leading to normalization of endothelium-dependent relaxations caused by both nitric oxide (NO) production or attributable to endothelium-dependent hyperpolarization (Fig. 4).

The facilitation of NO-dependent relaxations by chronic exposure to thymoquinone is explained, in the rat mesenteric artery, by chronic in vivo up-regulation of endothelial NO synthase (eNOS).

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at least to judge from the comparison of individual fluorescence intensities of the enzyme (Fig. 5)\(^{13}\).

In addition, acute exposure to the compound leads to \textit{in vitro} increased NO levels and eNOS activity in the rabbit aorta (exposed to pyrogallol to cause acute endothelial dysfunction)\(^ {9}\), in the rat aorta (Fig. 6A)\(^ {14}\) and in cultured human umbilical vein endothelial cells (Fig. 6B)\(^ {14}\).

The above discussed vasodilator and NO-promoting effects of thymoquinone suggest that the compound may reduce the risk for atherosclerosis, caused by endothelial dysfunction among other risk factors\(^ {15}\). In line with this suggestion, chronic treatment with thymoquinone of cholesterol-fed rabbits reduces the area of atherosclerotic lesions in their aortae\(^ {16}\).

### 3. Vasoconstrictor effects

In contrast to the relaxations observed in isolated rat arteries without endothelium, in contracted rings with endothelium of rat aortae, rat mesenteric arteries (Fig. 7A) and porcine coronary arteries (Fig. 7B), thymoquinone causes a sustained augmentation\(^ {12}\).

The augmentation by thymoquinone in preparations with endothelium is concentration-dependent (Fig. 8A) and requires previous activation of the contractile apparatus, as it is not present in quiescent preparations\(^ {12}\).

Endothelium-dependent contractions can be caused by increased production of vasoconstrictor prostanoids, endothelin-1 or augmented release of oxygen-derived free radicals\(^ {17-19}\). However, none of these mechanisms appear to account for the augmentation to thymoquinone\(^ {12}\). Counterintuitively, the vasoconstrictor responses to the quinone requires the presence of NO, since it is absent when endothelial NO production is inhibited (by endothelial removal or by inhibition of eNOS) and can be reinstalled by an exogenous NO donor in preparations without endothelium (Fig. 8B–C)\(^ {12}\).

The observation that thymoquinone augments the phosphorylation of eNOS at serine 1177, and hence increases the activity of the enzyme\(^ {15,20}\) in both isolated arteries and cultured endothelial cells of human umbilical veins (Fig. 6)\(^ {14}\), indicates that thymoquinone can stimulate the release of endothelium-derived NO needed for the vasoconstriction to occur.

Under most circumstances, NO is a potent endogenous vasodilator\(^ {15}\). Therefore, its involvement in an endothelium-dependent contraction seems paradoxical, to say the least. Soluble guanylyl cyclase (sGC) is the major downstream target of NO; its inhibition results in the lack of vasoconstrictor response to thymoquinone (Fig. 8B) and hence this enzyme must play a central role in the phenomenon\(^ {12}\). The canonical end-product of the enzymatic activity of sGC is guanosine 3’,5’-cyclic monophosphate (cyclic GMP). Production of the latter cannot explain the augmentations observed with thymoquinone, as its cell-permeable analogue, 8-bromo-cyclic GMP cannot restore the augmentations in preparations of either pig or rat arteries treated with a sGC-inhibitor\(^ {12}\). Soluble GC can synthetize cyclic nucleotides other than cyclic GMP; in particular inosine 3’,5’-cyclic monophosphate (cyclic IMP), using inosine triphosphate (ITP) as a substrate\(^ {21,22}\). With the use of ultra-high performance liquid chromatography (UPLC) coupled with tandem mass spectrometry (MS/MS), thymoquinone was found to increase the production of cyclic IMP (Fig. 9), but not that of cyclic GMP. The increase in intracellular cyclic IMP levels caused by the compound depends on prior eNOS- and sGC-activation (Figs. 9 and 10)\(^ {12}\). Several mechanisms appear to be involved in the cyclic IMP-mediated thymoquinone-induced augmentation; they differ depending on the blood vessel/species studied\(^ {12}\). In porcine coronary arterial smooth muscle, thymoquinone causes the activation of Rho-associated protein kinase (ROCK; an enzyme playing a key role in the phenomenon of calcium sensitization through inhibition of myosin light chain phosphatase)\(^ {23}\), therefore increasing the action of calcium ions on the contractile proteins; it also opens L-type calcium channels thereby increasing calcium influx.

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**Figure 4** Chronic effects of thymoquinone (TQ; 10 mg/kg/day by oral administration) on endothelium-dependent relaxations to acetylcholine in rat mesenteric arteries contracted to phenylephrine (PE) (From Idris-Khodja and Schini-Kerth\(^ {13}\), by permission). EDHF, endothelium-derived hyperpolarizing factor; NO, nitric oxide. Drug concentrations are given in M (mol/L).

**Figure 5** Chronic effects of thymoquinone (TQ; 10 mg/kg/day by oral administration) on endothelial nitric oxide synthase (eNOS) levels in rat mesenteric arteries, estimated from differences in individual fluorescence intensity (From Idris-Khodja and Schini-Kerth\(^ {13}\), by permission).
Figure 6 Western blotting of phosphorylated (at serine 1177, p-) eNOS and total (t-) eNOS in (A) rat aortae and (B) human umbilical vein endothelial cells exposed to $10^{-5}$ mol/L thymoquinone (TQ). A23, A23187 (calcium ionophore); ACh, acetylcholine (muscarinic receptor agonist); eNOS, endothelial nitric oxide synthase; Ctrl, negative control.

Figure 7 Original tracing of isometric tension recording in precontracted (A, with $10^{-6}$ mol/L phenylephrine) rat mesenteric arteries and (B, with $10^{-6}$ mol/L serotonin) porcine coronary arteries with endothelium exposed to thymoquinone ($3 \times 10^{-5}$ mol/L in rat mesenteric arteries, $10^{-5}$ mol/L in porcine coronary arteries). KCl, potassium chloride.

Figure 8 Experimental data of the study focusing on the mechanisms underlying thymoquinone-induced augmentations in isolated rat aortae during contractions to phenylephrine. Responses to increasing concentrations of thymoquinone in preparations (A, B) with and (A, C) without endothelium shown as (A) changes in tension as a percentage of the reference contraction to 60 mmol/L KCl or as (inset of A, B and C) areas under the curve of the contraction phase of the response. Areas under the curve were calculated for the contraction phase of the response to thymoquinone (i.e., $10^{-7}$–$10^{-4}$ mol/L) using Prism software (GraphPad software, San Diego, CA, USA). (Data from Detremmerie, by permission). DETA, DETA NONOate [nitric oxide (NO) donor]; E(+), with endothelium; E(−), without endothelium; L-NAME, $N\omega$-nitroarginine methyl ester [endothelial NO synthase (eNOS) inhibitor]; ODQ, 1H-[1,2,4]-oxadiazolo[4,3-a]quinazolin-1-one [soluble guanylyl cyclase (sGC) inhibitor]; YC-1, 1,3-(5ʹ-hydroxymethyl-2ʹ-furyl)-1-benzyl indazole (sGC activator). Drug concentrations are given in M (mol/L).
for the augmentation only\textsuperscript{12}. In the rat arterial smooth muscle, on the other hand, opening of T-type calcium channels contributes to calcium influx and hence contraction\textsuperscript{24}; thymoquinone causes augmentation by favoring this mechanism while activation of L-type calcium channels and ROCK do not seem to be involved (Fig. 11)\textsuperscript{12}. The difference in dependency on L-type (porcine coronary arteries) and T-type (rat aortae) calcium channels of the augmentation with thymoquinone confirms the different expression patterns of voltage-dependent calcium channels depending on the species or the vascular bed studied: L-type voltage-dependent calcium channels seem the major contributors to voltage-gated calcium entry in coronary myocytes\textsuperscript{25}, while in the rat aortae, both L-type and T-type voltage-dependent calcium channels are equally expressed\textsuperscript{26}.

4. Parallelism with hypoxic vasoconstriction

In addition to the pharmacological agent thymoquinone, an endothelium-dependent, NO-mediated augmentation of contraction has been demonstrated with acute hypoxia in isolated canine pulmonary, femoral and coronary arteries\textsuperscript{27–31} and in isolated porcine coronary arteries\textsuperscript{32,33}. There are several similarities in the characteristics of the augmentation by thymoquinone and of that in response to acute hypoxia (Table 1): augmentations to both stimuli (1) can be restored by exogenous NO and by stimulators of sGC in the absence of endothelium or eNOS activation\textsuperscript{12,30–33}; (2) do not necessitate the presence of the classical product of sGC, cyclic GMP; and (3) are associated with increased production of cyclic IMP\textsuperscript{12,32,33}, which is dependent on eNOS- and sGC-activation\textsuperscript{12,33}. It thus seems reasonable to conclude that cyclic IMP acts as the second messenger mediating the NO-dependent, biased sGC-dependent augmentation of vasoconstriction caused by both thymoquinone and acute hypoxia.

Although endogenous levels of ITP are lower than those of guanosine triphosphate under normal conditions\textsuperscript{34}, acute hypoxia
increases intracellular levels of ITP in smooth muscle cells of isolated porcine coronary arteries, as measured by UPLC–MS/MS. Therefore, as a mechanistic similarity exists between thymoquinone- and hypoxia-induced augmentations of contractions, thymoquinone also may increase the bioavailability of ITP in vascular smooth muscle cells, although actual measurements of the levels of the substrate remain to be performed. Despite the similarities, there are some differences between the augmentation by hypoxia and that by thymoquinone. While in porcine coronary arteries they both depend on calcium sensitization mediated by ROCK1,2,32,33, L-type calcium channels are involved only in thymoquinone-induced augmentation12. It is unknown whether or not T-type voltage-dependent calcium influx also plays a role in hypoxic vasoconstriction (Table 1).

5. Importance of the quinone moiety

Comparing the responses to thymoquinone with those to compounds with a similar chemical structure, namely 1,4-benzoquinone (Fig. 12) in rat arteries suggests that the quinone moiety is essential for evoking endothelium-dependent contractions that depend on activation of eNOS and sGC12. Quinones are substrates of NADPH:quinone oxidoreductase (NQO-1), an enzyme expressed abundantly in the vascular wall both at the endothelial15 and at the smooth muscle16 levels. The enzyme, by detoxifying endogenous and xenobiotic quinones and their derivatives, prevents the participation of these substances in redox cycling, hence playing an important role in the defense against oxidative stress17–19.

NQO-1 metabolizes thymoquinone, likely because of its structural resemblance to ubiquinone, the natural electron carrier in mitochondria20. Indeed, thymoquinone acts as an electron acceptor during the oxidation of nicotinamide adenine dinucleotide (NADH; an essential cofactor for NQO-1) to NAD1 + 21. NQO-1 regulates the NAD1 +/NADH ratio, the increase of which initiates a signalling cascade involving cluster of differentiation22 and cyclic adenosine diphosphate ribose (cADPR), resulting in calcium mobilization that in endothelial cells can activate eNOS by a protein kinase B/adenosine monophosphate-activated protein kinase-dependent mechanism23 or increase contractility of smooth muscle. Therefore, it is possible that the necessary eNOS-activation for the endothelium-dependent augmentation by thymoquinone to occur is caused at least in part by activation of endothelial NQO-1 upon binding with its substrate (Fig. 13). Whether or not, and how, activated NQO-1 in the vascular smooth muscle cells is involved in the biased activation of sGC remains to be resolved.

Table 1  Parallelism between thymoquinone-induced augmentation and hypoxic vasoconstriction.

| Mechanism          | TQ-induced augmentation | Hypoxic augmentation |
|--------------------|-------------------------|----------------------|
|                    | Rat arteries            | Porcine coronary arteries |
| Endothelium-dependency | ✓                     | ✓                        |
| eNOS-derived NO-dependency | ✓                     | ✓                        |
| sGC-dependency     | ✓                       | ✓                        |
| Cyclic IMP-dependency | ✓                     | ✓                        |
| ROCK-dependency    | ×                       | ✓                        |
| L-type VDCC-dependency | ×                     | ✓                        |
| T-type VDCC-dependency | ✓                     | ?                        |

eNOS, endothelial nitric oxide (NO) synthase; sGC, soluble guanylyl cyclase; ROCK, Rho-associated protein kinase; VDCC, voltage-dependent calcium channel. Adapted from Detremmerie et al.12, Chan et al.32 and Chen et al.33.

✓, involved; ×, not involved; ?, unknown.

Figure 12  Responses to increasing concentrations of (A) thymoquinone and (B) 1,4-benzoquinone in isolated rat aortae, precontracted with phenylephrine (1010–108 mol/L), shown as changes in tension as a percentage of the reference contraction to 60 mmol/L KCl. L-NAME, Nω-nitroarginine methyl ester (eNOS inhibitor); ODQ, 1H-[1,2,4]-oxadiazolo[4,3-a]quinoxalin-1-one (sGC inhibitor). (Data from Detremmerie et al.12, by permission.) Drug concentrations are given in M (mol/L).
6. Conclusions

Ex vivo studies in rat and porcine arteries reveal for quinones a novel and unique mechanism of action, favouring the occurrence of endothelium-dependent, NO- and sGC-mediated contractions, that depend on interference with calcium homeostasis, similar to that observed during hypoxic vasoconstriction. These contractions require a biased activation (by endogenous NO or synthetic activators) of sGC with subsequent production of cyclic IMP, as also demonstrated with acute hypoxia.

Quinone-induced augmentations likely involve activation of NQO-1, their metabolizing enzyme. The increase in NAD÷/NADH increases calcium mobility, favouring production of NO by eNOS which then diffuses to the underlying vascular smooth muscle cells to activate the biased sGC producing cyclic IMP, in turn facilitating contraction. In the absence of experiments performed with purified sGC, it is still uncertain whether or not the biased activity of the enzyme is due to a direct target effect of thymoquinone. Likewise, although the quinone moiety seems essential for thymoquinone-induced augmentations to occur, the link between NQO-1 activation by quinones and the alteration in sGC-activity resulting in contraction remains to be identified. Moreover, the importance (or not) of the augmenting effect of thymoquinone in vivo remains to be determined.

Hypoxic contractions may contribute to the increased prevalence of cardiovascular complications in cardiac patients with sleep apnea. By identifying the mechanisms underlying the endothelium-dependent augmentation caused by quinones and possibly other agents causing biased activity of sGC, the cellular targets involved in cardiovascular complications due to hypoxia can be studied, facilitating the development of novel strategies to prevent coronary hypoxic vasospasm.

Acknowledgments

This review is based in part on research conducted with financial support from the General Research Fund (17112914) of the Hong Kong Research Grant Council and from the Seed Funding Programme for Basic Research by University Research Committee of the University of Hong Kong (201511159163). The authors would like to thank Ms. Yee Har Chung and Mr. Godfrey Man for technical assistance and Ms. Ivy Wong for editorial assistance.

References

1. Ernster L, Dallner G. Biochemical, physiological and medical aspects of ubiquinone function. Biochim Biophys Acta 1995;1271:195–204.
2. Singh UP, Singh DP, Maurya S, Maheshwari R, Singh M, Dubey RS, et al. Investigation on the phenolics of some spices having pharmacotherapeutic properties. J Herb Pharmacother 2004;4:27–42.
3. Salem ML. Immunomodulatory and therapeutic properties of the Nigella sativa L. seed. Int Immunopharmacol 2005;5:1749–70.
4. Ali BH, Blunden G. Pharmacological and toxicological properties of Nigella sativa. Phytother Res 2003;17:299–305.
5. Butt MS, Sultan MT. Nigella sativa: reduces the risk of various maladies. Crit Rev Food Sci Nutr 2010;50:654–65.
6. Amin B, Hosseinzadah H, Black Cumin (Nigella sativa) and its active constituent, thymoquinone: an overview on the analgesic and anti-inflammatory effects. Plant Med 2016;82:8–16.
7. Khatab MM, Nagi MN. Thymoquinone supplementation attenuates hypertension and renal damage in nitric oxide deficient hypertensive rats. Phytother Res 2007;21:410–4.
8. El-Agamy DS, Nader MA. Attenuation of oxidative stress-induced vascular endothelial dysfunction by thymoquinone. Exp Biol Med 2012;237:1032–8.
9. Ghayur MN, Gilani AH, Janssen LJ. Intestinal, airway, and cardiovascular relaxant activities of thymoquinone. Evid Based Complement Altern Med 2012;2012:305319.
10. Darakshian S, Pour AB, Colagar AH, Sisakhtnezhad S. Thymoquinone and its therapeutic potentials. Pharmacol Res 2015;98–96:138–58.
11. Suddek GM. Thymoquinone-induced relaxation of isolated rat pulmonary artery. J Ethnopharmacol 2010;127:210–4.
12. Detremmerie CM, Chen Z, Li Z, Alkarhy KM, Leung SW, Xu A, et al. Endothelium-dependent contractions of isolated arteries to thymoquinone require biased activity of soluble guanylyl cyclase with subsequent cyclic IMP production. J Pharmacol Exp Ther 2016;358:558–68.
13. Idris-Khodja N, Schini-Kerth V. Thymoquinone improves aging-related endothelial dysfunction in the rat mesenteric artery. Naunyn-Schmiedeberg’s Arch Pharmacol 2012;385:749–58.
14. Detremmerie CM, Leung SW, Xu A, Gao Y, Vanhoutte PM. Biased activation of soluble guanylyl cyclase can cause contraction of isolated arteries. FASEB J 2016;30:11.
15. Vanhoutte PM, Zhao Y, Xu A, Leung SW. Thirty years of saying NO: sources, fate, actions, and misfortunes of the endothelium-derived vasodilator mediator. Curr Res 2016;119:375–96.
16. Nader MA, El-Agamy DS, Suddek GM. Protective effects of propolis and thymoquinone on development of atherosclerosis in cholesterol-fed rabbits. Arch Pharm Res 2010;33:637–43.
17. Tang EH, Leung FP, Huang Y, Feléoutou M, So KF, Man RY, et al. Calcium and reactive oxygen species increase in endothelial cells in response to releasers of endothelium-derived contracting factor. Br J Pharmacol 2007;151:15–23.
18. Vanhoutte PM, Tang EH. Endothelium-dependent contractions: when a good guy turns bad!. J Physiol 2008;586:5295–304.
19. Vanhoutte PM. Endothelium-dependent contractions in hypertension: when prostacyclin becomes ugly. Hypertension 2011;57:526–31.
20. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. Nature 1999;399:601–5.
21. Beste KY, Burchen H, Kaeve V, Stastch JP, Seifert R. Nucleotidyl cyclase activity of soluble guanylyl cyclase αβ, Biochemistry 2012;51:194–204.
22. Beste KY, Seifert R. cCMP, cUMP, cTMP, cIMP and cXMP as possible second messengers: development of a hypothesis based on studies with soluble guanylyl cyclase αβ, Biol Chem 2013;394:261–70.
23. Somlyo AP, Somlyo AV. Ca\(^{2+}\) sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase. *Physiol Rev* 2003;83:1325–58.
24. Duggan JA, Tabrizchi R. Influence of T-type Ca\(^{2+}\) (nifedipin) and Ca\(^{2+}\) (indanyloxycatic acid 94) channel antagonists on \(\alpha_1\)-adrenoceptor mediated contractions in rat aorta. *Can J Physiol Pharmacol* 2000;78:714–20.
25. Quignard JF, Frapier JM, Harricane MC, Albat B, Nargeot J, Richard S. Voltage-gated calcium channel currents in human coronary myocytes. Regulation by cyclic GMP and nitric oxide. *J Clin Investig* 1999;103:185–93.
26. Ball CJ, Wilson DP, Turner SP, Saint DA, Beltrame JF. Heterogeneity of L- and T-channels in the vasculature: rationale for the efficacy of combined L- and T-blockade. *Hypertension* 2009;53:654–60.
27. De Mey JG, Vanhoutte PM. Heterogeneous behavior of the canine arterial and venous wall. Importance of the endothelium. *Circ Res* 1982;51:439–47.
28. De Mey JG, Vanhoutte PM. Anoxia and endothelium-dependent reactivity of the canine femoral artery. *J Physiol* 1983;335:65–74.
29. Rubanyi GM, Vanhoutte PM. Hypoxia releases a vasoconstrictor substance from the canine vascular endothelium. *J Physiol* 1985;364:45–56.
30. Griseri T, Vanhoutte PM. Hypoxic contraction of canine coronary arteries: role of endothelium and cGMP. *Am J Physiol Heart Circ Physiol* 1991;261:H1769–77.
31. Pearson PJ, Lin PJ, Schaff HV, Vanhoutte PM. Augmented endothelium-dependent constriction to hypoxia early and late following reperfusion of the canine coronary artery. *Clin Exp Pharmacol Physiol* 1996;23:634–41.
32. Chan CK, Mak J, Gao Y, Man RY, Vanhoutte PM. Endothelium-derived NO, but not cyclic GMP, is required for hypoxic augmentation in isolated porcine coronary arteries. *Am J Physiol Heart Circ Physiol* 2011;301:H2313–21.
33. Chen Z, Zhang X, Ying L, Dou D, Li Y, Bai Y, et al. cIMP synthesized by sGC as a mediator of hypoxic contraction of coronary arteries. *Am J Physiol Heart Circ Physiol* 2014;307:H328–36.
34. Göttle M, Burhenne H, Sutcliffe D, Jinnah HA. Purine metabolism during neuronal differentiation: the relevance of purine synthesis and recycling. *J Neurochem* 2013;127:805–18.
35. Kim YH, Hwang JH, Noh JR, Gang GT, Kim DH, Son HY, et al. Activation of NAD(P)H:quinone oxidoreductase ameliorates spontaneous hypertension in an animal model via modulation of eNOS activity. *Cardiovasc Res* 2011;91:519–27.
36. Zhu H, Jia Z, Mahaney JE, Ross D, Misra HP, Trush MA, et al. The highly expressed and inducible endogenous NAD(P)H:quinone oxidoreductase 1 in cardiovascular cells acts as a potential superoxide scavenger. *Cardiovasc Toxicol* 2007;7:202–11.
37. Riley RJ, Workman P. DT-diaphorase and cancer chemotherapy. *Biochem Pharmacol* 1992;43:1657–69.
38. Ross D, Kepa JK, Winski SL, Beall HD, Anwar A, Siegel D. NAD(P)H:quinone oxidoreductase 1 (NQO1): chemoprotection, bioactivation, gene regulation and genetic polymorphisms. *Chem Biol Interact* 2000;129:77–97.
39. Han SJ, Kang ES, Kim HJ, Kim SH, Chun SW, Ahn CW, et al. The C609T variant of NQO1 is associated with carotid artery plaques in patients with type 2 diabetes. *Mol Genet Metab* 2009;97:85–90.
40. Sutton KM, Doucette CD, Hoksin DW, NADPH quinone oxidoreductase 1 mediates breast cancer cell resistance to thymoquinone-induced apoptosis. *Biochem Biophys Res Commun* 2012;426:421–6.
41. Staniek K, Gille L. Is thymoquinone an antioxidant?. *BMC Pharmacol* 2010;10: A9.