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Effects of phytoestrogens and 17β-estradiol on vasoconstriction elicited by reactive oxygen species

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To study the effects of different reactive oxygen species (ROS) on the resting tension of porcine coronary artery rings and to identify the effects of genistein (GEN), resveratrol (RES) and 17β-estradiol (EST) on ROS-elicited vasoconstriction, porcine coronary rings were prepared and mounted in an organ bath and, after an equilibration period, the changes induced by the drugs were observed. Rings with intact endothelium showed an obvious but slow contraction after treatment with xanthine (100 μM)/xanthine oxidase (20 mU · mL^{-1}) (X/XO) whereas endothelium-denuded rings showed no effects. H_2O_2 (200 μM) induced a fast and transient contraction in endothelium-denuded rings and failed to do so in intact-endothelium rings. Like superoxide dismutase (SOD, 200 U · mL^{-1}), GEN (1 μM) and RES (1 μM) significantly inhibited contractile response evoked by X/XO, however in contrast to GEN and RES, EST (1 μM) had no obvious effect. GEN (30 μM) and RES (30 μM), like catalase (CAT, 800 U · mL^{-1}), markedly attenuated the contraction elicited by H_2O_2. The results demonstrate that GEN and RES have distinct inhibitory effects on vasoconstriction induced by $O_2^{\cdot-}$ generated by X/XO and H_2O_2, and their actions are clearly greater than to that of EST.

1. Introduction

Some published studies have shown that reactive oxygen species (ROS) can stimulate signal transduction cascades, i.e., activation of transcription factor, gene expression, muscle contraction, cell growth, chemotaxis and apoptosis (Dalton et al. 1999; Shen et al. 1999), and ROS are probably mediators of physiological functions via their action as second messengers (Suzuki et al. 1997; Rhee et al. 1999). Also, there is evidence that ROS can contract blood vessels and increase the tension of blood vessels such as rat and rabbit aorta, canine and bovine coronary arteries, rat and bovine pulmonary arteries, human placental arteries, etc (Shen et al. 1999; Rodriguez et al. 1998; Mehta et al. 1991). ROS may therefore have very important effects in the regulation of vascular tone, but some unresolved questions still exist; for example, the precise physiological functions of ROS remain obscure. Moreover, generation of ROS can be triggered by various extracellular stimuli, which may have direct or indirect effects on cell functions (Finkel 1998). Some effects induced by ROS are harmful to the human body, and are believed to be associated with the pathogenesis of many diseases, particularly cardiovascular diseases (Shen et al. 1999). Therefore, it is very important to look for natural products which have a protective effect on the cardiovascular system and to study the relationship of their actions with ROS.

Genistein (GEN) and resveratrol (RES) are naturally occurring, plant-derived compounds, which are structurally and functionally similar to mammalian estrogens (EST). Many papers have reported that GEN, RES and EST can eliminate free radicals and display a strong antioxidant action (Leonard et al. 2003; Kim et al. 2002; Wei et al. 1993; Dantas et al. 2002). However, to date the effects of phytoestrogens and 17β-estradiol on reactive oxygen species-mediated vasoconstriction have not been reported. Therefore, the purpose of this work was to investigate the effects of GEN, RES and EST on arterial contractile response evoked by H_2O_2 and $O_2^{\cdot-}$ and the mechanisms underlying their actions.

2. Investigations and results

X/XO (X, 100 μM; XO, 20 U · mL^{-1}) caused a slow but obvious contraction in coronary arterial rings with intact-endothelium, the maximul tension was 678 ± 136 mg at 30 min after treatment with X/XO ($p < 0.001$, $n = 16$, Fig. 1 and Fig. 2),whereas endothelium-denuded rings showed no change.

Like SOD (200 U · mL^{-1}), GEN (1 μM) or RES (1 μM) were capable of inhibiting contractile responses induced by X/XO (100 μM), but EST (1 μM) had no evident effect (Fig. 2).

H_2O_2 (200 μM) induced a fast and transient contraction in endothelium-denuded rings. The maximum tension was 462 ± 71 mg ($p < 0.001$, $n = 12$, Fig. 3), However the tone of intact-endothelium rings only increased by 40 ± 10 mg after incubation with H_2O_2 ($p > 0.05$, $n = 10$).

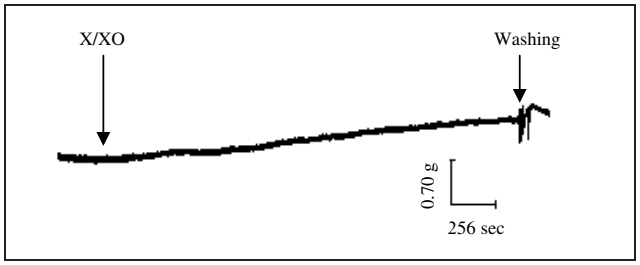

Fig. 1: Actual tracings to indicate the influence of xanthine/xanthine oxidase (X/XO, X: 100 μM; XO: 20 mU · mL^{-1}) on resting tension of porcine coronary arteries

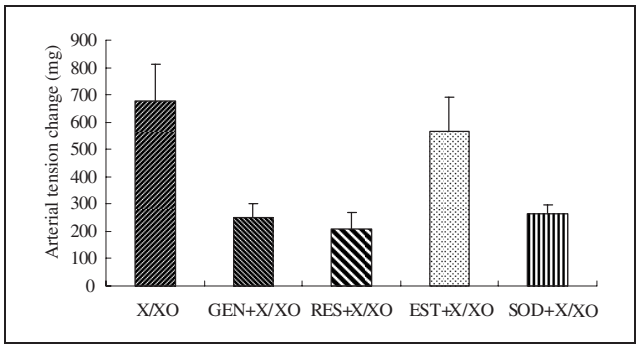

Fig. 2: Bar chart showing effects of genistein (GEN, 1 μM), resveratrol (RES, 1 μM) and 17β-estradiol (EST, 1 μM), superoxide dismutase (SOD, 200 U · mL^{-1}) on xanthine/xanthine oxidase (X/XO, X: 100 μM; XO: 20 mU · mL^{-1}) induced-contraction in porcine coronary arteries. Data are expressed as tension change (mg) (mean ± se, n = 12 ~ 18). ** $p < 0.01$ vs X/XO data

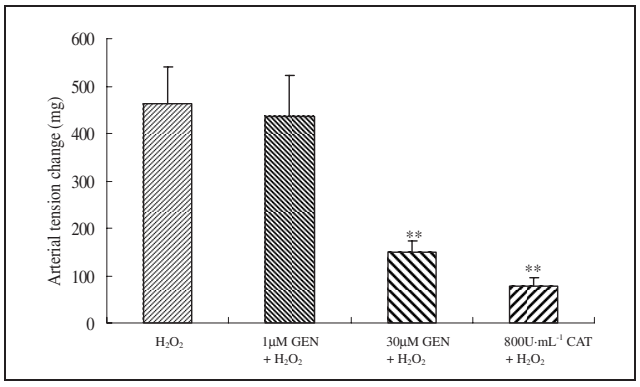

Fig. 3: Bar chart showing effects of genistein (GEN) and catalase (CAT) on H_2O_2 (200 μM) induced-contraction in porcine coronary arteries. Data are expressed as tension change (mg) (mean ± se, n = 12 ~ 18). ** $p < 0.01$ vs H_2O_2 data

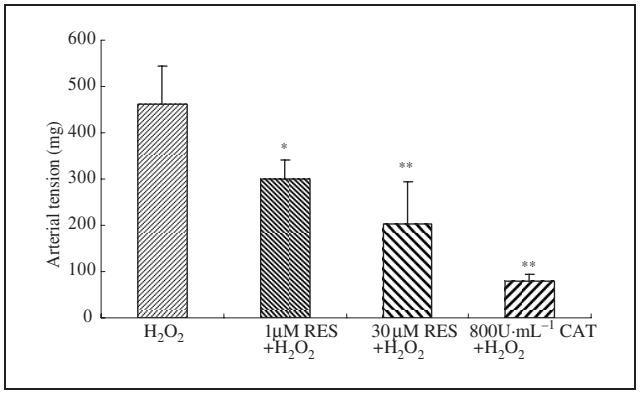

Fig. 4: Bar chart showing effects of resveratrol (RES) and catalase (CAT) on H_2O_2 (200 μM) induced-contraction in porcine coronary arteries. Data are expressed as tension change (mg) (mean ± se, n = 12 ~ 18). * $p < 0.05$, ** $p < 0.01$ vs H_2O_2 data

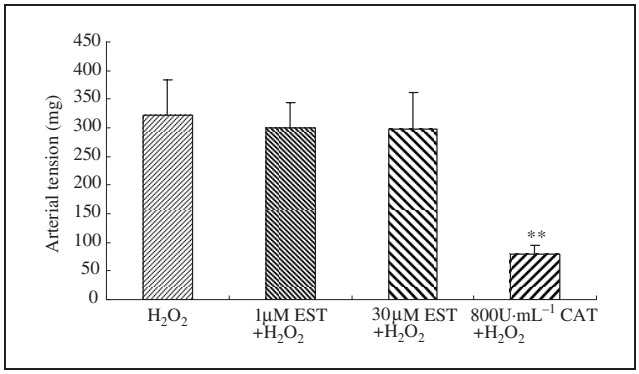

Fig. 5: Bar chart showing effects of 17β-estradiol (EST) and catalase (CAT) on H_2O_2 (200 μM) induced-contraction in porcine coronary arteries. Data are expressed as tension change (mg) (mean ± se, n = 12 ~ 18). ** $p < 0.01$ vs H_2O_2 data

Like CAT (800 U · mL^{-1}), GEN (30 μM, Fig. 3) and RES (1 μM and 30 μM, Fig. 4) respectively were able to remarkably inhibit contractile responses induced by H_2O_2 (200 μM) ($p < 0.01$, $n = 12$). However, GEN (1 μM, Fig. 3) and EST (1 μM and 30 μM, Fig. 5) had no obvious effect.

3. Discussion

Superoxide anion ($O_2^{\bullet-}$) can be generated from a system consisting of xanthine and xanthine oxidase, added to the bath solution simultaneously (Kimura et al. 2002; Elmoselhi et al. 1994; Grover and Samson 1987), and, accordingly, our experiment adopted this system as an $O_2^{\bullet-}$ donor. Some workers have reported that $O_2^{\bullet-}$ increases the arterial tone; however, it is not entirely clear if vessel contraction induced by $O_2^{\bullet-}$ is related to endothelium (Gryglewski et al. 1986). In our present study, the results showed that the contractile effect induced by $O_2^{\bullet-}$ was dependent on endothelium. We know, that the endothelium system is the biggest paracrine organ, releasing the endothelial-derived relaxing factor nitric oxide (NO), as well as the endothelial-derived contracting factor serotonin, both of which are thought to regulate the tone of blood vessels. $O_2^{\bullet-}$ can reduce the production of prostaglandin in endothelial cells, intensify thromboxane synthesis, eliminate NO, and cause arterial contraction (Peacock et al. 1990). At the same time, it may be pointed out that not only can $O_2^{\bullet-}$ reduce the effect of NO but also that the reactive products of the combination of $O_2^{\bullet-}$ and NO can cause multiple toxicity effects. Therefore, the proportion of NO and $O_2^{\bullet-}$ is crucial in controlling vessel physiological functions. Some workers consider that a high concentration of $O_2^{\bullet-}$ contracts blood vessels, or directly damages them, consequently induces piecemeal necrosis or vacuole necrosis and makes the activities of arterial rings lost (Miller et al. 1998; Kimura et al. 2002).

H_2O_2 can permeate membranes, stimulate the production of IP_3, and cause Ca^{2+} release from the endoplasmic reticulum. When the level of intracellular Ca^{2+} increases, contraction of vascular smooth muscle may occur. Studies have reported that H_2O_2 triggers vasoconstriction via the ATP receptor P_2 which includes two subtypes $P_{2\chi}$ and P_{2Y} (Shen et al. 2000; Michael et al. 1998). $P_{2\chi}$, a chemically-gated channel, distributes on the cellular membrane of vascular smooth muscle. It can open non-sensitive positive ion channels, make cells depolarized, then open voltage-dependent Ca^{2+} channels (VDCC) and elicit arterial contraction

which is not related to Gi/o protein and prostaglandin. On the other hand, P_{2Y} distributes on endothelium cells, and can not only couple with non-sensitive pertussis toxin sensitive (PTX) G protein, but also cause a vasorelaxant effect when it is activated by ATP (Michael et al. 1998). In the present study, it was found that there was a slight change in tension after treatment with H_2O_2 in arterial rings with endothelium. This phenomenon resembles results of Liu and Wang (2000) who found that 100 μM ATP failed to affect arterial tension in isolated rat aortic arteries with endothelium. Since ATP acts on $P_{2\chi}$ and P_{2Y} simultaneously, each subtype can cause oppsing changes in force which may counteract each other. Because H_2O_2 can act on either $P_{2\chi}$ or P_{2Y} receptors, it suggests that the same mechanisms probably mediate the effects of H_2O_2 and ATP on blood vessels.

GEN, RES and EST all have phenolic hydroxyl groups in their molecular structures, whose hydrogen atoms can break away from the oxygen atoms, forming hydrogen ions, exert deoxidization effects, and terminate chain reactions generated by free radicals. Furthermore, their ability to purge ROS is correlated with their content of phenolic hydroxyl group (Zhang et al. 2002) according to analysis of structure-activity relationships. Like SOD and CAT, GEN and RES can inhibit $O_2^{\bullet-}$ and H_2O_2 induced vasoconstriction, which is supported by an experiment by Wei et al. (1995) who found that GEN had a strong inhibitive effect on X/XO system since GEN (20 μM) can completely inhibit the X/XO system's ability to produce $O_2^{\bullet-}$. Therefore it is easy to understand that GEN may not only cut down $O_2^{\bullet-}$ production, but also in time scavenge $O_2^{\bullet-}$. RES is a lipid-soluble micromolecular substance which can pass through cellular membranes and scavenge intracellular ROS, and can also block the generation of ROS inside cells (Belguendouzl et al. 1998). Furthermore, RES (10 μg/ml) can increase endogenous SOD activity to 200.2%, which demonstrates that RES can possibly alter the intracellular oxidation-reduction status (Zhu et al. 2002). The above mechanisms may account for the inhibitory effects of the phytoestrogens GEN and RES on the contractile response induced by X/XO.

Antioxidant action may play an important role in the inhibitory effect of GEN on contractile responses to H_2O_2. Also, there is some evidence to indicate that there is phosphorization of tyrosine-receptors in H_2O_2-elicited vasoconstriction. A tyrosine-protein kinase inhibitor (AGI478) significantly reduced contraction induced by H_2O_2 in rat aortic rings (Yong-liang et al. 2002). The findings demonstrated that tyrosine-protein kinase system mediated contractile responses to H_2O_2. It is well known that GEN is an inhibitor of tyrosine-protein kinase (TPK), and therefore the present result of an inhibitory effect of GEN on H_2O_2-induced vasoconstriction may be related to its characteristic as a tyrosine-protein kinase inhibitor. Unfortunately there is a lack of direct evidence of the suppressing effect of RES on ROS-induced vasoconstriction, but in tumor investigations, RES shows a potent antitumour effect via TPK inhibition (Jayafilake et al. 1993; Meishiang et al. 1997). In addition, Slater et al. (2003) and Stewart et al. (1999) reported that RES was an inhibitor of protein kinase C (PKC). In view of the above discussion, one might speculate that RES probably reduced ROS-induced vasoconstriction via the inhibition of TPK and PKC, and its ROS scavenging characteristic.

In the present experiment, EST failed to affect the contractile responses elicited by X/XO and H_2O_2, which is probably accounted for by its lack of TPK inhibitory characteristic and the structural differences between EST, GEN, and RES, because the EST molecule only contains two phenolic hydroxyl groups, but the detail mechanisms involved are not completely understood yet, and need to be elucidated further. In conclusion, GEN and RES can decrease $O_2^{\bullet-}$ and H_2O_2 mediated-contractile responses, possibly by scavenging ROS, inhibiting their formation or on anti-oxidation effect; and therefore the phytoestrogens GEN and RES play a protective role in the cardiovascular system.

4. Experimental

4.1. ROS-generating systems and drugs

$O_2^{\bullet-}$ was generated from a system consisting of xanthine (X, 100 μM) and xanthine oxidase (XO, 20 mU · mL^{-1}), which were added to the bath solution simultaneously. The following drugs or chemicals were purchased from Sigma Chemical Co: genistein (GEN), 17β-estradiol (EST), resveratrol (RES), superoxide dismutase (SOD), catalase (CAT), xanthine (X), xanthine oxidase (XO), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and bradykinin. GEN, RES and EST were dissolved in dimethylsufoxide. Xanthine was dissolved in 5% NaOH solution, and then was titrated to an appropriate pH.

4.2. Tissue preparation

Hearts were collected from a local abattoir in cold, modified Krebs-Henseleit solution (composition in mM: 120 NaCl, 4.76 KCl, 1.18 $MgSO_4$, 1.25 $CaCl_2$, 25 $NaHCO_3$, 1.18 NaH_2PO_4 and 5.5 glucose). Left anterior descending coronary arteries were dissected free of fat and connective tissue and cut into 4 mm ring segments. Ring samples were then mounted on two stainless steel hooks in 5 ml organ baths at 37 °C, bubbled with 95% $O_2^{\bullet-}$ and 5% CO_2. Isometric tension generated by vascular smooth muscle was measured using a force transducer (JH-2) and recorded using the BL-420$^+$ Experimental System of Biological Function (TME, China) with an IBM computer.

4.3. Methods and protocols

Resting tension was set to 0.5–1.0 g in the preceding hour and 1.5 g during the following 30 min. After 1.5 h equilibration, rings were contracted with KCl (30 mM) in order to test their activities, and this viability test was repeated for each ring. In some rings, the endothelium was removed by gentle rubbing with a cotton cloth. Endothelial integrity was assessed pharmacologically by the ability of bradykinin (1 μM) to produce relaxation of tissues precontracted with $PGF_{2\alpha}$ (10 μM) (Li et al. 2006). The solution was changed every 20 min. After equilibration had stabilized, the experiments were performed as follows: (1) Intact-endothelium and endothelium-disrupted rings were subjected to H_2O_2 (200 μM) or X/XO (X, 100 μM; XO, 20 U · mL^{-1}), and then tension changes were observed. (2) After intact-endothelium rings were treated with GEN (1 μM), RES (1 μM), EST (1 μM) or SOD (100 U · mL^{-1}) separately, X/XO (X, 100 μM; XO, 20 U · mL^{-1}) was added to the bath, then changes of arterial tension were observed. (3) Endothelium-denuded rings were treated with GEN (1 μM and 30 μM), RES (1 μM and 30 μM), EST (1 μM and 30 μM) or SOD (800 U · mL^{-1}) separately, then H_2O_2 (200 μM) was added to the bath, and the changes of arterial tension were observed.

4.4 Statistical analysis

All of the data are expressed as mean ± se. Contractile responses induced by ROS are expressed as the value of tension (mg). Statistical analysis was performed using Student's t-test and analysis of variance (ANOVA). A probability level (P value) of less than 0.05 was considered significant.

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References

Belguendouz L, Fremont L et al. (1998) Interaction of transresveratrol with plasma lipoproteins. Biochem Pharmacol 55: 811–816.

Dalton TP, Shertzer HG et al. (1999) Regulation of gene expression by reactive oxygen. Pharmacol Toxicol 39: 67–101.

Dantas AP, Tostes RC et al. (2002) In vivo evidence for antioxidant potential of estrogen in microvessels of female spontaneously hypertensive rats. Hypertension 39: 405–411.

Darley-Usmar V, Weiseman H et al. (1995) Nitric oxide and oxygen radicals: a question of balance. FEBS Lett 369: 131–135

Elmoselhi A, Butcher A et al. (1994) Coronary artery contractility, Na (+)-pump and oxygen radicals. Gen Physiol Biophys 13: 247–256.

Finkel T. (1998) Oxygen radicals and signaling. Curr Opin Cell Biol 10: 248–253.
Grover AK, Samson SE (1987) Impurities in commercial xanthine oxidase inhibit Ca pump and interfere in contractility of pig coronary artery. Biochem Biophys Res Commun 143: 575–581.
Gryglewski RJ, Palmer RM et al. (1986) Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. Nature 320: 454–456.
Jayatilake GS, Jayasuriya H et al. (1993) Kinase inhibitors from *Polygonum cuspidatum*. J Nat Prod 56: 1805–1810.
Kim YK, Jang YY et al. (2002) Depressant effect of ambroxol on stimulated functional responses and cell death in rat alveolar macrophages exposed to silica in vitro. J Pharmacol Exp Ther 300: 629–637.
Kimura C, Cheng W et al. (2002) Superoxide anion impairs contractility in cultured aortic smooth muscle cells. Am J Physiol Heart Circ Physiol 283: H382–390.
Leonard SS, Xia C et al. (2003) Resveratrol scavenges reactive oxygen species and affects radical-induced cellular responses. Biochem Biophys Res Commun 309: 1017–1026.
Li HF, Tian ZF et al. (2006) A study of mechanisms involved in vasodilatation induced by resveratrol in isolated porcine coronary artery. Physiol Res 55: 365–372.
Liu SH, Wang H (2000) Pharmacological characteristics of P_2 purinoceptors in modulating vascular tone. Pharm J Chin PLA 16: 1–4.
Michael R, Boarder et al. (1998) The regulation of vascular function by P_2 receptors: multiple site and multiple receptors. Trends Pharm Sci 19: 99–107.
Mehta JL, Lawson DL et al. (1991) Role of superoxide radicals in anoxia reoxygenation-mediated vascular contraction. Life Sci 49: 1739–1746.
Meishiang J, Lining C (1997) Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275: 2188–2201.
Miller FJ, Gutterman DD et al. (1998) Superoxide production in vascular smooth muscle contributes to oxidative stress and impaired relaxation in atherosclerosis. Circ Res 82: 1298–1305.
Peacock AJ, Pickett C et al. (1990) Spontaneous hypoxaemia and right ventricular hypertrophy in fast growing broiler chicken reared at sea level. Comp Biochem Physiol 97(A): 537–541.
Rhee SG (1999) Redox signaling: hydrogen peroxide as intracellular messenger. Exp Mol Med 31: 53–59.
Rodriguez-Martinez MA, Garcia-Cohen EC et al. (1999) Changes in plasma oxidative state with age and their influence on contractions elicited by noradrenaline in the rat tail artery. Life Sci 65: 915–924.
Shen JZ, Zhang XF et al. (2000) Differential contractile actions of reactive oxygen species on rat aorta: Selective activation of ATP receptor by H_2O_2. Life Science 66: 291–296.
Slater SJ, Seiz JL et al. (2003) Inhibition of protein kinase C by resveratrol. Biochim Biophys Acta. 1637: 59–69.
Stewart JR, Ward NE et al. (1999) Resveratrol preferentially inhibits protein kinase C-catalyzed phosphorylation of a cofactor-independent, arginine-rich protein substrate by a novel mechanism. Biochemistry 38: 13244–13251.
Suzuki YJ, Forman HJ et al. (1997) Oxidants as stimulators of signal transduction, Free Radic Biol Med 22: 269–285.
Zhang HD, Zhang SH et al. (2001) Structure identification and antioxidative activity study on flavonoids in soy sauce. Journal of South China University of Technology 29: 86–89.
Zhang Y L, Shen J ZH et al. (2002) Role of EGF in the H_2O_2-induced aorta contraction of rat. Zhejiang Medicine 24: 407–409.
Zhu ZQ, Zhang XY et al. (2002) Free radical biological mechanism involved in the inhibition of tumor activity of human cervix cancer Hela cells in vitro, J East China Normal University 2: 97–103.
Wei H, Bowen R et al. (1995) Antioxidant and antipromotional effects of the soybean isoflovone genistein. Proc Soc Exp Biol Med 208: 124–130.
Wei H, Wei L et al. (1993) Inhibition of tumor promoter-induced hydrogen peroxide formation in vitro and in vivo by genistein. Nutr Cancer 20: 1–12.