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Baicalein impairs vascular tone in normal rat aortas: Role of superoxide anions

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Abstract

Acute exposure to the flavonoid baicalein inhibited endothelium-dependent relaxation in physiological arteries, although the mechanisms are not fully understood. We investigated the effect of baicalein on vascular tone in Wistar–Kyoto (WKY) rat isolated aortic rings in the presence and absence of oxidative stress to further determine the underlying mechanisms. Exposure to baicalein (10 μM) completely abolished endothelium-dependent relaxation induced by acetylcholine and attenuated significantly the endothelium-independent relaxation induced by sodium nitroprusside. Baicalein, similar to *N*ω-nitro-L-arginine methyl ester (L-NAME, 10 μM), potentiated significantly the contractile response of aortic rings to α_1-adrenoceptor agonist phenylephrine. In the presence of L-NAME the baicalein effect on phenylphrine contraction or acetylcholine relaxation was unaltered, suggesting that these effects of baicalein are (like L-NAME effect) endothelial nitric oxide synthase (eNOS)/endothelium-derived nitric oxide-dependent. Inhibition of cyclooxygenase activity with indomethacin (10 μM) or scavenging of superoxide anions with superoxide dismutase (150 units/ml), but not scavenging of hydrogen peroxide with catalase (800 units/ml), enhanced significantly by an essentially similar extent the relaxation to acetylcholine in baicalein-pretreated aortic rings. Relaxant effect to acetylcholine was significantly attenuated in control aortic rings, but was completely abolished in baicalein-pretreated aortic rings in the presence of reduced form of β-nicotinamide adenine di-nucleotide (β-NADH, 300 μM). Baicalein blocked β-NADH (300 μM)-induced transient contractions, suggesting that baicalein may have inhibited activity of NADH/NADPH-oxidase. Baicalein did not alter the failure of acetylcholine to induce relaxation in the presence of pyrogallol (300 μM). In summary, acute exposure to baicalein impairs eNOS/endothelium-derived nitric oxide-mediated vascular tone in rat aortas through the inhibition of endothelium-derived nitric oxide bioavailability coupled to reduced bioactivity of endothelium-derived nitric oxide and to cyclooxygenase-mediated release of superoxide anions.
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Keywords: Baicalein; Endothelium-derived nitric oxide; Superoxide anions; Vascular tone; Oxidative stress

1. Introduction

There is a growing interest in the antioxidant characteristics and use of dietary flavonoids in the management of vascular complications associated with several cardiovascular diseases (Hertog et al., 1993; Knekt et al., 1996). The vascular protective mechanisms of flavonoids are not completely understood. A significant line of evidence suggests that these beneficial effects may include an increased bioavailability of endothelium-derived nitric oxide, achieved *via* a decreased inactivation of endothelium-derived nitric oxide by superoxide anions and its reactive metabolites and/or an increased synthesis of nitric oxide from endothelium (Cook and Samman, 1996; Heijnen et al., 2000; Kris-Etherton and Keen, 2002). Baicalein exists extensively in plant-based human diets and is a potent free radical scavenger and antioxidant (Hamada et al., 1993; Shieh et al., 2000; Ajay et al., 2005). It exerts anti-hypertensive effects in renin-dependent hypertension (Takizawa et al., 1998), reduces hyperglycemia-induced proliferation and migration of vascular

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smooth muscle cells (Nishio and Watanabe, 1997), prevents oxidative stress-induced cell injury in cardiomyocytes (Shao et al., 2002), and reduces progression of angiogenesis *in vivo* (Liu et al., 2003). Recently, we also demonstrated that chronic oral administration of baicalein improved endothelium-dependent relaxation in spontaneously hypertensive rat aortas (Machha and Mustafa, 2005). In contrast, acute exposure to baicalein inhibited endothelium-dependent relaxation and enhanced receptor-mediated contractions in normal rat arteries, although the mechanisms are not fully understood (Chen et al., 1999; Tsang et al., 2000; Huang et al., 2004).

The aim of the present study was to examine the effect of baicalein on vascular tone in aortic rings isolated from Wistar–Kyoto (WKY) rats, and to characterize the mechanisms underlying these responses. For that purpose, the effects of baicalein on endothelium-dependent and -independent vascular relaxation induced by acetylcholine and sodium nitroprusside, respectively, and on α_1-adrenoceptor-mediated vascular contraction induced by phenylephrine were recorded in the presence and absence of various pharmacological interventions. In addition, the effect of baicalein on vascular tone in WKY rat isolated aortic rings in the presence of oxidative stress induced by superoxide anions was also investigated.

2. Materials and methods

2.1. Drugs and chemicals

The following drugs were used: acetylcholine chloride, baicalein, catalase, indomethacin, *N*ω-nitro-L-arginine methyl ester, phenylephrine-HCl, pyrogallol, reduced form of β-nicotinamide adenine di-nucleotide, superoxide dismutase (Sigma-Aldrich Co., St. Louis, Mo., USA), and sodium nitroprusside (BDH Limited, Poole, England). All the drugs were dissolved in distilled water with the exception of baicalein, which was dissolved in dimethyl sulfoxide (DMSO, final concentration <0.05% v/v), and indomethacin, which was dissolved in 0.5% w/v sodium carbonate.

2.2. Aortic ring preparation

All the experimental procedures were subjected to The University of Malaya Animal Experimentation Ethics Committee approval. Male WKY rats (20–21 weeks) were anaesthetized with pentobarbital (60 mg/kg of body weight, i.p.). The descending thoracic aorta was dissected out *via* a midline incision. After the surrounding fat and connective tissue had been carefully cleaned off, the aorta was cut into small rings (3–5 mm in width) and suspended between two L-shaped stainless steel hooks in a 5 ml organ bath. The organ bath was filled with normal Krebs physiological salt solution (KPSS) of the following composition (mM): NaCl 118.2, KCl 4.7, $CaCl_2{\cdot}2H_2O$ 2.5, KH_2PO4 1.2, $MgCl_2$ 1.2, glucose 11.7, $NaHCO_3$ 25.0, and EDTA 0.026. The bathing solution was gassed continuously with 95% oxygen and 5% carbon dioxide at 37 °C (pH=7.4). Isometric tension (g) was measured using a force displacement transducer connected to a Mac Lab recording system (ADI Instruments, Australia) coupled to a display monitor. Aortic rings were then progressively stretched to an optimal basal tension of 1 g and allowed to equilibrate for 45 min. During this period, the bathing solution was replaced every 15 min and, if needed, the basal tone was readjusted to 1 g. Aortic rings were then repeatedly stimulated with the high KCl solution (high K^+, 80 mM) for 5 min at 10 min intervals until two consecutive equal contractions were attained — evidence of tissue stability.

2.3. Experimental protocol

Following washout of high K^+ responses, the aortic rings were incubated for 20 min with baicalein (10 μM) or its vehicle, which served as control, and cumulative concentration–response curves to the endothelium-dependent and -independent relaxant agonists acetylcholine (10^{-10} to 10^{-5} M) and sodium nitroprusside (10^{-11} to 10^{-6} M), respectively, were performed on aortic rings which were pre-contracted with phenylephrine (1 μM). In another set of experiments the baicalein or its vehicle-treated tissues were exposed to graded concentrations of the α_1-adrenoceptor agonist phenylephrine (10^{-10} to 10^{-5} M). The baicalein concentration of 10 μM was chosen based on previous studies which examined the acute effects of baicalein on vascular responses in physiological rat arteries (Chen et al., 1999; Tsang et al., 2000; Huang et al., 2004). In addition, the physiological range of plasma concentration of flavonoids is reported to be usually between 0.1–10 μM (Scalbert and Williamson, 2000). Preliminary studies showed that baicalein in concentration of 10 μM or its vehicle did not modify basal tension of aortic rings during the time course of incubation, *i.e.*, before addition of the agonists.

In experiments to characterize the mechanism involved in the effects of baicalein, aortic rings were exposed to various pharmacological agents for 5 min before the incubation with baicalein or its vehicle. To examine the possible role of endothelium-derived nitric oxide, concentration–response curves to acetylcholine and phenylephrine were performed in aortic rings in which the activity of endothelial nitric oxide synthase (eNOS) was inhibited with *N*ω-nitro-L-arginine methyl ester (L-NAME, 10 μM). To characterize the involvement of cyclooxygenase products, superoxide anions and hydrogen peroxide, concentration–response curves to acetylcholine were performed in aortic rings pretreated with indomethacin (10 μM), superoxide dismutase (150 units/ml) and catalase (800 units/ml), respectively.

Lastly, to examine the effect of baicalein on endothelium-dependent relaxations in the presence of oxidative stress, concentration–response curves to acetylcholine were performed in aortic rings incubated with baicalein or its vehicle in the presence of reduced form of β-nicotinamide adenine di-nucleotide (β-NADH, 300 μM) or pyrogallol (300 μM). Pyrogallol or β-NADH was added to tissue bath 5 min before constricting the aortic rings with 1 μM phenylephrine.

2.4. Data presentation and statistical analysis

The concentrations indicated in the text or in the figures represent the final tissue-bath concentrations of the various

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