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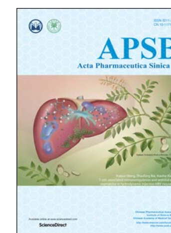

ORIGINAL ARTICLE

Mitochondrial uncoupler triclosan induces vasorelaxation of rat arteries

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KEY WORDS

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Abstract Our previous studies found that mitochondrial uncouplers induced vasodilation. Triclosan, the broad spectrum antibacterial agent, is the active ingredient in soaps and toothpastes. It was reported that triclosan induced mitochondrial uncoupling, so we aim to investigate the effects of triclosan on vascular function of rat mesenteric arteries and aorta. The isometric tension of rat mesenteric artery and thoracic aorta was recorded by multi-wire myograph system. The cytosolic $[Ca^{2+}]_i$, mitochondrial reactive oxygen species (mitoROS), and mitochondrial membrane potential of smooth muscle cells (A10 cells) were measured using laser scanning confocal microscopy. Triclosan treatment relaxed phenylephrine (PE)- and high K^+ (KPSS)-induced constriction, and pre-treatment with triclosan inhibited PE- and KPSS-induced constriction of rat mesenteric arteries. In rat thoracic aorta, triclosan also relaxed PE- and KPSS-induced constriction. Triclosan induces vasorelaxation without involving K_{ATP} channel activation in smooth muscle cells of arteries. Triclosan treatment increased cytosolic $[Ca^{2+}]_i$, mitochondrial ROS production and depolarized mitochondrial membrane potential in A10 cells. In conclusion, triclosan induces mitochondrial uncoupling in vascular smooth muscle cells and relaxes the constricted rat mesenteric arteries and aorta of rats. The present results suggest that triclosan would indicate vasodilation effect if absorbed excessively *in vivo*.

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1. Introduction

Triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl), a broad spectrum antimicrobial agent, has been widely used in soaps, mouthwashes, toothpastes and other products in household personal care and hospital applications. In addition to their antimicrobial effects, triclosan has multiple biological functions, including disrupting endocrine function[1] and suppressing the function of natural killer cell[2] and mast cells[3], and was reported inducing the mitochondrial uncoupling[4]. Because of the ubiquitous use, contamination of triclosan has been detected in different environmental matrices including terrestrial, aquatic and biosolids[5], thus the implication of triclosan pollution for human and environmental health has been concerned.

Mitochondrial uncoupling is a process of proton leaking from the intermembrane space into the mitochondrial matrix which prevents the development of a proton electrochemical gradient and reduces ATP production. Mitochondrial uncoupling is generally induced by mitochondrial uncoupling proteins (UCPs) or chemical mitochondrial uncouplers. Our previous studies found that mitochondrial uncouplers carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and niclosamide induced vasodilation of constricted arteries[6,7]. We put forward an opinion that the chemical mitochondrial uncouplers hold the properties of vasoactivity in common, in other words, chemical mitochondrial uncouplers relax the constricted arteries and prevent stimuli-induced artery constriction. Weatherly et al.[4] reported that triclosan induced mitochondrial uncoupling in living rat and human mast cells and in primary human keratinocytes. Based on our opinion, triclosan would induce vasorelaxation. Therefore the aim of the present work is to investigate the effects of triclosan on vascular function of rat mesenteric arteries and aorta.

2. Materials and methods

2.1. Animals and agents

The adult Sprague–Dawley rats (male, body weight 320–350 g, 8–10 weeks) were purchased from Charles River (Charles River Laboratory Animal, Beijing, China). All the experimental procedures were approved by the Institutional Animal Care and Use Committee of Harbin Medical University, China. Phenylephrine (PE) was purchased from Shanghai Harvest Pharmaceutical Co., Ltd., China. Triclosan were purchased from Shanghai Dibai biological technology Co., Ltd. (Shanghai, China). PE and acetylcholine (Ach) were dissolved in distilled water, and others were dissolved in DMSO (Tianjin Fuyu Fine Chemical Co., Ltd.).

2.2. Cell culture

Arterial smooth muscle cells (A10) were purchased from American Type Culture Collection. A10 cells were cultured in DMEM medium (high glucose) containing 15% FBS and 1% penicillin/streptomycin at 37 °C/5% CO_2. Cells were used within 8 passages.

2.3. Rat mesenteric artery and thoracic aorta preparation

Adult male Sprague–Dawley rats were sacrificed after anesthetized by sodium pentobarbitone (40 mg/kg, i.p.). The entire mesentery and thoracic aorta was removed quickly, then transferred into cold (4 °C) modified physiological salt solution (PSS) with the following composition (mmol/L): NaCl, 130; KCl, 4.7; $MgSO_4 \cdot 7H_2O$, 1.17; KH_2PO_4, 1.18; $NaHCO_3$, 14.9; $CaCl_2$, 1.6; D-glucose, 5.5 (pH 7.35–7.45). Fat tissues of mesenteric artery and thoracic aorta were separated. The mesenteric arteries and thoracic aorta were dissected into 2-mm and 3–4-mm rings, respectively.

2.4. Isometric tension recording of mesenteric artery and thoracic aorta

The experiments were carried out according to our previous work[6–9]. The mesenteric artery and thoracic aorta rings were randomized for different treatments. Mesenteric arterial ring was mounted between two wires, and fixed in the bath filled with 5 mL PSS and were continuously bubbled with gas (95% O_2 + 5% CO_2). Thoracic aortic rings were mounted in triangle-shape hook and then suspended in the bath filled with 10 mL PSS and were continuously bubbled with gas (95% O_2 + 5% CO_2). The isometric contractions of mesenteric arterial rings were measured by using multi wire myograph system (model 620 DMT, Danish Myo Technology, Denmark), and the isometric contractions of thoracic aortic rings were measured by using multi-channel myograph system (BL-420S, Chengdu Taimeng Software Co., Ltd., China). The arterial rings were equilibrated for 60 min before the experiment. The resting tension of mesenteric arterial ring was 0.6 mN and this value of thoracic aorta ring was 1.96 mN, which were the optimal preload for force development of the vessels determined in preliminary studies. Then a wake-up protocol was performed to reactivate the mechanical, function, and signaling properties of the vessels by using high K^+ PSS (KPSS) and phenylephrine (PE) stimuli. The KPSS (60 mmol/L K^+) solution inducing vasoconstriction was composed of (mmol/L): NaCl, 74.7; KCl, 60; $MgSO_4 \cdot 7H_2O$, 1.17; KH_2PO_4, 1.18; $NaHCO_3$, 14.9; $CaCl_2$, 1.6; D-glucose, 5.5; EDTA, 0.026. In order to avoid the error induced by natural rundown of the artery tension, we calculated the relaxation ratio of triclosan by subtracting the relaxation ratio of corresponding control (DMSO).

2.5. Measurement of mitochondrial reactive oxygen species

The methods in detail were described as in our previous studies[6–8]. Cultured arterial smooth muscle cells (A10) were loaded with MitoSOX (2 μmol/L) for 20 min and Hoechst (1 μg/mL) for 15 min at 37 °C and then the fluorescence was measured by using confocal microscopy. Confocal microscope images were collected by using a Zeiss LSM 700 with the Zeiss LSM software (Zeiss, Oberkochen, Germany). Images of MitoSOX fluorescence were obtained using a 40 × oil objective with an excitation at 555 nm and an emission of 585 nm. Images of Hoechst staining were obtained by using excitation at 405 nm and an emission of 435 nm. The levels of mitochondria ROS were represented by the relative intensity of fluorescence.

2.6. Measurement of cytosolic $[Ca^{2+}]_i$ of smooth muscle cells

The methods in detail were described as in our previous studies[6–8]. Cultured arterial smooth muscle cells (A10) were loaded with 5 μmol/L fluo-3/AM for 15 min (37 °C) and rinsed four times with warm PBS (37 °C). The fluorescence was measured by using confocal microscopy (Zeiss LSM 700). The excitation and

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