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Effect of celecoxib on cyclooxygenase-1-mediated prostacyclin synthesis and endothelium-dependent contraction in mouse arteries

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ABSTRACT

This study aimed to determine whether a cyclooxygenase-2 (COX-2) inhibitor celecoxib influences endothelium-dependent contraction independent of its action on COX-2 and, if so, the underlying mechanism(s). Abdominal aortas and/or carotid arteries from C57BL/6 mice or those with genetic COX-2 deficiency (COX-$2^{-/-}$) were isolated for functional and/or biochemical analyses. Result showed that following NO synthase inhibition celecoxib not only reduced the contraction evoked by acetylcholine in C57BL/6 abdominal aorta, but also that in COX-2 $^{-/-}$ mice showing a comparable magnitude. Notably, the IC_{50} of celecoxib obtained in COX-2 $^{-/-}$ abdominal aorta was only ~0.364 μM. Also, celecoxib exhibited a similar effect on COX-2 $^{-/-}$ carotid arteries. Interestingly, celecoxib was not only found to inhibit the production of the prostacyclin (PGI_2) metabolite 6-keto-PGF $_{1\alpha}$ in COX-2 $^{-/-}$ aortas, but also caused a reduction in the contraction evoked by PGI_2, by the α_1-adrenergic agonist phenylephrine, or by 30 mM K^+-induced depolarization in COX-2 $^{-/-}$ and/or C57BL/6 abdominal aorta. Moreover, N-[2-(cyclohexyloxy)-4-nitrophenyl]-methanesulfonamide (NS398), another COX-2 inhibitor, also reduced the contraction evoked by acetylcholine or by 30 mM K^+-induced depolarization in COX-2 $^{-/-}$ mice. These results demonstrate explicitly that in mouse arteries celecoxib not only inhibits COX-1-mediated synthesis of PGI_2 and probably some other prostanoids, but also causes a reduction in vessel contractility that is independent of either COX-2 or COX-1, leading to an inhibition of COX-1-mediated endothelium-dependent contraction with an IC_{50} value far below that of it considered for COX-1 . Also, our data suggest that such effects of celecoxib could be possibly shared by some other COX-2 inhibitors, such as NS398.

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1. Introduction

Cyclooxygenase-2 (COX-2) inhibitors have been designed to relieve symptoms of pain and inflammation in arthritis while minimize the gastrointestinal adverse effects associated with the traditional COX inhibitors, including indomethacin (Hinz et al., 2007). However, some COX-2 inhibitors, such as rofecoxib, have been suggested to cause increased incidence of cardiovascular events, due to their selective inhibition on endothelial prostacyclin (PGI_2) synthesis (FitzGerald and Patrono, 2001; Grosser et al., 2006; Hinz et al., 2007). On the contrary, celecoxib, a COX-2 inhibitor that is still in clinical usage, has been suggested to be less detrimental to the cardiovascular system (Hinz et al., 2007) or even to improve the outcome of cardiovascular event (Zhao et al., 2012). In fact, celecoxib has been found to improve endothelial dysfunction associated with diseases, such as hypertension (Chenevard et al., 2003; Hermann et al., 2003; Soloviev et al., 2011; Widlansky et al., 2003); however, the exact underlying mechanism behind this still remains to be elucidated clearly.

Endothelium-dependent vasoconstrictor activity, which is commonly associated with COX mediated metabolism of arachidonic acid and normally found in variety of species and vascular beds (Ansari et al., 2007; Derkach et al., 2000; Ihara et al., 2000, 2001; Okon et al., 2002; Traupe et al., 2002; Zhou et al., 2005), has been suggested to be implicated in endothelial dysfunction of diseases, such as hypertension (Gluais et al., 2005; Mundy et al., 2007; Traupe et al., 2002; Vanhoutte, 2011). Interestingly, PGI_2, a commonly recognized vasodilator, has been found to be the major endothelial COX-derived product produced in the vessel wall (Bolego et al., 2009; Bunting et al., 1976; Gluais et al., 2005; Liu et al., 2012b; Wong et al., 2009). Also, our recent studies suggest that both the PGI_2 (IP) and thromboxane-prostanoid (TP) receptors, which mediate the dilator or constrictor activity, respectively, concomitantly modulate the vasomotor reaction to PGI_2 (Liu et al., 2012a). Moreover, in some arteries, such as the mouse abdominal aorta where the expression or functional presence of IP receptor is limited, the process of PGI_2 synthesis itself is suggested to play an important part in endothelium-derived vasoconstrictor activity that blunts the dilation evoked by endothelial NO in health and disease

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condition (Gluais et al., 2005; Liu et al., 2012b; Vanhoutte, 2011). In addition, a major involvement of COX-1 in endothelium-dependent contraction or endothelial PGI_2 synthesis has been recognized in a variety of vascular beds, including small or large arteries from humans and animal models (Ansari et al., 2007; Gluais et al., 2005; Liu et al., 2012b,c; Mundy et al., 2007; Niwa et al., 2001; Sun et al., 2006; Tang et al., 2005; Traupe et al., 2002).

Interestingly, celecoxib has been reported to reduce endothelium-dependent contraction at 1–3 μM via inhibiting COX-2 mediated metabolism (Wong et al., 2009). Also, there are studies suggesting that this compound is able to decrease smooth muscle contractility (Brueggemann et al., 2009). The present study was thus to determine whether a similar concentration of celecoxib influences endothelium-dependent contraction independent of its action on COX-2 and if so, the underlying mechanism(s).

2. Material and methods

2.1. Chemicals and solution

N^{ω}-nitro-L-arginine methyl ester (L-NAME), phenylephrine, acetylcholine, the traditional COX inhibitor indomethacin, which has IC_{50} values of 0.018 and 0.026 μM for COX-1 and COX-2, respectively (Riendeau et al., 1997), and the COX-2 inhibitor celecoxib, which has IC_{50} values of 0.04 and 15 μM for COX-2 and COX-1, respectively (Penning et al., 1997), were purchased from Sigma (St Louis, MO). PGI_2, 6-keto-PGF $_{1\alpha}$ and the COX-1 inhibitor 1-[[4,5-bis(4-methoxyphenyl)-2-thiazolyl]carbonyl]-4-methyl-piperazine monohydrochloride (FR122047), which has IC_{50} values of 0.028 and 65 μM for COX-1 and COX-2, respectively (Ochi et al., 2000), and another COX-2 inhibitor N-[2-(cyclohexyloxy)-4-nitrophenyl]-methanesulfonamide (NS398), which has IC_{50} values of 0.004–0.006 and 1.9–3.2 μM for COX-2 and COX-1, respectively (Range et al., 2000; Riendeau et al., 1997), were purchased from Cayman Chemical (Ann Arbor, MI). L-NAME, phenylephrine, acetylcholine, and FR122047 were dissolved in distilled water, while PGI_2 was dissolved in carbonate buffer (50 mM; pH 10.5). Celecoxib, NS398, and indomethacin were dissolved in DMSO at 2000-fold of the final working concentration.

The compositions of PSS and 60 mM K^+-PSS (K^+) were as described previously (Liu et al., 2012b; Zhou et al., 2005).

2.2. Mice and tissue preparation

All procedures were in conformance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85–23 , revised 1996), and approved by the Institutional Animal Research and Use Committee of Shantou University.

C57BL/6 mice were obtained from SLAC (Shanghai, China). The heterozygous COX-2 deficient mice (COX-$2^{+/-}$; Mixed P129 and B6 background), kindly provided by Dr. Youfei Guan (Beijing University College of medicine), were cross-bred with C57BL/6 mice (for 9 generations) into C57BL/6 background. COX-$2^{-/-}$ mice were produced by cross-breeding of male COX-$2^{-/-}$and female COX-$2^{+/-}$ mice. Genotyping was performed by PCR of tail biopsy as described elsewhere (Liu et al., 2012b; Morham et al., 1995). All mice for experimental purpose were normal in appearance and did not show overt cardiovascular abnormality.

Male C57BL/6, or COX-$2^{-/-}$ mice (8–12 weeks) were euthanized by CO_2 inhalation. The aortas or carotid arteries where COX-1 has been found to mediate most prominent endothelium-dependent vasoconstrictor activities were isolated for experimental purposes (Liu et al., 2012b). With the assistance of binocular microscope, vessels were dissected free of adherent tissues. For functional analysis, the abdominal sections of aortas and carotid arteries were further cut into 1 mm rings as described previously (Liu et al., 2012b; Zhou et al., 2005).

2.3. Analyses of vascular response

The in vitro analyses of vascular function were performed with isometric force measurement as described elsewhere (Liu et al., 2012b; Zhou et al., 2005). Briefly, the vascular ring was mounted between two tungsten wires in a 37 °C water-circulating tissue bath. One wire was stationary, whereas the other was connected to a force transducer (AE 801, Horten, Norway). In some experiments, the endothelium was denuded by rotating the vascular ring around the tungsten wires with a passive tension kept at 100–150 mg (Liu et al., 2012b; Zhou et al., 2005). Thereafter, vessels were stimulated with 60 mM K^+ every 15 min, and the resting tension increased in a stepwise manner. After the equilibration, the resting tension was adjusted to an optimal level (~300 mg for abdominal aorta and 250 mg for carotid arteries), at which point the response to 60 mM K^+ was maximal and reproducible.

Unless otherwise indicated, experiments were performed in the presence of NO synthase (NOS) inhibitor L-NAME (1 mM), which we previously showed to modify the response of mouse artery in a similar manner to endothelial NOS deficiency (Zhou et al., 2005). Inhibitors (L-NAME, celecoxib, indomethacin, FR122047, or NS398) were added 30 min before the vessel was contracted with an agent, and were kept in the solution throughout the experiment. The contraction elicited by an agent was expressed relative to that evoked by 60 mM K^+.

2.4. Assay of 6-keto-$PGF_{1\alpha}$

The in vitro production of the PGI_2 metabolite 6-keto-$PGF_{1\alpha}$ was determined by high performance liquid chromatograph–mass spectroscopy (HPLC-MS) slightly modified from the protocol we described previously (Liu et al., 2012b,c). Briefly, aortas were cut open and rinsed with PSS. Aortic strips (2 whole sections of aortas were pooled for each single measurement) were incubated with PSS at 37 °C for 30 min, and then sequentially exposed to 1 ml PSS and that containing 10 μM acetylcholine (37 °C) for 15 min (5 times of the half-life time for PGI_2 to convert to 6-keto-$PGF_{1\alpha}$) each. The solvent or celecoxib was added during 30 min incubation, and was kept in the solution throughout the subsequent 15-min exposure to PSS and that containing 10 μM acetylcholine. Samples or standards (25 ng dissolved in 1 ml PSS) were extracted by solid phase extraction cartridges (SPE, ProElut™C18, size 100 mg; Dikma Technologies, Lake Forest, CA, USA) followed by the detection with HPLC–MS . The HPLC system (Agilent 1100, Agilent Technologies, Santa Clara, CA, USA) was equipped with an HPLC column (Symmetry® C18, 3.5 μm, 2.1 × 100 mm^2, Waters, Milford, MA, USA) with the column chamber set at 40 °C. The mobile phase was methanol/10 mM ammonium acetate (50/50, pH 7.5) with a flow rate of 0.2 ml/min. The mass spectrometer (Q Trap; Applied Biosystems, Carlsbad, CA, USA) was equipped with an electrospray ionization ion source. The amount of 6-keto-PGF $_{1\alpha}$ was calculated from the area of signal relative to that of standards, and was expressed in nanogram per milligram of dried tissues.

2.5. Western blot

The expressions of COX-1, COX-2, PGI_2 synthase (PGIS), and β-actin were detected with Western blot. For protein preparation, abdominal aortas or lung tissues were minced in ice-cold RIPA buffer containing proteinase inhibitor cocktail (Roche Applied Science; Germany) followed by homogenizing using a glass homogenizer. Anti-COX-1 (polyclonal; rabbit; 1:1000 dilution),

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