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Short communication

Cyclooxygenase-independent inhibition of smooth muscle cell mitogenesis by ibuprofen

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Abstract

The aryl-propionic acid derivative, ketoprofen, has been shown to inhibit fibroblast growth by a cylooxygenase-dependent mechanism [Sánchez, T., Moreno, J.J., 1999. *S*(+) enantiomer inhibits prostaglandin production and cell growth in 3T6 fibroblast cultures. Eur. J. Pharmacol. 370, 63–67]. The present study demonstrates that ibuprofen, another aryl-propionic acid derivative, inhibited platelet-derived growth factor-BB (20 ng/ml)-induced mitogenesis of cultured bovine coronary artery smooth muscle cells in a stereo-independent manner. In addition, pretreatment of the cells with indomethacin (3 μM) did not affect the inhibitory effects of ibuprofen enantiomers on smooth muscle cell mitogenesis. Thus, aryl-propionic acid-type cyclooxygenase inhibitors can inhibit cell proliferation by both, cyclooxygenase-dependent and -independent ways.

Keywords: Ibuprofen; Cyclooxygenase; Smooth muscle cell; Proliferation

1. Introduction

Prostaglandins are important modulators of cell proliferation (reviewed in Schrör and Weber, 1997). The generation of prostaglandins is regulated by the activity of cyclooxygenase (prostaglandin endoperoxide H synthase; 1.14.99.1) which exists in two isoforms referred to as cyclooxygenase-1 and -2, respectively (Otto and Smith, 1995). In vascular smooth muscle cells, cyclooxygenase-2 can be upregulated by growth factors, such as platelet-derived growth factor (Weber et al., 1998).

In a recent paper, Sánchez and Moreno (1999) demonstrated that inhibition of fibroblast mitogenesis by the non-steroidal anti-inflammatory drug, ketoprofen, is related to its cyclooxygenase-inhibitory effects. The present study demonstrates that ibuprofen, another aryl-propionic acid derivative, inhibits vascular smooth muscle cell mitogenesis by a cyclooxygenase-independent mechanism.

2. Materials and methods

2.1. Materials

Cell culture media were from Life Technologies (Karlsruhe, Germany). [^{3}H]thymidine was from DuPont NEN (Dreieich, Germany). *R*(−), *S*(+), and racemic ibuprofen were gifts from Dolorgiet-Arzneimittel (St. Augustin, Germany). All other chemicals were from Sigma (Deisenhofen, Germany).

2.2. Cell culture

Coronary artery smooth muscle cells were isolated enzymatically from the left anterior descending coronary artery of adult cattle and cultured as previously described (Zucker et al., 1998). The cells were cultured in a 80% Ham's F-12/20% Dulbecco's modified Eagle's medium supplemented with 15% fetal calf serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin. The media were exchanged twice a week. Smooth muscle cells were passaged once a week using trypsin- ethylenediaminetetraacetic acid (0.05%/0.5 mM).

2.3. DNA synthesis

DNA synthesis was measured as described previously (Bönisch et al., 1998). Briefly, smooth muscle cells were

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seeded in 24-well plates and cultured for 72 h under standard conditions. Cells were serum-deprived for 72 h in order to allow defined stimulation with platelet-derived growth factor-BB (PDGF, 20 ng/ml). Cells were incubated with indomethacin or ibuprofen for 15 min prior to stimulation with PDGF, as indicated. After 20 h, cells were pulsed with [^{3}H]thymidine (0.5 μCi/well) for 4 h. Subsequently, the media were removed and the cells were washed twice with 1 ml of ice-cold phosphate buffered saline, 0.3 ml of ice-cold perchloric acid (0.3 M) and again with ice-cold phosphate buffered saline. The cells were solubilized with 0.3 ml NaOH (0.1 M) for 30 min at 37°C. [^{3}H]thymidine incorporation was determined by liquid scintillation counting.

2.4. Measurement of 6-keto-prostaglandin $F_{1\alpha}$

Smooth muscle cells were serum-deprived for 72 h and then stimulated with PDGF for 4 h in the absence and presence of indomethacin (3 μM), respectively. 6-keto-prostaglandin $F_{1\alpha}$ was determined in the supernatants by radioimmunoassay as previously described (Schrör and Seidel, 1988).

2.5. Cytotoxicity assays

Possible cytotoxic effects of ibuprofen (24 h incubation) were studied using ethidium homodimer-1 and calcein acetoxymethyl ester fluorescence assays (LIVE/DEAD Viability/Cytotoxicity Kit for animal cells, Molecular Probes, Eugene, OR, USA) according to the manufacturer's instructions. Membrane-permeant calcein acetoxymethyl ester is cleaved by esterases in live cells to yield green fluorescence, and membrane-impermeant ethidium homodimer-1 labels nucleic acids of membrane-compromised cells with red fluorescence. Cells were analyzed using a fluorescence microscope (IX-50, Olympus Optical, Hamburg, Germany). At the maximal concentration used, no cytotoxic effects were observed (not shown).

2.6. Statistics

Data are means ± S.E.M. from n experiments. Statistical analysis was performed using one-way analysis of variance followed by Bonferroni multiple comparisons test. $P < 0.05$ was considered significant.

3. Results

In the absence of indomethacin, ibuprofen concentration-dependently inhibited platelet-derived growth factor-BB-induced DNA synthesis in cultured vascular smooth muscle cells. $R(-)$ enantiomer, $S(+)$ enantiomer, and racemic ibuprofen were equally effective in inhibiting smooth muscle cell mitogenesis. In the presence of indomethacin (3 μM), platelet-derived growth factor-induced DNA synthesis was not changed. Indomethacin did not affect the inhibitory effects of ibuprofen enantiomers on smooth muscle cell mitogenesis. The data are summarized in Fig. 1.

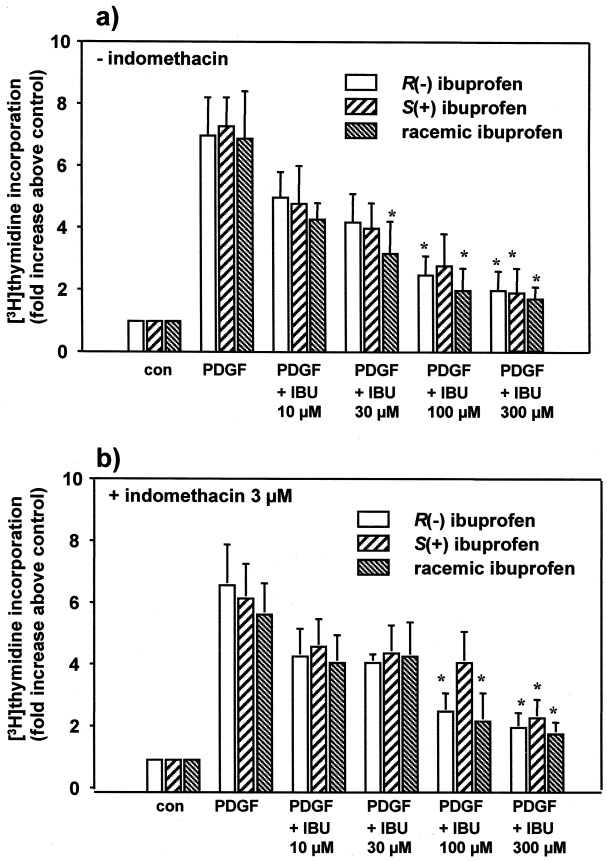

Fig. 1. Effects of $R(-)$, $S(+)$ and racemic ibuprofen (10–300 μM) on platelet-derived growth factor-BB (PDGF, 20 ng/ml)-induced DNA synthesis in cultured bovine coronary artery smooth muscle cells in the absence (a) and presence of indomethacin (3 μM) (b). Basal [^{3}H]thymidine incorporation in control cells (1231 ± 366 dpm/well) was not altered by indomethacin treatment (1558 ± 471 dpm/well). Data are means ± S.E.M. of $n = 4$ independent experiments, $^*P < 0.05$ vs. PDGF.

Under basal conditions, 6-keto prostaglandin $F_{1\alpha}$ accumulated to a concentration of 139 ± 40 pg/ml in cell supernatants ($n = 3$). Platelet-derived growth factor-BB significantly increased the concentration of 6-keto prostaglandin $F_{1\alpha}$ to 505 ± 73 pg/ml ($n = 3$, $P < 0.05$). In the presence of indomethacin (3 μM), the concentration of 6-keto prostaglandin $F_{1\alpha}$ was reduced by > 90% (29 ± 19 pg/ml, $n = 3$, $P < 0.05$).

4. Discussion

This study provides evidence for cyclooxygenase-independent inhibition of vascular smooth muscle cell mitogenesis by ibuprofen.

The aryl-propionic acid derivative, ibuprofen, is a chiral compound. Inhibition of cyclooxygenase resides almost

exclusively in the *S*(+) enantiomer (Adams et al., 1976; Geisslinger et al., 1989; Kean et al., 1991; Neupert et al., 1997). Accordingly, *S*(+) ibuprofen was about 15-fold more potent in inhibiting platelet thromboxane B_2 formation as compared to its *R*(−)enantiomer (Villanueva et al., 1993). In vascular smooth muscle cells, however, the inhibitory effects of ibuprofen on platelet-derived growth factor-BB-induced DNA synthesis were not stereoselective. These findings are in line with our previously published data, demonstrating non-stereoselective inhibition of neutrophil function by ibuprofen (Villanueva et al., 1993). Similarly, both *R*(−) and *S*(+) ibuprofen were effective in inhibiting nuclear factor-κB activity (Scheuren et al., 1998). In addition, neither the stimulatory effects of platelet-derived growth factor-BB on smooth muscle cell mitogenesis nor the inhibitory effects of ibuprofen were affected by indomethacin (3 μM), i.e., at a concentration which significantly (> 90%) inhibited prostaglandin formation. Therefore, the inhibitory effects of ibuprofen on platelet-derived growth factor-BB-induced DNA synthesis appear not to be related to cyclooxygenase inhibition.

In a recent study, Sánchez and Moreno (1999) demonstrated that ketoprofen, which is also an aryl-propionic acid-type cyclooxygenase inhibitor, inhibited fibroblast mitogenesis in a stereoselective manner. Thus, different cell types (e.g., fibroblasts vs. smooth muscle cells) may differ with respect to the involvement of endogenous prostaglandins in growth control. Non-steroidal anti-inflammatory drugs, such as aryl-propionic acid derivatives, inhibit cell proliferation by both, cyclooxygenase-dependent and -independent ways.

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