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Short communication

Selective cyclooxygenase inhibitors prevent the growth of *Chlamydia pneumoniae* in HL cells

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Abstract

The effects of the selective cyclooxygenase (COX) inhibitors SC-560 and PTPBS were studied in *Chlamydia pneumoniae*-infected HL cell cultures. *Chlamydia pneumoniae* growth and viability were assessed by quantifying inclusions and re-passages. COX-1 and COX-2 mRNA expression in HL cells during chlamydial infection was quantified with real-time polymerase chain reaction. SC-560 (10 μg/mL) and PTPBS (18 μg/mL) completely inhibited the growth of *C. pneumoniae* and the effect was dose-dependent between 4–9 μg/mL and 2–16 μg/mL, respectively. Inclusion size was reduced from 11.5 ± 1.3 μm to 1.9 ± 0.7 μm in the presence of the drugs. Removing the drugs returned the size to normal and increased the number of inclusions. Selective COX inhibitors appear to have a chlamydiostatic but not chlamydiacidic effect; they inhibit the growth of *C. pneumoniae* in vitro but do not prevent infection or eradicate *C. pneumoniae* from host cells.

Keywords: *Chlamydia pneumoniae*; Cyclooxygenase (COX); Real-time PCR

1. Introduction

Cyclooxygenase (COX) catalyses the biosynthesis of prostacyclin and thromboxane A_2, which are associated with atherosclerosis [1]. COX has two isoforms: COX-1 is constitutively expressed under normal physiological conditions, but the expression of COX-2 is induced by inflammation [2,3]. Both isoforms have been found expressed or overexpressed in human atherosclerotic lesions, and COX-2 is expressed only in atheromatous but not in normal arteries [3,4], implying that inhibition of COX-2 may be beneficial in atherosclerosis [3]. Selective COX inhibitors suppress the two isoforms respectively, thus reducing the production of prostacyclin and thromboxane A_2. This might consequently reduce the progression of atherosclerosis by decreasing vascular inflammation [5]. Clinical applications of COX inhibitors in the treatment of atherosclerosis and the prevention of cardiac events are still under debate [5,6]. Several lines of evidence suggest that chronic *Chlamydia pneumoniae* infection is associated with coronary heart disease and atherosclerosis [7]. Very little is known about the effects of the COX inhibitors on *C. pneumoniae* infection. Here, we studied the effects of selective COX inhibitors on *C. pneumoniae* in vitro.

2. Materials and methods

2.1. Cell line and organisms

HL cells were used for the propagation of *C. pneumoniae* and the experiments with different drugs. *Chlamydia pneumoniae* Kajaani 7 (K7) [8] was propagated in HL cells and purified through Urografin gradients. Finally, the obtained pellet was re-suspended in sucrose–phosphate–glutamic acid buffer (0.2 M sucrose, 3.8 mM KH_2PO_4, 6.7 mM Na_2HPO_4, 5 mM L-glutamic acid (pH 7.4)) and stored at −70 °C in aliquots until used.

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2.2. Reagents

The selective COX-1 inhibitor SC-560 and the selective COX-2 inhibitor PTPBS were obtained from Calbiochem (Darmstadt, Germany). The inhibitors were dissolved in dimethyl sulfoxide (DMSO) to make stock solutions (5 mg/mL) and kept in the dark. Phosphate-buffered saline (PBS) (pH 7.2) was used to dilute the stock solution.

2.3. Inhibition assays of infectivity and growth

Approximately 3×10^6 HL cells were grown on sterile glass coverslips (diameter 13 mm) in 24-well culture plates (Nunc) for 24 h to reach confluent cell monolayers before inoculation. All wells were inoculated with the same amount of chlamydial elementary bodies (EBs) to achieve ca. 200–300 inclusions on each coverslip. SC-560 or PTPBS at concentrations of 0.01–20 μg/mL was added to the cells. The plates were incubated at 4 °C for 2 h without centrifuging and then washed three times with ice-cold PBS. Fresh culture medium supplemented with 0.5 μg/mL of cycloheximide and drugs in the abovementioned concentrations was added to the wells. The cells were then incubated for 72 h at 35 °C in 5% CO_2 and the culture medium was refreshed every 24 h.

2.4. Recovery of C. pneumoniae after treatment

After 72 h incubation, culture medium was refreshed with medium without any COX inhibitors and incubation was extended for another 72 h. The presence of viable EBs after 72 h of COX inhibitor treatment was studied by re-passaging the infected cells into fresh HL cell monolayers with centrifuging ($480 \times g$ for 1 h) and culturing for another 72 h in medium without COX inhibitors. The inclusions were counted after both the first and the second 72-h period and compared with each other.

Control infections were performed without COX inhibitors for each experiment, but the effect of the same amount of diluted DMSO was tested.

2.5. Chlamydia inclusion counts

Infected cells were fixed with 100% methanol and stained with a fluorescein isothiocyanate (FITC)-conjugated murine monoclonal antibody genus-specific to *Chlamydia* (Pathfinder Chlamydia Culture Confirmation System; Bio-Rad S.A., Redmond, WA). All inclusions on the coverslips were counted using a fluorescent microscope (Axioskop 2; Carl Zeiss, Jena, Germany). Inclusion counts on the control infection coverslips were considered as 100% and were compared with the inclusion counts in other cells.

2.6. Confocal microscopy of the morphology of inclusions

Morphological differences of the inclusions stained as above were examined under a confocal microscope (LSM 510; Carl Zeiss).

2.7. Quantification of COX-1 and COX-2 mRNA expression

2.7.1. RNA purification and cDNA construction

Chlamydial EBs (3–4 inclusion-forming units/cell) were inoculated into HL cell monolayers in a 24-well culture plate by centrifuging for 1 h with 7 μg/mL SC-560, 12 μg/mL PTPBS or without inhibitors. Control cells were left uninfected. After inoculation, the cells were washed and incubated as described above for 6 h (the expression of COX-2 may crest at this time point [9]) but without cycloheximide and with or without the abovementioned drug concentrations. Both infected and uninfected cells were collected with RNA*later* RNA Stabilization Reagent, and RNase-Free DNase Set and RNeasy Kit (Qiagen, Hilden, Germany) were used to isolate total RNA according to the manufacturer's instructions. cDNA was constructed by reverse transcription of the isolated RNA with the Omniscript RT Kit (Qiagen) according to the manufacturer's protocol. From each well, 10 μL of RNA was isolated and 6 μL was transcribed into cDNA.

Table 1
Primers and probes used in the polymerase chain reaction

Primer/probe	COX-1	COX-2	PBGD[a]
Forward primer	5′-CCAGGAGCTCGTAGGAG AGAAG-3′	5′-CGACTCCCTTGGGTGTC AAAGGTAA-3′	5′-AGAGTGATTCGCGTGG GTACC-3′
Reverse primer	5′-GTGGCCGTCTTGACAA TGTTAA-3′	5′-AAAGACCTCCTGCCCCACAGCAA-3′	5′-GGCTCCGATGGTGA AGCC-3′
Probes			
Fluorescein	5′-GCACTTTTCAAGAAGCAGTCC AGGGTAG-FL-3′	5′-CCTCCCACAGTCAAAGATACTCAG GCAGAGATG-FL-3′	5′-AGTGGACCTGGTTGTTC ACTCCTTGAA-FL-3′
LC-Red640	5′-LC Red640-ACTCCAACGCATCAATGTC TCCATACAA-p-3′	5′-LC Red640-CTACCCTCCTCAAGTCCCTG AGCATCTACG-p-3′	5′-LC Red640-ACCTGCCCACTGTGCTTCCTCCT-p-3′

[a] Housekeeping porphobilinogen deaminase gene.