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15-Deoxy-delta12,14-prostaglandin-J_2 up-regulates cyclooxygenase-2 but inhibits prostaglandin-E_2 production through a thiol antioxidant-sensitive mechanism

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ABSTRACT

15-Deoxy-delta12,14-prostaglandin-J_2 (15d-PGJ_2) has potent anti-inflammatory effects including the inhibition of interleukin-1β (IL-1β)-induced expression of cyclooxygenase-2 (COX-2) and prostaglandin E_2 (PGE_2) production in several cell types. 15d-PGJ_2 contains an α,β-unsaturated electrophilic ketone and several evidences suggest that thiol reducing agents prevent or revert the cellular effects of 15d-PGJ_2. The present study was devoted to analyze the effect of 15d-PGJ_2 on COX-2 expression in cultured human mesangial cells (HMC). 15d-PGJ_2 induced an increase in the reduced glutathione (GSH) content and up-regulated COX-2 protein expression, but not COX-1, in a manner which was unaffected by selective peroxisome proliferator-activated receptor γ (PPARγ) blockade nor mimicked by ciglitazone, a PPARγ agonist. *N*-acetylcysteine (NAC), a thiol reducing agent, but not reactive oxygen species scavengers, prevented 15d-PGJ_2-induced COX-2 up-regulation. Depletion of GSH by buthionine sulfoximine, which diminishes thiol antioxidant activity, cooperated with 15d-PGJ_2 to accumulate COX-2. Therefore, 15d-PGJ_2 up-regulated COX-2 through a thiol antioxidant-sensitive mechanism. Interestingly, NAC did not inhibit the COX-2 expression induced by the electrophilic α,β-unsaturated compound PGA_2. Up-regulation of COX-2 by 15d-PGJ_2 did not result in increased PGE_2 production. Furthermore, preincubation with 15d-PGJ_2 inhibited IL-1β-induced PGE_2 production although IL-1β-induced COX-2 expression remained unaffected by the treatment with 15d-PGJ_2. On the contrary, PGA_2 elicited an increase in PGE_2 production and it acted synergistically with IL-1β to enhance PGE_2 production. These results indicate for the first time that 15d-PGJ_2 inhibits PGE_2 production independently of its effect on COX-2 expression.

1. Introduction

Glomerular cells and especially mesangial cells play an active part in the inflammatory response to glomerular injury. On activation, mesangial cells are able to proliferate, produce extracellular matrix proteins and release numerous cytokines, growth factors and prostaglandins (PGs) [1].

Cyclooxygenase (COX) is a key enzyme in PG synthesis. COX catalyzes the conversion of arachidonic acid to PGG_2 by its cyclooxygenase activity and, of PGG_2 to PGH_2, by its peroxidase activity. Two COX isoforms called COX-1 and COX-2 are currently known. The COX-1 isoenzyme is constitutively expressed in many tissues and is assumed to be responsible for the physiological functions of PGs such as maintenance of the integrity of gastric mucosa. In contrast COX-2 is an immediate early response gene that is undetectable in most mammalian tissues, but not all. For instance, COX-2 is constitutively expressed in several kidney segments [2] and in cultured renal cells such as human mesangial cells and Madin-Darby canine kidney cells [3,4]. COX-2 is rapidly induced by several stimuli, being inflammatory mediators among them [5]. Particularly relevant for our work, up-regulation of COX-2 by the inflammatory cytokine interleukin-

Abbreviations: BSO, buthionine sulfoximine; COX, cyclooxygenase; COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2; DCFH-DA, 2′,7′-dichloro-fluorescein diacetate; DMSO, dimethyl sulfoxide; ERK1/2, extracellular-regulated 1/2 kinase; FBS, fetal bovine serum; GSH, reduced glutathione; H_2O_2, hydrogen peroxide; HMC, human mesangial cells; IL-1β, interleukin-1β; JNK, c-Jun N-terminal kinase; NAC, *N*-acetylcysteine; p-38, p38 MAP kinase; PBS, phosphate-buffered saline; PG, prostaglandin; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; 15d-PGJ_2, 15-deoxy-delta12,14-prostaglandin J_2; PPARγ, peroxisome-proliferator activated receptor γ; ROS, reactive oxygen species.

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1β (IL-1β) has also been evidenced in cultured mesangial cells [6].

In the kidney, an uncontrolled inflammatory response to an acute insult may lead to chronic inflammation, permanent tissue damage and progressive renal insufficiency. The cyclopentenone 15-deoxy-delta12,14-prostaglandin J_2 (15d-PGJ_2) has been shown to display protective effects against injury or inflammation [7]. 15d-PGJ_2 is the dehydration end product of prostaglandin D_2 (PGD_2). No specific receptor for 15d-PGJ_2 has been identified to date [8]. Instead, 15d-PGJ_2 is a ligand for the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) [9]. However, it has been realized that 15d-PGJ_2 may also modulate multiple cellular functions by mechanisms independent of receptors. Part of the biological effects of 15d-PGJ_2 are likely to be caused by its ability to lower intracellular reduced glutathione (GSH) levels and/or by its binding to free thiol groups of critical cellular proteins [10–14] and, interestingly, several evidences suggest that thiol reducing agents prevent or revert the cellular effects of 15d-PGJ_2 [11,15–18].

COX-2 is one of the targets for the anti-inflammatory effects of 15d-PGJ_2. Both COX-2 expression and PGE_2 production induced by IL-1β, bacterial lipopolysaccharide or phorbol 12-myristate 13-acetate plus calcium ionophore have been previously shown to be inhibited by 15d-PGJ_2 in cultured cells, including rat fetal hepatocytes [19], human osteoarthritic chondrocyes [20], human colon carcinoma cell [16], human synovial fibroblasts [21] and rat mesangial cells [6,13]. However, even though 15d-PGJ_2 is likely to play a role in the prevention and/or resolution of glomerular inflammation, there are no data on the effect of 15d-PGJ_2 in the expression and activity of COX-2 in cultured human mesangial cells. Therefore the present study was devoted to analyze the effect of 15d-PGJ_2 in the expression and activity of COX-2 in cultured human mesangial cells either in basal conditions or after being up-regulated in response to the inflammatory cytokine IL-1β.

2. Materials and methods

2.1. Chemicals

The prostaglandins 15-deoxy-delta12,14-15d-PGJ_2 (indicated as 15d-PGJ_2 along the manuscript), PGE_2, PGA_2, 9,10-dihydro-15d-PGJ_2, $PGF_{2\alpha}$, iloprost, ciglitazone and PPARγ antagonist, GW9662, were purchased from Cayman Chemical (Cayman Chemical Company, Ann Arbor, MI). Cycloheximide, catalase, *N*-acetylcysteine (NAC), buthionine sulfoximine (BSO), Tiron and allopurinol were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). 2′,7′-dichloro-fluorescein diacetate (DCFH-DA) was supplied by Molecular Probes (Oregon, USA).

2.2. Cell culture

Human mesangial cells (HMC) were obtained from adult specimens, as we previously described [22]. The identity of the cells was confirmed by morphologic and functional criteria. Under phase-contrast microscopy, all cells appeared large and stellate and no cells with epithelial or endothelial morphologic characteristics were seen. HMC showed histochemical evidence of containing actomyosin fibres, and they did not stain for factor VIII, unlike endothelial cells. In addition, all cells examined contracted after incubation with platelet-activating factor, angiotensin II and arginin-vasopresin. The culture medium was Roswell Park Memorial Institute 1640 supplemented with 10% fetal bovine serum (FBS), 20 mM L-glutamine and antibiotics (penicillin 100 U ml^{-1} and streptomycin 100 μg ml^{-1}). Confluent cells between 6th and 15th passages were used and they were made quiescent when appropriate by 24 h incubation with medium supplement with 0.5% FBS.

2.3. Determination of ROS production

DCFH-DA is a nonpolar substance and freely diffuses through the cell membrane; within the cell it is hydrolysed to fluorescein (DCFH), a polar compound that is trapped within the cell. DCFH is a substrate that is easily oxidized to DCF by hydrogen peroxide (and others substances generated by oxidative metabolic burst) emitting fluorescence at 560 nm. DCFH-DA (5 μM) was incubated with mesangial cells for 45 min at 37 °C and washed before the addition of experimental treatments. Afterwards cells were detached by trypsinization, washed and cellular fluorescence intensity was quantified by flow cytometry (FACScan, Becton Dickinson). Each assay was repeated three times and all experiments were performed in quadruplicate wells.

2.4. Western blot analysis

Cells were homogenized in Tris–HCl buffer (pH 7.4) containing 150 mM NaCl, 5 mM EDTA, 1% deoxycholic acid, 0.1% SDS, 1% Triton X-100 and protease inhibitors 1 mM phenylmethylsufonylfluoride, 10 μg/ml aprotinin, 2 μg/ml leupeptin and the phosphatase inhibitor 0.2 mM $NaVO_4$. Cell proteins (30–40 μg) were run in a 8–10% SDS-polyacrilamide gel, transferred onto a nitrocellulose membrane (Trans-Blot Transfer Medium, BioRad, CA, USA) and incubated overnight at 4 °C with antibodies recognizing specifically COX-1 or COX-2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) as described previously [10]. This incubation was followed by a second incubation with a peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and immunoreactive products were detected by chemiluminiscence using the ECL Western Blotting Detection Reagents (Amersham Biosciences, Little Chalfont, UK) following the protocol provided by the manufacturer. As a loading control, blots probed were subsequently re-probed with anti-α-actin (Sigma–Aldrich Inc., St. Louis, MO, USA).

2.5. Determination of PGE_2 formation

PGE_2 in the medium was determined in triplicate using an EIA kit (Cayman Chemical Company, Ann ARbor, MI) following the manufacture's protocol. The culture medium of HMC cells grown in six well-plates and treated as described in the legend of Fig. 3 was collected and diluted two times with PGEM Assay Buffer. The assay was performed in a total volume of 150 μl, with the following components being added in 50-μl volumes: standards or biological samples, enzymatic tracer and specific antiserum. After overnight incubation at 4 °C, the plates were washed, and 200 μl Ellman's reagent was added into each well. After 1–2 h, the absorbance at 405 nm of each well was measured. Standard curve from 50 to 0.39 pg ml^{-1} was used to evaluate the concentrations of PGE_2. Results were calculated by using the nonlinear regression of a four-parameter logistic model.

2.6. Determination of reduced glutathione (GSH) content

Cells for reduced glutathione content measurement were lysed in a cold room. Cellular proteins were precipitated with 0.9 ml perchloric acid. After neutralization with 0.3 ml 1 M $KOH/KHCO_3$ and centrifugation, 0.15 ml of supernatant were collected on plastic tubes, *o*-phtaldialdehyde was added (0.15 ml of a 7.46 μM solution) and tubes were incubated at room temperature for 15 min.