COX-2 dependent PGE₂ downregulates αᵥ integrin expression via the EP₃ receptor in cultured mesangial cells

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Background: In experimental glomerulonephritis, inhibition of cyclooxygenase 2 (COX-2) enhances the renocortical expression of pathogenic αᵥ integrins.

Aims: To study whether this effect is mediated by prostaglandin E₂ (PGE₂) acting through its EP₃ receptor in cultured rat mesangial cells (MCs).

Methods: MCs were incubated with lipopolysaccharide (LPS), celecoxib, PGE₂, or the selective EP₃ agonist, MB28767. The expression of COX-2, EP₃, and αᵥ integrin mRNA was measured by reverse transcriptase polymerase chain reaction.

Results: LPS upregulated COX-2 expression 2.8-fold and αᵥ integrin expression twofold. The COX-2 inhibitor celecoxib increased αᵥ integrin mRNA expression twofold. Both exogenous PGE₂ and the specific EP₃ receptor agonist, MB28767, reduced constitutive αᵥ integrin mRNA expression to half normal values. COX-2 dependent PGE₂ suppressed the expression of αᵥ integrin mRNA mediated by the EP₃ receptor in MCs.

Conclusions: These results suggest that COX-2 suppresses the expression of αᵥ integrins by an increased production of PGE₂ activating its EP₃ receptor in glomerulonephritis.

Cyclooxygenase 2 (COX-2) is the inducible isoform of the cyclooxygenases and is upregulated in various inflammatory renal diseases in humans and animal models that produce prostaglandins. One function of enhanced COX-2 expression in experimental glomerulonephritis is the suppression of renal αᵥ integrin expression. αᵥ Integrins, especially the subgroup αᵥβ₃, play a key role in angiogenesis. Inhibition of COX-2 impairs mesangial capillary healing after injury in experimental glomerulonephritis. Dormond et al were able to show that the effect of COX-2 inhibitors on angiogenesis in endothelial cells is mediated by αᵥβ₃ integrins. Thus, COX-2 is thought to influence the progression of experimental nephritis by controlling the expression of αᵥ integrins. We previously found that inhibition of COX-2 resulted in the upregulation of COX-2, the EP₃ receptor for prostaglandin E₂ (PGE₂), and αᵥ integrin expression in experimental glomerulonephritis. In our present study, we investigated whether COX-2 derived PGE₂, acting through its EP₃ receptor, regulates the expression of αᵥ integrins in renal mesangial cells (MCs).

"αᵥ Integrins, especially the subgroup αᵥβ₃, play a key role in angiogenesis"

Abbreviations: COX-2, cyclooxygenase 2; LPS, lipopolysaccharide; MC, mesangial cell; PGE₂, prostaglandin E₂; RT-PCR, reverse transcriptase polymerase chain reaction

Figure 1 (A) Expression of αᵥ integrin mRNA in cultured rat mesangial cells (MCs) after incubation with 5μM celecoxib for three hours. The bar chart shows the densitometric analysis and a representative reverse transcription polymerase chain reaction (RT-PCR) gel is shown on the right. (B) Expression of cyclooxygenase 2 (COX-2) mRNA in cultured MCs after incubation with 1μg/ml lipopolysaccharide (LPS) for three hours. The bar chart shows the densitometric analysis and a representative RT-PCR gel is shown on the right. (C) Expression of EP₃ receptor mRNA in cultured MCs after incubation with 1μg/ml LPS for three hours. The bar chart shows the densitometric analysis and a representative RT-PCR gel is shown on the right. *p < 0.05 of three independent experiments. GAPDH, glyceraldehyde 3-phosphate dehydrogenase gene; n.s., not significant.
METHODS

Materials
Celecoxib (Celebrex®) was provided by Pfizer (New York, USA) and cell culture reagents were purchased from Gibco BRL (Karlsruhe, Germany). Ready To Go™ reverse transcription polymerase chain reaction (RT-PCR) beads were from Amersham Pharmacia Biotech (Freiburg, Germany) and all other chemicals were from Sigma-Aldrich (Deisenhofen, Germany).

Cell culture
Rat MCs were a kind gift from Professor Pfeilschifter (Frankfurt, Germany). They were cultured in Dulbecco’s modified Eagle’s medium containing 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 10% fetal calf serum under 5% CO2. After the indicated incubation times total RNA was isolated and used for RT-PCR with Ready To Go beads. RT-PCR products were fractionated on a 1.5% agarose gel and the band width of the single products was determined by densitometry and normalised to the band width of the fragment specific for the constitutively expressed glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene, which had been coamplified in the same reaction.

Statistical analysis
All values are means (SEM) of n experiments. The Mann-Whitney U test was used for statistical analysis. A p value < 0.05 was considered significant.

RESULTS

To study the influence of celecoxib on mesangial αv integrin expression, we incubated MCs with 5μM celecoxib. A significant twofold increase in the expression of αv subunit mRNA was seen after three hours (fig 1A). When incubated with lipopolysaccharide (LPS), MCs showed a twofold increase in the expression of αv subunit mRNA (fig 2C) and a 2.3-fold increase in COX-2 mRNA expression (fig 1B) after three hours of incubation. The expression of the EP3 receptor remained unchanged (fig 1C).

We incubated MCs with exogenous PGE2 to investigate whether the effect of celecoxib on the expression of αv integrins was mediated by the inhibition of prostaglandin synthesis or by cyclooxygenase independent mechanisms. Incubation with 1μM PGE2 reduced physiological αv integrin expression after three and 24 hours (fig 2A), indicating that

Take home messages

- In glomerulonephritis, cyclooxygenase 2 appears to suppress the expression of αv integrins by the increased production of prostaglandin E2, which acts via its EP3 receptor.
- This mechanism can explain the previous finding that celecoxib upregulates renal cortical αv integrin expression in experimental nephritis.
celecoxib effects the expression of $\alpha_v$ integrins via inhibition of prostaglandin synthesis.

To investigate the involvement of the EP$_3$ receptor in the suppressive effects of PGE$_2$ on the expression of $\alpha_v$ integrins, we incubated MCs with the selective EP$_3$ receptor agonist, MB28767. The results showed a significant reduction of $\alpha_v$ integrin mRNA expression to half the normal values after three and 24 hours of incubation with 0.1 $\mu$M MB28767 (fig 2B). This suppressive effect was also evident in MCs that were co-incubated with LPS to simulate the inflammatory state (fig 2C).

Thus, selective activation of the EP$_3$ receptor suppressed the expression of $\alpha_v$ integrins in MCs.

DISCUSSION

In our study, we found increased production of $\alpha_v$ integrin mRNA in MCs after incubation with the COX-2 inhibitor celecoxib. This effect is probably mediated by inhibition of COX-2 dependent PGE$_2$ formation, because PGE$_2$ alone reduced physiological $\alpha_v$ integrin mRNA expression in cultured MCs. This observation agrees with the in vivo finding that COX-2 inhibition reduced renal PGE$_2$ formation but in parallel enhanced renal $\alpha_v$ integrin expression in rats with passive Heymann nephritis.

“Prostaglandin E$_2$ alone reduced physiological $\alpha_v$ integrin mRNA expression in cultured mesangial cells”

When incubated with LPS (to simulate renal inflammation), MCs showed an enhanced expression of COX-2 and $\alpha_v$ integrin mRNA, suggesting a pathological role for these proteins in the mesangium. The expression of EP$_3$ receptor mRNA was not significantly affected by LPS, although we previously saw upregulation of EP$_3$ receptor expression in the renal cortex of rats with experimental nephritis. Nevertheless, we studied EP$_3$ receptor function by stimulating MCs with the specific EP$_3$ receptor agonist MB28767. MB28767 reduced $\alpha_v$ integrin mRNA expression in untreated and LPS treated cells also. Overall, COX-2 dependent PGE$_2$ reduced $\alpha_v$ integrin mRNA expression in MCs, acting through its EP$_3$ receptor. This mechanism can explain the previous finding that celecoxib upregulates renal cortical $\alpha_v$ integrin expression in experimental nephritis.

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