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

C Waldner, K Schrör, P Heering

Background: In experimental glomerulonephritis, inhibition of cyclooxygenase 2 (COX-2) enhances the renocortical expression of pathogenic $\alpha_v$ 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 $\alpha_v$ integrin mRNA was measured by reverse transcriptase polymerase chain reaction.

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

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

Cyclooxygenase 2 (COX-2) is the inducible isofrom 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 $\alpha_v$ integrin expression. $\alpha_v$ Integrins, especially the subgroup $\alpha_v\beta_3$, play a key role in angiogenesis.

Inhibition of COX-2 impairs mesangial capillary healing after injury in experimental glomerulonephropathy. Dormond et al were able to show that the effect of COX-2 inhibitors on angiogenesis in endothelial cells is mediated by $\alpha_v\beta_3$ integrins. Thus, COX-2 is thought to influence the progression of experimental nephritis by controlling the expression of $\alpha_v$ integrins. We previously found that inhibition of COX-2 resulted in the upregulation of COX-2, the EP3 receptor for prostaglandin E2 (PGE2), and $\alpha_v$ 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 $\alpha_v$ integrins in renal mesangial cells (MCs).

"$\alpha_v$ Integrins, especially the subgroup $\alpha_v\beta_3$, 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 $\alpha_v$ 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 the effect of celecoxib on αv integrin expression was cyclooxygenase dependent.

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

Figure 2
(A) Expression of αv integrin mRNA in cultured rat mesangial cells (MCs) after incubation with 1μM prostaglandin E₂ (PGE₂) for 24 hours. The bar chart shows the densitometric analysis, with a representative reverse transcription polymerase chain reaction (RT-PCR) gel above. (B) Expression of αv integrin mRNA in cultured MCs after incubation with 0.1μM MB28767 for 24 hours. The bar chart shows the densitometric analysis, with a representative RT-PCR gel above. (C) Expression of αv integrin mRNA in cultured MCs after co-incubation with 1μg/ml lipopolysaccharide (LPS) and 0.1μM MB28767 for 24 hours. The bar chart shows the densitometric analysis, with a representative RT-PCR gel to the right. *p < 0.05 of three independent experiments. GAPDH, glyceraldehyde 3-phosphate dehydrogenase gene.

www.jclinpath.com
celecoxib effects the expression of αv integrins via inhibition of prostaglandin synthesis.

To investigate the involvement of the EP3 receptor in the suppressive effects of PGE2 on the expression of αv integrins, we incubated MCs with the selective EP3 receptor agonist, MB28767. The results showed a significant reduction of αv integrin mRNA expression to half the normal values after three and 24 hours of incubation with 0.1 μM 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 EP3 receptor suppressed the expression of αv integrins in MCs.

DISCUSSION

In our study, we found increased production of αv integrin mRNA in MCs after incubation with the COX-2 inhibitor celecoxib. This effect is probably mediated by inhibition of COX-2 dependent PGE2 formation, because PGE2 alone reduced physiological αv integrin mRNA expression in cultured MCs. This observation agrees with the in vivo finding that COX-2 inhibition reduced renal PGE2 formation but in parallel enhanced renal αv integrin expression in rats with passive Heymann nephritis.1

“Prostaglandin E2 alone reduced physiological αv integrin mRNA expression in cultured mesangial cells”

When incubated with LPS (to simulate renal inflammation), MCs showed an enhanced expression of COX-2 and αv integrin mRNA, suggesting a pathological role for these proteins in the mesangium. The expression of EP3 receptor mRNA was not significantly affected by LPS, although we previously saw upregulation of EP3 receptor expression in the renal cortex of rats with experimental nephritis. Nevertheless, we studied EP3 receptor function by stimulating MCs with the specific EP3 receptor agonist MB28767. MB28767 reduced αv integrin mRNA expression in untreated and LPS treated cells also. Overall, COX-2 dependent PGE2 reduced αv integrin mRNA expression in MCs, acting through its EP3 receptor. This mechanism can explain the previous finding that celecoxib upregulates renal cortical αv integrin expression in experimental nephritis.3

REFERENCES