

SHORT REPORT

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

C Waldner, K Schrör, P Hering

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

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

Conclusions: These results suggest that COX-2 suppresses the expression of α_v 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 humans¹ and animal models² that produce prostaglandins. One function of enhanced COX-2 expression in experimental glomerulonephritis is the suppression of renal α_v integrin expression.³ α_v Integrins, especially the subgroup $\alpha_v\beta_3$, play a key role in angiogenesis.⁴ Inhibition of COX-2 impairs mesangial capillary healing after injury in experimental glomerulonephritis.⁵ 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.⁶ Thus, COX-2 is thought to influence the progression of experimental nephritis by controlling the expression of α_v integrins. We previously found that inhibition of COX-2 resulted in the upregulation of COX-2,⁷ the EP₃ receptor for prostaglandin E₂ (PGE₂), and α_v integrin³ expression in experimental glomerulonephritis. In our present study, we investigated whether COX-2 derived PGE₂, acting through its EP₃ receptor, regulates the expression of α_v integrins in renal mesangial cells (MCs).

“ α_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

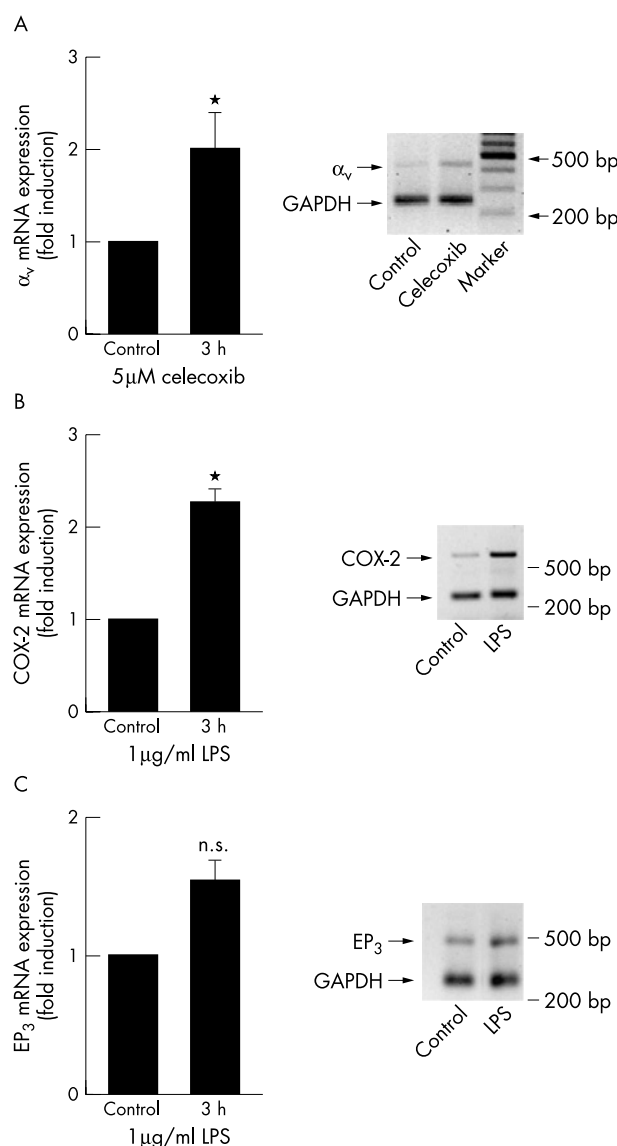


Figure 1 (A) Expression of α_v integrin mRNA in cultured rat mesangial cells (MCs) after incubation with 5 μ M celecoxib for three hours. The bar chart shows the densitometric analysis and a representative reverse transcription polymerase chain reaction (RT-PCR) gel is shown on the right. (B) Expression of cyclooxygenase 2 (COX-2) mRNA in cultured MCs after incubation with 1 μ g/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 1 μ g/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.

Take home messages

- In glomerulonephritis, cyclooxygenase 2 appears to suppress the expression of α_v 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 α_v integrin expression in experimental nephritis

RESULTS

To study the influence of celecoxib on mesangial α_v integrin expression, we incubated MCs with 5 μ M celecoxib. A significant twofold increase in the expression of α_v subunit mRNA was seen after three hours (fig 1A). When incubated with lipopolysaccharide (LPS), MCs showed a twofold increase in the expression of α_v 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 α_v integrins was mediated by the inhibition of prostaglandin synthesis or by cyclooxygenase independent mechanisms. Incubation with 1 μ M PGE₂ reduced physiological α_v integrin expression after three and 24 hours (fig 2A), indicating that

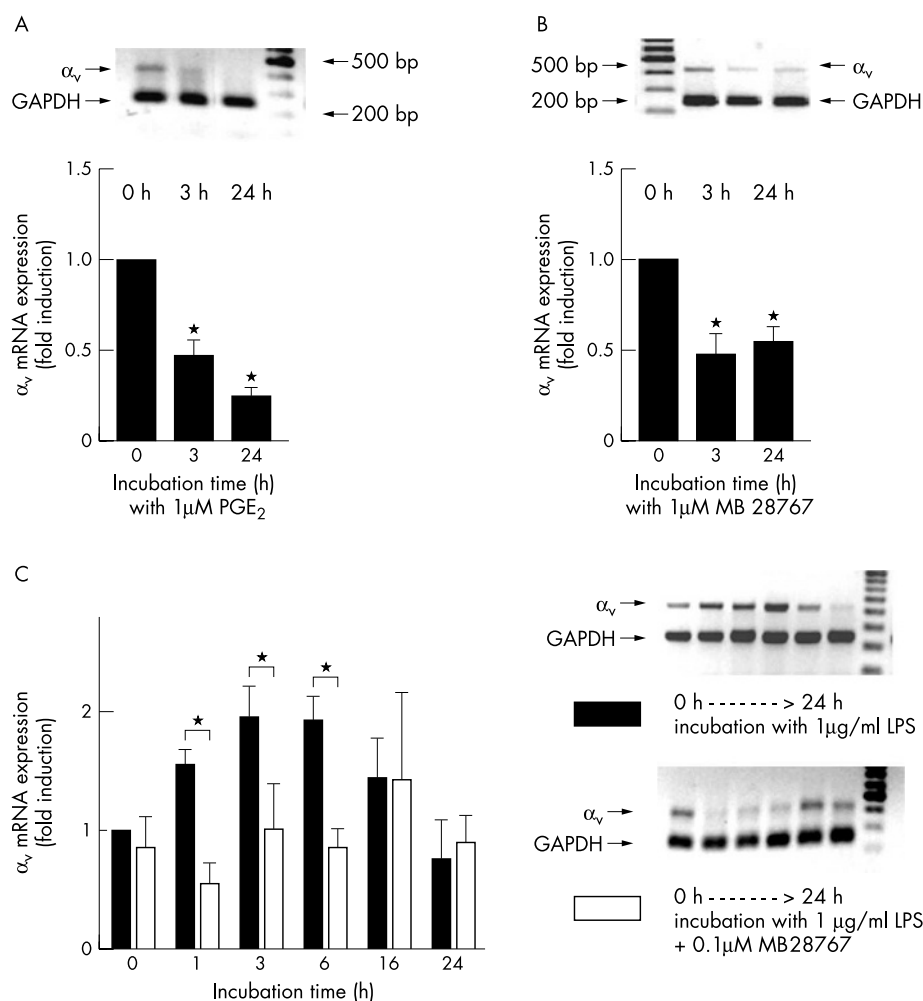


Figure 2 (A) Expression of α_v integrin mRNA in cultured rat mesangial cells (MCs) after incubation with 1 μ M prostaglandin E₂ (PGE₂) for 24 hours. The bar chart shows the densitometric analysis, with a representative reverse transcription polymerase chain reaction (RT-PCR) gel above. (B) Expression of α_v integrin mRNA in cultured MCs after incubation with 0.1 μ M MB28767 for 24 hours. The bar chart shows the densitometric analysis, with a representative RT-PCR gel above. (C) Expression of α_v integrin mRNA in cultured MCs after co-incubation with 1 μ g/ml lipopolysaccharide (LPS) and 0.1 μ M 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.

celecoxib effects the expression of α_v integrins via inhibition of prostaglandin synthesis.

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

DISCUSSION

In our study, we found increased production of α_v 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 α_v 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 α_v integrin expression in rats with passive Heyman nephritis.³

“Prostaglandin E₂ alone reduced physiological α_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 α_v 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 α_v integrin mRNA expression in untreated and LPS treated cells also. Overall, COX-2 dependent PGE₂ reduced α_v integrin mRNA expression in MCs, acting through its EP₃ receptor. This mechanism can explain the previous finding that celecoxib upregulates renal cortical α_v integrin expression in experimental nephritis.³

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