The Effect of COX-2 Inhibition on Podocyte Injury Induced by Puromycin in Nephrin-Driven COX-2 Transgenic Mice

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Although the exact mechanisms of podocyte injury resulting in proteinuria are still unclear, results of recent experiments suggested that COX-2 activation might be involved in podocyte injury and COX-2 inhibitor treatment reduced proteinuria in some of renal disease models. But, the role of COX-2 in the pathogenesis of podocyte injury and the effect of selective COX-2 inhibition on podocyte injury has not been fully established.

Several experimental animal models including puromycin aminonucleoside (PAN)-induced nephropathy are widely used to study the mechanism of podocyte injury *in vivo*. It has been well known that podocytes are the primary target in the experimental models of PAN-induced nephropathy, closely mimicking minimal change disease in human both morphologically and functionally. Interestingly, while PAN readily induces a massive proteinuria in the rat, the mouse has usually been resistant to PAN. Therefore, we hypothesized that if COX-2 play an important role in podocyte injury COX-2 transgenic mice with over-expression of COX-2 in glomeruli would be susceptible to PAN. In addition, we hypothesized that selective COX-2 inhibition would ameliorate podocyte injury induced by PAN in COX-2 transgenic mice.

To test our hypothesis, we used nephrin-driven COX-2 transgenic mice (male B6/D2/F6 mice). The animals were randomly assigned to each experimental group : ① wild-type control mice, ② wild-type mice treated with PAN alone, ③ COX-2 transgenic control mice, ④ COX-2 transgenic mice treated with PAN alone and ⑤ COX-2 transgenic mice treated with PAN and a selective COX-2 inhibitor (SC58236). PAN was injected retro-orbitally twice at day-1 (15 mg/100 g BW) and day-3 (30 mg/100 g BW). SC58236 (6 mg/L) was administered in drinking water through the present study immediately after PAN injection. The animals from each group were sacrificed at the end of day-3, 5, and 10 after PAN injection.

Albuminuria was measured daily as the ratio of urinary albumin (μg/mL) to creatinine (mg/mL) using 24-hours urine samples collected daily from day-0 to day-10. To analyze the structural changes, light microscopic and electron microscopic studies were performed. To detection of COX-2 expression, rabbit anti-murine COX-2 polyclonal antibody was used in IHC, IF and Western blot analysis. Immunoreactive COX-2 expression of glomeruli and renal cortex was determined in glomerular homogenized protein and renal cortical microsome samples, respectively. COX-2 mRNA levels were detected by real-time PCR.

The expression of podocyte proteins (nephrin, WT-1, CD2AP, ZO-1, podocin, and podocalyxin)

in renal cortex or isolated glomeruli was also analyzed. To isolate glomeruli, Dynabeads® M-450 tosylactivated was used immediately after sacrifice.

Only COX-2 transgenic mice developed significant albuminuria after PAN injection, whereas wild-type mice showed no albuminuria despite of PAN administration. In addition, down-regulation of nephrin protein and mRNA was observed also only in COX-2 transgenic mice after PAN treatment. These findings suggest that selective over-expression of COX-2 in glomeruli may be involved in the susceptibility to PAN. Interestingly, the change of glomerular COX-2 expression showed similar manner with one of albuminuria in COX-2 transgenic mice treated with PAN. In addition, the extent of glomerular COX-2 expression correlated weakly with the level of albuminuria in COX-2 transgenic mice treated with PAN. In contrast to COX-2 transgenic mice treated with PAN, albuminuria did not develop in COX-2 transgenic mice with COX-2 inhibition in spite of PAN administration. The effacement of foot process induced by PAN was improved partially by selective COX-2 inhibition. Down-regulation of podocyte protein nephrin in COX-2 transgenic mice treated with PAN was also recovered by selective COX-2 inhibitor treatment. In the other hand, there was no significant alteration of CD2AP, ZO-1, podocin or podocalyxin after PAN alone or with selective COX-2 inhibitor treatment.

The present study demonstrates that COX-2 may play an important role to increase susceptibility of podocytes to injury induced by PAN, and selective COX-2 inhibitor treatment has beneficial effect on proteinuria and podocyte injury in nephrin-driven COX-2 transgenic mice.