Protective Effect of KW-3635, a Specific Thromboxane A2-Receptor Antagonist, on Experimental Glomerulonephritis in Mice

Michiyo Kawakage1, Kiyoshi Sato2 and Akira Karasawa1

1Department of Pharmacology and 2Department of Toxicology, Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Suntogun, Shizuoka 411, Japan

Received February 8, 1994 Accepted April 1, 1994

ABSTRACT—We studied the effect of KW-3635, a selective thromboxane A2 (TXA2)-receptor antagonist, on experimental glomerulonephritis. The glomerulonephritis was induced in mice by the administration of rabbit anti-mouse glomerular basement membrane (GBM) antibody. It was characterized as proteinuria, changes of serum biochemical parameters and glomerular histopathological abnormalities. The administration of KW-3635 (30 mg/kg/day, p.o.) significantly ameliorated the proteinuria, elevation of serum urea nitrogen and the thickening of GBM. These data suggest that TXA2 may play an important pathogenic role in the development and progression of glomerulonephritis.

Keywords: Thromboxane A2-receptor antagonist, KW-3635, Nephritis

Immune glomerulonephritis has been characterized by proteinuria and changes of the renal function in many animal models. Many mediators have been reported to play roles in the progression of pathophysiologic changes following the immunologic insult (1). In the kidney, thromboxane A2 (TXA2) exerts multiple actions, such as vasoconstriction, contraction of glomerular mesangial cells and intraglomerular platelet aggregation, which may contribute to the progression of immune-mediated renal disease (2). Increased renal TXA2 biosynthesis has been documented in nephrotoxic serum (NTS) nephritis in rats, suggesting that TXA2 is an important mediator of the renal damage (3). Moreover, treatment with a TXA2 synthetase inhibitor is reported to be beneficial in the established glomerulonephritis in dogs (4). In the present study, we determined the effect of KW-3635, sodium (E)-11-[2-(5,6-dimethyl-1-benzimidazolyl)-ethylidene]-6,11-dihydrodibenz[b,e]oxepin-2-carboxylate monohydrate, a specific TXA2 receptor antagonist (5), on glomerulonephritis in mice to clarify the role of TXA2.

Male ddY mice weighing 18 to 20 g (Shizuoka Laboratory Animal Center, Inc., Hamamatsu) were used. Food and water were provided ad libitum. NTS was produced in rabbits by repeated immunization with glomerular basement membrane (GBM)-rich fractions according to the previously reported method (6, 7). Mice were immunized by an intraperitoneal injection of 0.5 mg rabbit IgG (RGG) emulsified with 0.25 ml of Freund complete adjuvant. Five days later, NTS in a volume of 0.075 ml was injected intravenously (day 0). The urine was collected for 6 hr under a saline load (50 ml/kg, p.o.) on days 3, 5, 10, 15, 20, 24 and 26, and the blood samples were collected on day 26. The amount of urinary total protein (TP) and albumin (ALB), serum ALB, total cholesterol (T-CHO) and urea nitrogen (UN) were measured by an autoanalyzer (AU510; Olympus, Tokyo). Pathological changes in the kidney slices on day 26 were evaluated after staining with hematoxylin and eosin. The thickening of GBM and the proliferation of mesangial cells were graded on a scale from 0 to 4+, where 0 indicated no abnormality, and 1+, 2+, 3+ and 4+ represented mild, moderate, moderately-severe and severe changes, respectively. As an index of the severity of renal abnormalities, the average histopathologic score was calculated and analyzed.

KW-3635 was synthesized in our laboratories and suspended in 0.3% sodium carboxymethylcellulose (0.3% CMC). KW-3635 at 10, 30 or 100 mg/kg/day was orally given to mice once a day, starting on the day of NTS injection (day 0). In the control group, the nephritic mice were treated with 0.3% CMC. In the normal group, mice were administered saline in place of NTS and treated with 0.3% CMC. These doses were selected by a preliminary experiment examining the effect of KW-3635 on the sudden death induced by U-46619 (0.5 mg/kg, i.v.) in mice. The mortalities were significantly decreased in the 30 and 100
mg/kg (p.o.) of KW-3635-treated groups as compared with that in the vehicle-treated group. Results are expressed as means ± S.E. Intergroup comparisons were performed by Student’s t-test, Dunnett’s test or Steel’s test. The renal histopathologic data were assessed for statistical significance by the χ²-test using rank scores of the data.

Figure 1 shows the effect of KW-3635 on the excretion of total protein and albumin in urine. As the NTS nephritis developed, excretions of urinary protein and albumin increased significantly. The administration of KW-3635 tended to, or significantly ameliorated the proteinuria and albuminuria. As shown in Table 1, serum ALB significantly decreased, and T-CHO and UN increased in the control (vehicle-treated nephritic) group on day 26, as compared with those in the normal group. The changes of biochemical parameters tended to be improved in the KW-3635-treated mice. At a dose of 30 mg/kg/day, KW-3635 significantly reduced the elevated serum UN level. Figure 2 shows the representative photomicrographs of the kidney from the vehicle- or KW-3635 (30 mg/kg/day)-treated nephritic mouse. The kidneys in the control group showed severe thickening of GBM and proliferation of mesangial cells (Fig. 2A). At a dose of 10 mg/kg/day, KW-3635 had no effect on these renal histological changes. By contrast, the score of GBM thickening in mice given a large dose of KW-3635 (30 mg/kg/day) was significantly lower than that in the control (Table 1, Fig. 2B). A similar result was obtained with a high dose (100 mg/kg/day) of KW-3635, although it was not statistically significant (Table 1). KW-3635 (30, 100 mg/kg/day) tended to reduce the proliferation of mesangial cells as compared with the control (Table 1).

It was reported (8) that in the NTS nephritic rats, there is enhanced synthesis of TXA₂, as measured by TXB₂ (a degradation product of TXA₂), in the glomerulus, and a significant correlation was found between glomerular

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**Table 1. Effects of KW-3635 on serum parameters and renal histopathology in mice on day 26**

| Group     | Serum parameters | Histopathological scores*   |
|-----------|------------------|-----------------------------|
|           | ALB (g/dl)       | T-CHO (mg/dl)               | GBM  | Mesangial cells |
| Control   | 2.22±0.16        | 298.32±24.67                | 3.0±0.5 | 2.4±0.4         |
| KW-3635   | 2.33±0.25        | 249.76±24.91                | 2.9±0.2 | 3.1±0.2         |
| 10 mg/kg  | 2.41±0.30        | 204.49±29.82                | 2.0±0.3 | 2.0±0.3         |
| KW-3635   | 2.46±0.21        | 268.25±16.69                | 1.9±0.4 | 1.3±0.2         |
| 30 mg/kg  |                  | 122.23±9.93**               | 0.0±0.0*| 0.0±0.0*        |
| KW-3635   | 3.03±0.07*       | 122.23±9.93**               | 0.0±0.0*| 0.0±0.0*        |
| 100 mg/kg |                  | 122.23±9.93**               | 0.0±0.0*| 0.0±0.0*        |

Values are means ± S.E. (n=7-10). *P<0.05 and **P<0.01, when compared with the control value by Student’s t-test, Steel’s test or the χ²-test. Histopathological abnormality was graded on a scale from 0 to 4+ with, according to the severity. ALB: albumin, T-CHO: total choleseterol, UN: urea nitrogen, GBM: thickening of GBM, Mesangial cells: proliferation of mesangial cells.
A. Control

B. KW-3635 (30 mg/kg/day)

Fig. 2. Effects of KW-3635 on the renal changes in mice with glomerulonephritis. A. Severe diffuse and/or nodular thickening of glomeruli with mesangial proliferation, and proliferation of capsular endothelium with crescentic or glandular formation were observed in the control (vehicle-treated nephritic) mouse. B. Minimal change of glomeruli, and non-remarkable tubulo-interstitial tissue were observed in the KW-3635 (30 mg/kg/day)-treated mouse. The magnification is 150 ×.
TXA2 synthesis and urinary protein excretion. On the other hand, Takahashi et al. (3) have demonstrated that glomerular filtration rate (GFR) decreases in NTS nephritic rats and that this fall in GFR can be prevented by the TXA2-receptor antagonist. The present study using experimental nephritic mice also revealed a beneficial effect of the TXA2-receptor antagonist on the development of nephritis. Thus, these results support the notion that TXA2 is one of the mediators responsible for the pathogenesis of glomerulonephritis. The anti-nephritic effect of KW-3635 is probably due to inhibition of the various actions of TXA2, including TXA2-dependent platelet aggregation and contraction of renal vascular and glomerular mesangial cells.

In the present study, KW-3635 at a high dose (100 mg/kg/day) did not significantly improve the NTS nephritis. It is reported that TXA2 might be important for maintaining GFR in some cases and that TXA2 could contribute to the increase of GFR in hypoperfused kidney (9). It is, therefore, possible that the high dose of KW-3635 might have completely inhibited even the beneficial action of TXA2 in the kidney, resulting in the bell-shaped effect of KW-3635. The present results are in accordance with our previous observation that chronic administration of KW-3635 (30 mg/kg/day) markedly attenuated the lupus nephritis in NZB × NZW F1 mice, whereas the high dose (100 mg/kg/day) of this drug did not improve the nephritis (10).

In summary, KW-3635 showed the improvement of NTS nephritis in mice. Short-term treatment with KW-3635 decreased urinary protein and suppressed the changes in serum biochemical parameters and the severity of the renal histologic abnormalities. These data suggest that TXA2 may play an important pathogenic role in the development and progression of glomerulonephritis.

Acknowledgments

We are grateful to Mr. Kozo Yao and Ms. Toyoko Kashiwagi for support in the experiment and to Dr. Hiroshi Nishimura for his advice as a pathologist.

REFERENCES

1 Couser WG: Pathogenesis of glomerulonephritis. Kidney Int Supp 42, S19–S26 (1993)
2 Stahl RAK, Thaiss F, Kuhl S, Schoeppe W and Helmchen UM: Immune-mediated mesangial cell injury. Biosynthesis and function of prostanooids. Kidney Int 38, 273–281 (1990)
3 Takahashi K, Schreiner GF, Yamashita K, Christman BW, Blair I and Badr KF: Predominant functional roles for thromboxane A2 and prostaglandin E2 during late nephrototoxic serum glomerulonephritis in the rat. J Clin Invest 85, 1974–1982 (1990)
4 Grauer GF, Fritsbe DD, Longhofer SL, and Cooley AJ: Effects of a thromboxane synthetase inhibitor on established immune complex glomerulonephritis. Kidney Int 37, 415 (1990)
5 Karasawa A, Kawakage M, Shirakura S, Higo K, Kubo K, Ohshima E and Obase H: Antiplatelet effects of the novel thromboxane A2 receptor antagonist sodium (E)-11-[2-(5,6-dimethyl-1-benzimidazolyl)-ethylidene]-6,11-dihydrodibenzo[b,e]oxepin-2-carboxylate monohydrate. Arzneimittel For schung 41, 1230–1236 (1991)
6 Nagai H, Takizawa T, Nishiyori T, and Koda A: Experimental glomerulonephritis in mice as a model for immunopharmacological studies. Jpn J Pharmacol 32, 1117–1124 (1982)
7 Spiro RG: Studies on the renal glomerular basement membrane. J Biol Chem 242, 1915–1922 (1967)
8 Llanos EA, Andres GA and Dunn MJ: Glomerular prostaglandin and thromboxane synthesis in rat nephrototoxic serum nephritis. J Clin Invest 72, 1439–1448 (1983)
9 Goto F, Jackson EK, Ohnishi A, Herzer W and Branch RA: Effect of cyclooxygenase and thromboxane synthase inhibition on the response to angiotensin II in the hypoperfused canine kidney. J Pharmacol Exp Ther 243, 799–803 (1987)
10 Kawakage M, Mizumoto H, Nukui E, Sato S, and Karasawa A: Effects of KW-3635, a specific thromboxane A2 receptor antagonist, on the development of lupus nephritis in NZB × NZW F1 mice. Jpn J Pharmacol 63, 433–438 (1993)