Effects of KW-3635, a Specific Thromboxane A2-Receptor Antagonist, on the Development of Lupus Nephritis in NZB × NZW F1 Mice

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ABSTRACT—We examined the effect of KW-3635, a specific thromboxane A2 (TXA2)-receptor antagonist, on the development of lupus nephritis in NZB × NZW F1 mice. KW-3635 was orally given once a day for 33 weeks beginning at eight weeks of age. In the control group, the mice began to die at 39 weeks of age, showing severe proteinuria and histopathologic abnormality in the renal glomeruli. Administration of KW-3635 (30 mg/kg/day) significantly reduced urinary protein excretion (1.7±0.9 vs. 8.5±2.4 mg/6 hr/mouse, P<0.01), mortality (1/18 vs. 6/19, P<0.05) and the histopathologic score of the kidney examined at 41 weeks of age. Thus, chronic administration of KW-3635 markedly attenuated the renal disease in NZB × NZW F1 mice, suggesting that TXA2 is an important mediator of the pathogenesis in this murine model of lupus nephritis.

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

Thromboxane A2 (TXA2) is a labile derivative of arachidonic acid metabolism and exerts multiple actions within the kidney. These actions include constriction of glomerular afferent and efferent arterioles, intraglomerular platelet aggregation, contraction of glomerular mesangial cells and increase of mesangial cell matrix synthesis (1, 2). Increased renal TXA2 biosynthesis has been documented in a variety of animal models of renal disease (3–5) and in patients with systemic lupus erythematosus (SLE) (6). These results suggest that TXA2 plays an important role in the progression of renal diseases.

It was demonstrated that patients with SLE had significantly higher urinary TXB2 (stable product of TXA2) and lower 6-keto-PGF1α (stable product of PGI2) as compared with those in normal subjects. In the patients with SLE, a significant inverse correlation was found between the urinary TXB2/6-keto-PGF1α ratio and creatinine clearance (Ccr) (6). Moreover, recent studies have shown that a thromboxane A2-receptor antagonist (TXRA) improves renal function in patients with lupus nephritis (7, 8). Renal TXA2 production is also increased in NZB × NZW F1 mice that spontaneously develop an autoimmune disease with glomerulonephritis, which resembles human SLE (9). The effects of thromboxane synthase inhibitors (TXSIs) have been investigated in NZB × NZW F1 mice. Most of the studies (10, 11) indicated that TXSI did not protect NZB × NZW F1 mice against the development of lupus nephritis, although Parbtani et al. (12) reported that a TXSI had some beneficial effects in this strain of mice. On the other hand, few researchers have investigated the possible protective effects of TXRA in NZB × NZW F1 mice (13).

KW-3635 is a novel TXRA (14, 15). We reported that KW-3635 potentiates the diuretic action of furosemide in saline-loaded normal rats, suggesting that it inhibits TXA2 within the kidney (16). In the present study, we determined the effect of KW-3635 on the development of murine lupus nephritis in NZB × NZW F1 mice to clarify the role of TXA2 in this renal disease.

MATERIALS AND METHODS

Animals

Female NZB × NZW F1 mice (Charles River Japan, Inc., Yokohama) were used. Food and water were provided ad libitum.

Experimental protocols

Beginning at 8 weeks of age, eighteen NZB × NZW F1 mice in each group were randomized to receive either KW-3635 (30, 100 mg/kg/day) or its vehicle (0.3% sodium carboxymethylcellulose). KW-3635 at 30 or 100 mg/kg/day was orally given to mice once a day. These doses were selected by a preliminary experiment examining the
effect of the drug on the sudden death induced by the TXA₂ analogue U-46619 (0.5 mg/kg, i.v.) in mice. The mortalities in the 30 and 100 mg/kg (p.o.) of KW-3635-treated groups decreased to 75% (15/20) (P<0.05) and 55% (11/20) (P<0.01), respectively, as compared with 100% (20/20) in the vehicle-treated group. The mortality was not improved by 10 mg/kg of KW-3635 (19/20). The doses that blocked the response to U-46619 were chosen for the chronic treatment studies.

Effects of KW-3635 were determined by measuring urinary protein excretion, renal histopathologic changes, anti-DNA antibody levels in sera and the mortality. For the measurement of urinary protein, groups consisting of 9 mice in each were used. The urine was periodically collected for 6 hr following oral administration of saline (0.5 ml/10 g), and the concentration of urinary protein was measured by an autoanalyzer (AU510; Olympus, Tokyo).

Histopathologic changes in the glomeruli were evaluated at 41 weeks. The kidney was removed and fixed with 10% buffered-formalin (pH 7.25). Transverse kidney slices were embedded in paraffin, sectioned and stained with hematoxylin and eosin. The specimens of the kidney were examined by light microscopy. Individual abnormalities in tissue sections were graded on a scale from 0 to 4 +, where 0 was no abnormality, and 1 +, 2 +, 3 +, and 4 + represented mild, moderate, moderately-severe, and severe abnormalities, respectively, according to the report of Spurney et al. (17).

Anti-DNA antibody levels in sera were measured by ELISA according to the method of Mihara et al. (18). Briefly, after coating with ssDNA at a concentration of 2 μg/ml overnight at 4°C, the plates were washed 3 times with PBS containing Tween 20 and BSA (PBS-T). Serial dilutions of sera in PBS-T were added to each well and incubated. The plates were again washed, followed by the addition of peroxidase-conjugated goat anti-mouse Ig diluted 1/500 in PBS-T. After washing with PBS-T, the plates were incubated with a substrate solution of o-phenylenediamine. The absorbance at 490 nm was measured using an Immuno Reader NJ-2000 (Inter Med Japan Co., Ltd., Tokyo). Data are expressed as optical density (OD) units calculated.

**Drugs used**

Sodium (E)-11-[2-(5,6-dimethyl-1-benzimidazolyl)ethylidene]-6,11-dihydroidibenzen[b,e]oxepine-2-carboxylate monohydrate (KW-3635) was synthesized in our laboratories. Single strand DNA (ssDNA) (Sigma Chemical Co., St. Louis, MO, USA) and peroxidase-conjugated goat anti-mouse immunoglobulins (Ig) (Cappel Research Products, Durham, NC, USA) were used to measure anti-DNA antibody levels in sera.

**Statistical analyses**

Data are presented as means±S.E. Results were statistically evaluated with a Scheffe-type test following the Kruskal-Wallis test. For histopathologic studies and incidence of mortality, the statistical significance was tested by the χ²-test.

**RESULTS**

At the beginning of the experiment, eighteen NZB × NZW F1 mice were used in each group. In the vehicle treated group, the mice began to die from 39 weeks of age. The mice treated with KW-3635 at 30 mg/kg/day had a significantly reduced mortality at 40 and 41 weeks of ages (Fig. 1). In the group treated with a high dose (100 mg/kg/day) of KW-3635, the mortality was sig-
Table 1. Biochemical parameters in sera at the age of 41 weeks

|       | N   | TG (mg/dl)     | TC (mg/dl)     | TP (g/dl) | ALB (g/dl) | UN (mg/dl) |
|-------|-----|----------------|----------------|-----------|------------|------------|
| Vehicle    | 12  | 119.2±27.2 | 302.9±67.2 | 5.74±0.30 | 2.47±0.22 | 61.3±20.4  |
| KW-3635 30 mg/kg | 17  | 87.6±16.4 | 174.7±16.2 | 6.49±0.20 | 3.02±0.13 | 29.3±5.4   |
| KW-3635 100 mg/kg | 10  | 110.8±41.0 | 156.7±14.7 | 6.66±0.28 | 2.87±0.23 | 36.5±11.7  |

Values are means±S.E. TG: triglyceride, TC: total cholesterol, TP: total protein, ALB: albumin, UN: urea nitrogen.

Fig. 3. Microphotographs of the kidney from NZB×NZW F1 mice. A: vehicle, B: KW-3635 (30 mg/kg/day). The magnification is 100×.
significantly higher at 36 to 38 weeks of age as compared with that in the control mice (Fig. 1). Proteinuria occurred from 35 weeks of age, although there were individual variations. At 40 and/or 41 weeks of age, the mice treated with KW-3635 at 30 and 100 mg/kg exhibited significantly decreased proteinuria as compared with the vehicle-treated control (Fig. 2). The mice in all the groups showed similar urine volume (data not shown). Table 1 shows the biochemical parameters in the sera at 41 weeks of age. In the vehicle group, triglyceride and urea nitrogen exhibited higher values. KW-3635 tended to reduce these parameters, although the effects were not statistically significant.

Thereafter, we evaluated the effect of KW-3635 on renal histologic abnormalities at 41 weeks of age. Figure 3 shows the representative photomicrographs of the kidney from NZB × NZW F1 mice given either the vehicle or KW-3635 (30 mg/kg/day). The kidneys from the vehicle treated mice typically showed severe proliferative glomeruli, glomerulosclerosis and lymphoid cell infiltration, which are compatible with the histopathologic changes in SLE. The histopathologic abnormality in the KW-3635 (30 mg/kg/day)-treated group was significantly decreased as compared with that in the vehicle control (Table 2).

Anti-ssDNA antibody levels in the sera are shown in Table 3. There was no significant difference in anti-ssDNA antibody levels between the KW-3635 treated mice and the vehicle control, indicating that immuno-suppressive action is not involved in the beneficial effect of KW-3635.

DISCUSSION

NZB × NZW F1 mice serve as an excellent animal model for the study of SLE (9). In NZB × NZW F1 mice, renal TXA2 production is increased, and the enhanced renal TXA2 production correlates with urinary protein excretion as well as the severity of renal histopathology (9). In the present study, we examined the effects of KW-3635, a TXRA, on urinary protein excretion, histopathologic changes in glomeruli and mortality in this model. KW-3635 at a dose of 30 mg/kg/day decreased urinary protein excretion and reduced the histopathologic changes and the mortality. The reduced mortality, however, cannot entirely be ascribed to the ameliorated renal injury, since it may also be related to the systemic blockade by KW-3635 of various TXA2 responses. Stegmeier et al. reported, in an abstract, that BM13,505, another TXRA, delayed the manifestation of lupus nephritis in NZB × NZW F1 mice (13). Our results, as well as the result reported by Stegmeier et al., suggest that TXA2 is an important mediator of the development of lupus nephritis in NZB × NZW F1 mice.

Cyclooxygenase inhibitor (9) and most of the TXSI (10, 11) failed to show any beneficial effects on lupus nephritis in NZB × NZW F1 mice. For example, TXSI had no apparent effect on urinary protein excretion and mortality in spite of a 40% reduction of urinary TXB2 excretion (11). This may be due to the incomplete suppression of renal synthesis of TXA2. Indeed, since urinary TXB2 is derived from both the kidney and the extrarenal sources in lupus nephritis (6, 19), a 40% reduction of urinary TXB2 may not reflect the suppression of renal synthesis of TXA2. Alternatively, prostaglandin endoperoxides, which cannot be attenuated by TXSI, might have aggravated the lupus nephritis. Even if TXSI can completely inhibit renal synthesis of TXA2, it can not inhibit the synthesis of prostaglandin endoperoxides, which may act as an agonist on the thromboxane receptor (20). Therefore, TXRA has some therapeutic advantages over TXSI for the treatment of thromboxane-dependent disorders.

Inhibition of TXA2 (and prostaglandin endoperoxides) may improve renal injury by a variety of direct and indirect mechanisms. In the kidney, TXA2 induces the decrease of glomerular filtration rate via the reduction of renal blood flow (21) and the contraction of mesangial cells (2, 22). Thus, TXRA could improve the depressed renal function induced by TXA2, the synthesis of which is increased in NZB × NZW F1 mice (9). On the other hand, TXA2 causes intraglomerular platelet aggregation and releases a variety of growth factors which stimulate mesangi-
al cells proliferation (22). Moreover, TXA₂ can enhance immune complex deposition (3), stimulate production of extracellular matrix protein (23) and increase glomerular capillary permeability, leading to the renal degeneration and inflammation. KW-3635 might have inhibited these deleterious actions of TXA₂, resulting in the improvement of lupus nephritis in NZB × NZW F1 mice.

In the present study, KW-3635 at a high dose (100 mg/kg/day) did not improve or rather aggravated the lupus nephritis in NZB × NZW F1 mice. TXA₂ is one of the factors modulating the tubuloglomerular feedback response (24) and may be important to maintain glomerular filtration rate in some cases. In fact, it has been reported (25) that TXA₂ could contribute to the increase of glomerular filtration rate in the hypoperfused kidney. Therefore, it is possible that KW-3635 at the high dose might have completely inhibited even the beneficial renal response of TXA₂, resulting in the decrease in glomerular filtration rate. Thus, KW-3635 at the high dose might not have improved lupus nephritis in NZB × NZW F1 mice. The exact mechanism for the ineffectiveness at the high dose of KW-3635 awaits further investigation.

In summary, chronic TXA₂ receptor blockade showed the improvement of lupus nephritis in NZB × NZW F1 mice. Treatment with KW-3635 decreased urinary protein excretion, histopathologic changes and the mortality. These data suggest that TXA₂ is an important mediator of the development of lupus nephritis in NZB × NZW F1 mice.

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