TACROLIMUS (FK506)-INDUCED NEPHROTOXICITY IN SPONTANEOUS HYPERTENSIVE RATS

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ABSTRACT — To clarify the profile of the tacrolimus (FK506)-induced nephrotoxicity and its mechanism, 1, 2 and 4 mg/kg/day of tacrolimus was administered intramuscularly (i.m.) to spontaneous hypertensive rats (SHR) for 2 weeks, and biochemical and pathological parameters were studied in the animals. The acute nephrotoxicity of tacrolimus was characterized as increase of blood urea nitrogen (BUN) and plasma creatinine (P-Cr) levels in the groups of 1 mg/kg/day and more, decrease of creatinine clearance (CCr) value in the groups of 2 mg/kg/day and more, and histopathologically luminal narrowing of the arteriole adjacent the glomerulus in the groups of 1 mg/kg/day and more. These changes were associated with an increase of plasma renin activity (PRA) and urinary thromboxane B2 content and decrease of 6-keto-prostaglandinF1α (6-keto-PGF1α) content. Nilvadipine, which is one of the Ca2+ antagonist and is known to have renal vasodilating activity, prevented both biochemical and histopathological changes due to tacrolimus. The results indicated that the acute nephrotoxicity of tacrolimus was derived from impairment of glomerular function associated with the constriction of the renal arteriole brought about by the drug.

All of these renal disorders induced by tacrolimus recovered completely or partially when the drug was withdrawn for 2 or 4 weeks. Consequently, the acute nephrotoxicity of tacrolimus in SHR was considered to be reversible.

KEY WORDS: Tacrolimus, FK506, Nephrotoxicity, SHR, Vasoconstriction, Reversibility.

INTRODUCTION

Tacrolimus is a new macrolide entity consisting of 23 aliphatic heterocyclic rings (Fig. 1), which is isolated from the culture broth of Streptomyces tsukubaensis, and possesses similar but more potent immunosuppressive properties to cyclosporine A, inhibiting cell-mediated and humoral immune responses (Kino et al., 1987 a, b).

Tacrolimus is clinically effective for treatment of allograft rejection of the liver, kidney and pancreas (Starzl et al., 1989). Although nephrotoxicity is a major side effect of tacrolimus, its mechanism and its reversibility in animals have not been well studied or proven. Since nephrotoxicity of cyclosporine A was induced more clearly in spontaneous hypertensive rats (SHR) than in Sprague-Dawley (SD) strain rats (Ryffel et al., 1985), this study was conducted to investigate the functional and morphological profile of the nephrotoxicity occurring when tacrolimus is given to SHR.

Additionally it was examined whether the nephrotoxic findings recover or not when the
drug is stopped.

MATERIALS AND METHODS

1. animals

Spontaneous hypertensive male rats (SHR), (Japan Charles River Inc.) with blood pressure of 161 to 208 mmHg, aged 11 weeks and weighing 254 to 326 g were used. During the acclimation and study periods, the animals were allowed free access to standard diet (CE-2, CLEA Co., Ltd., Na content: 0.39%) and tap water.

2. test substances

On present study, tacrolimus (FK506) formulation for intramuscular injection (Fujisawa Pharmaceutical Co., Ltd.) each vial contains 20 mg of tacrolimus hydrate, 4 mg of polyoxymethylene hydrogenated oil and 50 mg of D-mannitol), its placebo formulation and nilvadipine (Fujisawa Pharmaceutical Co., Ltd.) were used, and suspended in physiological saline for dosing.

3. assay

Urinalysis: Urine volume, N-acetyl-β-D-glucosamidase activity (NAG, NAG test kit; Shionogi Co., Ltd.), creatinine (U-Cr) content and Na (U-Na) content were measured for each urine specimen. In addition, urinary thromboxane B₂ (TXB₂) and urinary 6-keto-prostaglandinF₁α (6-keto-PGF₁α) contents were measured by radio immunoassay (Amersham, Japan). Biochemistry: Plasma creatinine (P-Cr), blood urea nitrogen (BUN) and plasma-Na (P-Na) levels were measured with an autoanalyser (Hitachi 7150). Creatinine clearance (CCR) was calculated from urinary creatinine and P-Cr values. Plasma renin activity (PRA) was measured by radio immunoassay (Dinabot Co., Ltd.).

Tacrolimus concentration in blood; Tacrolimus concentration in blood was measured by ELISA with a monoclonal anti-tacrolimus antibody (Tamura et al., 1987).

Histopathology; The kidneys were fixed in phosphate-buffered 10% formalin and its paraffin specimens were prepared and these sections were stained with hematoxylin and eosin, or by Bowies’ method (Ishikawa et al., 1991) for detecting juxtaglomerular apparatus. Luminal narrowing of the arterioles were classified as following criteria in consideration of the incidence.

Slight(±) inside diameter diminished to about 1/2.

Moderate (+) inside diameter diminished to less than 1/2.

4. experimental protocol

In all experiments, the animals were used in group of 7, the allocation was carried out so that initial group mean body weight was approximately equalized and non-treated and placebo group were provided.

Protocol-1: Tacrolimus was given intramuscularly (i. m.) to the animals in a daily dose of 1, 2 and 4 mg/kg/day for 2 weeks, 24-hour urine for urinalysis was collected after the last dosing. During urine collection, no food was allowed but tap water was given.

Blood was withdrawn from the abdominal aorta under either anesthesia 24 hours after last dosing, and serum for biochemical analysis was separated by centrifugation. The animals were bled to death after blood collection and kidneys for histopathological examination were removed immediately.

Protocol-2: Tacrolimus was given i. m. to the animals in a daily dose of 4 mg/kg/day and nilvadipine was given subcutaneously concomitantly in divided doses of 0.1, 0.5 and 1.0 mg/kg/day 2 times a day (just before and 4 hours after dosing with tacrolimus) for 2 weeks.

Subsequent procedures were the same as Protocol-1 and a part of blood sample was used for measuring tacrolimus concentration.
Protocol-3: For examining the recovery of nephrotoxicity by tacrolimus, three groups were set in this protocol. Animals in the first group were given i.m. in a daily dose of 4 mg/kg/day for 2 weeks. Animals in the second or third group were withdrawn the drug for 2 or 4 weeks after 2 weeks administration, respectively.

Nephrotoxic parameters were examined on 2, 4 or 6 weeks after the commencement of first dosing in respective group.

5. statistics

Results were represented as the mean±S. D. value. Statistical comparison between treatment group and non-treatment group or placebo group was performed by the use of Student's or Welch's t-test after analysis of variances.

RESULTS

1. Profile of nephrotoxicity induced by tacrolimus (Protocol-1)

Increase of BUN and P-Cr were observed in the groups of 1 mg/kg/day and more in a dose dependent manner. Ccr was decreased in the groups of 2 mg/kg/day and more. Increase of TXB2 and decrease of 6-keto-PGF1α were observed in the groups of 2 mg/kg/day and more, 1 mg/kg/day and more respectively (Table 1). Histopathologically, slight to moderate luminal narrowing of periglomerular arterioles and slight to moderate basophilic cortical tubules in which there were scarcely regenerating findings but degenerating ones were predominant were observed in the groups of 1 mg/kg/day and more dose-dependently (Table 2, Photo. 1-a, b). In contrast, both Na urine excretion ratio (data not shown) and NAG content scarcely differed in the animals treated with tacrolimus from those in placebo group.

2. Preventive effect of nilvadipine on the nephrotoxicity induced by tacrolimus (Protocol-2)

Decrease of BUN and P-Cr and increase of Ccr in the groups concomitantly treated with nilvadipine of 1.0 mg/kg/day, 0.1 mg/kg/day and more, 0.1 mg/kg/day and more, respectively, compared with those of 4 mg/kg/day of tacrolimus without nilvadipine group (Fig. 2-a, b). Histopathologically, decrease or disappearance of luminal narrowing of the arterioles and decrease of basophilic cortical tubules were observed in the groups of concomitantly treated with nilvadipine of 0.5 mg/kg/day and more (Photo. 1-c). The tacrolimus concentration (Cminum) in the blood were almost the same level in all groups including nilvadipine treated group (Fig. 2-b).

3. Reversibility of nephrotoxicity induced by tacrolimus (Protocol-3)

BUN, P-Cr and Ccr of the tacrolimus treated groups were clearly different from those of non-treatment control group on day 15. These parameters showed marked and time-dependent trend toward recovery by the withdraw of tacrolim-

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non-treatment</th>
<th>Placebo</th>
<th>FK506 (mg/kg/day, i. m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1)</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>29.6±2.6</td>
<td>28.0±2.2</td>
<td>42.0±4.3 **</td>
</tr>
<tr>
<td>P-Cr (mg/dl)</td>
<td>0.51±0.04</td>
<td>0.51±0.04</td>
<td>0.69±0.04 **</td>
</tr>
<tr>
<td>P-Na (mEq/l)</td>
<td>141.9±0.7</td>
<td>143.9±0.9 **</td>
<td>141.0±0.8 **</td>
</tr>
<tr>
<td>P-K (mEq/l)</td>
<td>5.7±0.3</td>
<td>5.1±0.4 **</td>
<td>5.4±0.2 *</td>
</tr>
<tr>
<td>Urine volume (ml/24hrs/100g)</td>
<td>3.93±2.25</td>
<td>7.10±4.08</td>
<td>4.93±2.83</td>
</tr>
<tr>
<td>Ccr (ml/hr/100g)</td>
<td>16.8±3.2</td>
<td>19.5±4.9</td>
<td>15.2±2.2</td>
</tr>
<tr>
<td>NAG index (U/g-Cr)</td>
<td>64.0±7.6</td>
<td>60.4±12.0</td>
<td>67.8±22.3</td>
</tr>
<tr>
<td>U-Na (μEq/24hrs/100g)</td>
<td>174.9±36.7</td>
<td>151.8±40.0</td>
<td>144.2±21.8</td>
</tr>
<tr>
<td>TXB2 (ng/24hrs/100g)</td>
<td>N.T.</td>
<td>35.9±11.6</td>
<td>44.5±12.1</td>
</tr>
<tr>
<td>6-keto PGF1α (ng/24hrs/100g)</td>
<td>N.T.</td>
<td>7.32±1.34</td>
<td>4.10±0.96**</td>
</tr>
</tbody>
</table>

Values represent mean±S. D. of 7 rats. N.T.: Not Tested
* : P<0.05, ** : P<0.01 versus non-treatment control group
# : P<0.05, ## : P<0.01 versus placebo control group
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Table 2. Histopathological findings in kidney of SHR dosed with FK506.

<table>
<thead>
<tr>
<th>Findings (Grade)</th>
<th>Incidence (No. of rats with findings/rats used)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-treatment</td>
</tr>
<tr>
<td>Luminal narrowing of periglomerular arterioles</td>
<td></td>
</tr>
<tr>
<td>(±)</td>
<td>0/7</td>
</tr>
<tr>
<td>(+)</td>
<td>0/7</td>
</tr>
<tr>
<td>Basophilic cortical tubules</td>
<td></td>
</tr>
<tr>
<td>(±)</td>
<td>0/7</td>
</tr>
<tr>
<td>(+)</td>
<td>0/7</td>
</tr>
</tbody>
</table>

±: slight, +: moderate

Fig. 2. Effect of Nilvadipine on FK506 induced nephrotoxicity in SHR. Nilvadipine was dosed s.c. with 4 mg/kg/day of FK506 to SHR for 2 weeks. Panel a) shows BUN and P-Cr, and b) shows CGr and Cmin of FK506 in blood. Values are expressed as the mean±S.D. of 7 rats.

**; P<0.05, ***; P<0.01 versus non-treatment control groups
#; P<0.05, ##; P<0.01 versus FK506 4 mg/kg/day without nilvadipine group.

The key index of the clinical adverse effect on the kidney of the liver transplant recipients induced by tacrolimus is the increase of plasma creatinine level and decrease in glomerular filtration rate (GFR) (McDiarmid et al., 1993).

Characteristic toxicities, such as decrease of body weight gain and pancreatic toxicity, were observed in the repeated oral toxicity study in SD strain rats for 13 weeks, but no increase of P-Cr, which is the indicator of nephrotoxicity, was observed even in the 3.2 mg/kg/day high dose group (Ohara et al., 1990). On the other hand, cyclosporine A was reported that its nephrotoxicity was induced more severely and clearly in SHR than SD strain rats (Ryffel et al., 1985). Therefore we selected SHR to examine the nephrotoxicity of tacrolimus. Intramuscular injection can produce higher concentration of tacrolimus than oral administration. In dose finding study for

mus (Fig. 3–a, b). PRA and urinary TXB2/6-keto-PGF1α ratio of the tacrolimus treated group increased remarkably after 2 weeks of treatment, and returned to those of control levels after 4 weeks without drug treatment (Fig. 3–d, 4).

There were luminal narrowing of the arterioles adjacent to the glomerulus, basophilic cortical tubules and increase of number of granules in the juxtaglomerular apparatus in the animals treated with tacrolimus, and these changes were also improved after 4 weeks without drug treatment (Table 3, Photo. 2–a, b).

DISCUSSION

The key index of the clinical adverse effect on the kidney of the liver transplant recipients induced by tacrolimus is the increase of plasma creatinine level and decrease in glomerular filtration rate (GFR) (McDiarmid et al., 1993).

Characteristic toxicities, such as decrease of body weight gain and pancreatic toxicity, were observed in the repeated oral toxicity study in SD strain rats for 13 weeks, but no increase of P-Cr, which is the indicator of nephrotoxicity, was observed even in the 3.2 mg/kg/day high dose group (Ohara et al., 1990). On the other hand, cyclosporine A was reported that its nephrotoxicity was induced more severely and clearly in SHR than SD strain rats (Ryffel et al., 1985). Therefore we selected SHR to examine the nephrotoxicity of tacrolimus. Intramuscular injection can produce higher concentration of tacrolimus than oral administration. In dose finding study for
Nephrotoxicity of tacrolimus in SHR.

Photo. 1. Histopathological findings of the arterioles adjacent to the glomerulus. 
Hematoxylin eosin staining; original magnification \( \times 50 \)

\[\begin{align*}
a & : \text{Kidney of SHR dosed i.m. with placebo formulation of FK506} \\
& \quad - \text{No noteworthy changes -} \\
b & : \text{Kidney of SHR dosed i.m. with 4 mg/kg/day of FK506.} \\
& \quad - \text{Narrowing of the arterioles -} \\
c & : \text{Kidney of SHR dosed i.m. with 4 mg/kg/day of FK506 and 1.0 mg/kg/day of Nilvadipine} \\
& \quad - \text{No noteworthy changes -}
\end{align*}\]

Table 3. Histopathological findings in Kidney of SHR after withdrawal of FK506.

<table>
<thead>
<tr>
<th>Findings (Grade)</th>
<th>With FK506 (4 mg/kg/day)</th>
<th>Withdrawal of FK 506</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 weeks</td>
<td>2 weeks</td>
</tr>
<tr>
<td>Luminal narrowing of periglomerular arterioles (No. of rats)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-)</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>(±)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>(+)</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Basophilic cortical tubules (No. of rats)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(±)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>(+)</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Increase of the number of granules in juxtaglomerular appratus (No. of rats)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(±)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>(+)</td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

7 rats in each group. -: no change, ±: slight, +: moderate
Fig. 3. Changes in BUN, P-Cr, Ccr and PRA on withdrawal of FK506. 4 mg/kg/day of FK506 was dosed i.m. to SHR for 14 days, followed by 14 or 28 days recovery period. ○—○: non-treatment control group, ●—●: FK506 (4 mg/kg/day) group, Panel a), b), c) and d) shows BUN, P-Cr, Ccr, and PRA, respectively. Values are expressed as the mean±S.D. of 7 rats.

* ; P<0.05, ** ; P<0.01 versus non-treatment control group.
Nephrotoxicity of tacrolimus in SHR.

Fig. 4. Changes in TXB2/6-keto-PGF1α ratio on withdrawal of FK506. 4 mg/kg/day of FK506 was dosed to SHR for 2 weeks, followed by 4 weeks recovery period.
□ : non-treatment control group, ■ : FK506 (4 mg/kg/day) group.
Values are expressed as the mean±S.D. of 7 rats.
** : P＜0.01 versus non-treatment control group.

examining a nephrotoxicity of tacrolimus, increase of P-Cr and BUN were noted in SHR injected intramuscularly with 4 mg/kg/day of tacrolimus for 2 weeks. We have another reason that SHR was chosen and SD strain rat was abandoned in the present study for tacrolimus-induced nephrotoxicity. We have experience that SD rat died by intramuscular injection of 2 mg/kg/day for 2 weeks and did not have nephrotoxicity in dose group less than 2 mg/kg/day (data not shown). Accordingly, if 4 mg/kg/day of tacrolimus could be injected intramuscularly to SD strain rat for 2 weeks without death, this strain may also have tacrolimus-induced nephrotoxicity.

The profile of tacrolimus-induced nephrotoxicity in SHR was decrease of glomerular filtration rate (GFR) revealed by decrease of CCR, and histopathologically, luminal narrowing of the arteriole adjacent to the glomerulus and basophilic cortical tubules.

Since Na excretion ratio in the urine, which is one of the parameter of tubular function, was not changed, tacrolimus could scarcely have had a distinct injurious effect on tubular function. Basophilic cortical tubules were observed, but since neither necrosis nor increase of urine NAG activity were noted, it was considered that this tubular change was not serious and it was presumed to have been caused by circulatory dysfunction.

Above findings suggested that the decrease of glomerular filtration rate (GFR) was induced by vasoconstriction of arteriole adjacent to the glomerulus.

To confirm this hypothesis, several vasoactive factors were also investigated and the ameliorative effect on renal dysfunction by tacrolimus was examined through concomitant treatment with nilvadipine, which is a calcium antagonist.
and has a potent renal vasodilating activity (Ohtsuka et al., 1988).

Urinary TXB₂ (stable metabolite of thromboxane A₂ and urinary 6-keto-PGF₁α (stable metabolite of prostacyclin) were measured as the representative eicosanoide, i.e. vasoconstriction and vasodilating respectively.

Tacrolimus increased TXB₂ dose-dependently and counterwise decreased 6-keto-PGF₁α in the same way.

Increase of numbers of granules in the juxtaglomerular apparatus supports the role of tacrolimus in the acceleration of renin synthesis, and actually plasma renin activity, which affects contraction of the arterioles, increased in the rats dosed with the tacrolimus.

These findings suggest that the nephrotoxicity of tacrolimus is associated with the release of vasoactive factors (thromboxane and renin).

Furthermore, concomitant treatment with nilvadipine prevented the increase of BUN and P-Cr and decrease of Ccr induced by tacrolimus. In addition, histopathologically, basophilic cortical tubules and luminal narrowing of the arteriole adjacent to the glomerulus were almost completely prevented by concomitant treatment with nilvadipine. Thus, nilvadipine countered tacrolimus-induced nephrotoxicity. Since the concentration of tacrolimus in blood was not affected by the administration of nilvadipine, these ameliorating effects were considered to be based on the pharmacological effect of nilvadipine.

Therefore, tacrolimus-induced nephrotoxicity would be induced through renal vascular change.

From these results, tacrolimus caused the vasoconstriction of arterioles adjacent to the glomerulus and decrease the glomerular filtration rate followed by decrease BUN and P-Cr and induction of basophilic cortical tubules.

The reversibility of the tacrolimus nephrotoxicity was confirmed in stopping the drug for 2 or 4 weeks.

After the withdrawal period of 2 or 4 weeks, all renal changes induced by tacrolimus were demonstrated either complete or partial recovery during this period, the nephrotoxicity of tacrolimus was considered to be reversible.

REFERENCES


