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New morphological changes induced by FK506 in a short period in the rat kidney and the effect of superoxide dismutase and OKY-046 on THEM: the relationship of FK506 nephrotoxicity to lipid peroxidation and change in production of thromboxane A_2 in the kidney

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Abstract. Juxtaglomerular (JG) hyperplasia and tubular damage along with a decrease in the urine creatinine level induced by FK506 in rat kidney have already been reported in previous paper by us [6]. In this paper, we document the relationship of FK506 nephrotoxicity to the change in the production of thromboxane (Tx) A_2 and the lipid peroxidation of the cellular membrane in the rat kidney in order to clarify its morphogenesis. The urinary excretion of TxB_2 increased with FK506 administration even on day 1 ($P < 0.02$). Histologically, OKY-046 (thromboxane synthetase inhibitor) decreased tubular damage, although JG hyperplasia was not eradicated, while biochemically the excretion of TxB_2 decreased significantly ($P < 0.02$), and both the decrease in the urine creatinine level and the increase in the *N*-acetyl-β,D-glucosaminidase (NAG) index were relatively smaller. Although the FK506-induced morphological and biochemical changes could not be prevented by the continuous administration of superoxide dismutase (SOD) 30000 U/kg daily, the malondialdehyde content in renal tissue removed 1 h after FK506 administration had increased. These data suggest that FK506 nephrotoxicity is related to the change in the production of TxA_2 and lipid peroxidation of the cellular membrane. However, other mechanisms such as the involvement of sympathomimetic effects of FK506 and other vasoconstrictive factors cannot be rules out.

Key words: FK506 – Nephrotoxicity – JG hyperplasia – Thromboxane – Tubular damage – Vasoconstriction

FK506 is a potent immunosuppressive agent in vitro and in vivo in animals and shows great promise as a powerful means of inducing long-term allograft acceptance in the clinical setting [1–3]. However, there is a paucity of reports concerning morphological studies on nephrotoxicity, although some reports suggest that FK506 induces nephrotoxicity [4, 5]. In a previous paper, we reported that FK506 induced morphological changes [juxtaglomerular (JG) hyperplasia and tubular damage] in the rat kidney [6]. In this paper, we discuss the relationship of FK506 nephrotoxicity to the change in the production of thromboxane (Tx) A_2 and the lipid peroxidation of the cellular membrane in the rat kidney.

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Materials and methods

In total, 33 8-week-old female SPF Wistar rats, purchased from a commercial breeder, were used. FK506 freezed-dry powder was supplied by Fujisawa Pharmaceutical, Osaka, Japan. It was dissolved in saline for intraperitoneal injection. OKY-046 (thromboxane synthetase inhibitor) was supplied by Ono Pharmaceutical, Osaka, Japan).

It was dissolved in water for oral administration. Recombinant human superoxide dismutase (SOD) was supplied by Nippon Kayaku, Osaka, Japan. It was dissolved in saline for intravenous administration.

Rats were randomly divided into 5 groups, with 4 rats each in groups 1, 2, and 3; 18 in group 4; and 3 in group 5. Animals in group 1 were given FK506 10 mg/kg daily intraperitoneally for 7 days. Animals in group 2 were given OKY 100 mg/kg daily orally in combination with FK506 10 mg/kg daily intraperitoneally for 7 days. Animals in group 3 were administered SOD 30000 U/kg daily continuously with an osmotic pump (ALZET; model 2001) in combination with FK506 10 mg/kg daily intraperitoneally for 7 days. The osmotic pump was set in the subcutaneous tissue and connected with the polyethylene catheter. The other side of the catheter was inserted into the left iliac vein, and its tip was kept in the inferior vena cava. In group 4, animals were administered intraperitoneally with FK506 10 mg/kg only once. As a control for group 4, group 5 animals were not given any drug. The first day of drug administration is classified hereafter as day 0.

Each kidney was removed 24 h after the last injection of FK506 for morphological examination in groups 1, 2, and 3; and 0.5, 1, 2, 4, 8, and 24 h after FK506 injection for measuring of malondialdehyde (MDA) content in group 4. The kidneys in group 5 animals were removed, too. For morphological examination, H & E, PAS (periodic acid Schiff), Masson trichrome and PAM (periodic acid methenamine silver) stains were employed, and some kidneys were examined under the electron microscope. Renal tissue for the measuring of MDA was frozen at −80 °C. Urine samples over 24 h were collected on day 0 (from 24 h to just before the first administration of FK506), as well as on days 1, 3 and 7 to check the creatinine level, *N*-acetyl-β,D-glucosaminidase (NAG) activity, and TxB_2 content. The

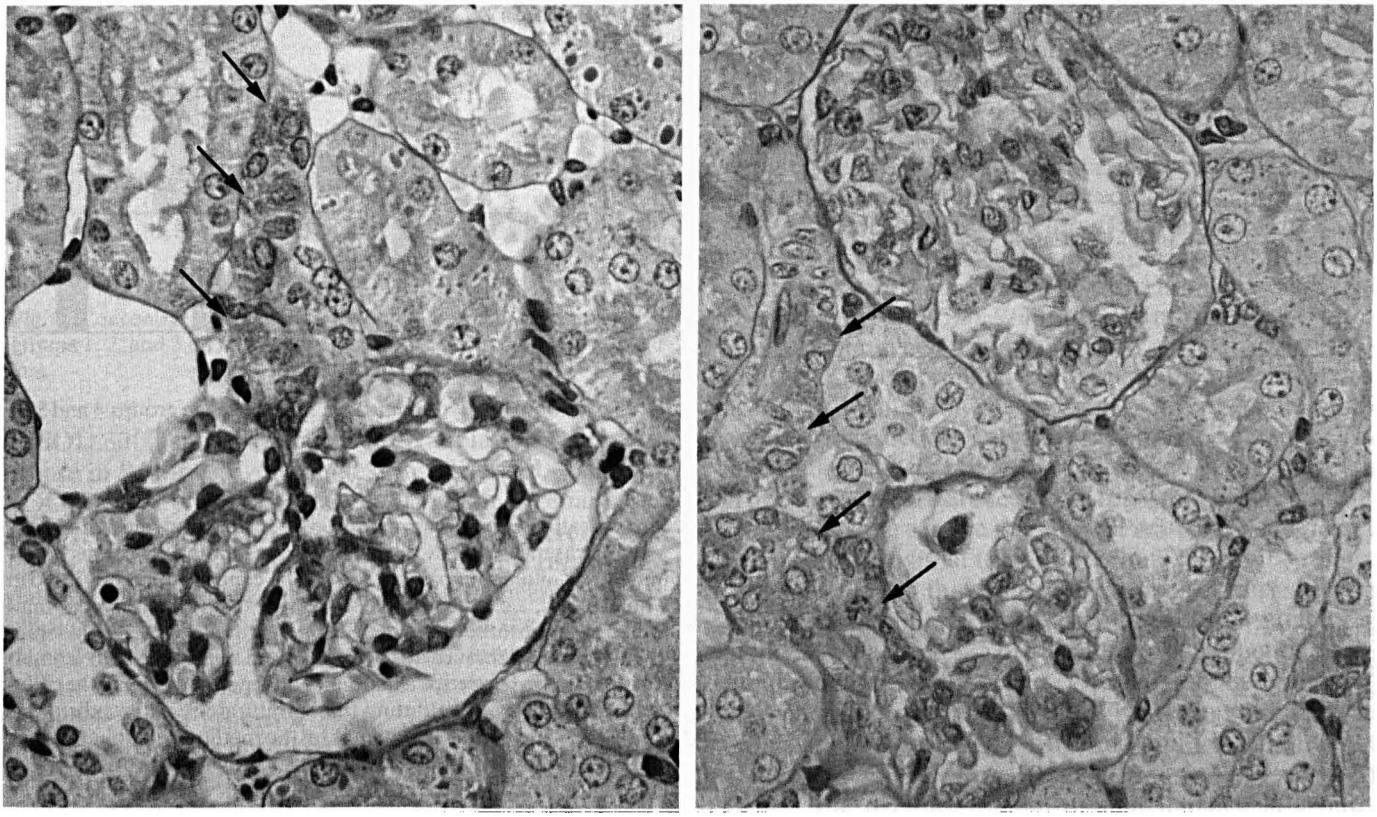


Fig. 1 A, B. Juxtaglomerular (JG) hyperplasia with a lot of renin granules in JG cell *(arrow)*. No significant differences between group 1 (**A**) and group 2 (**B**) (PAS, original magnification × 100)

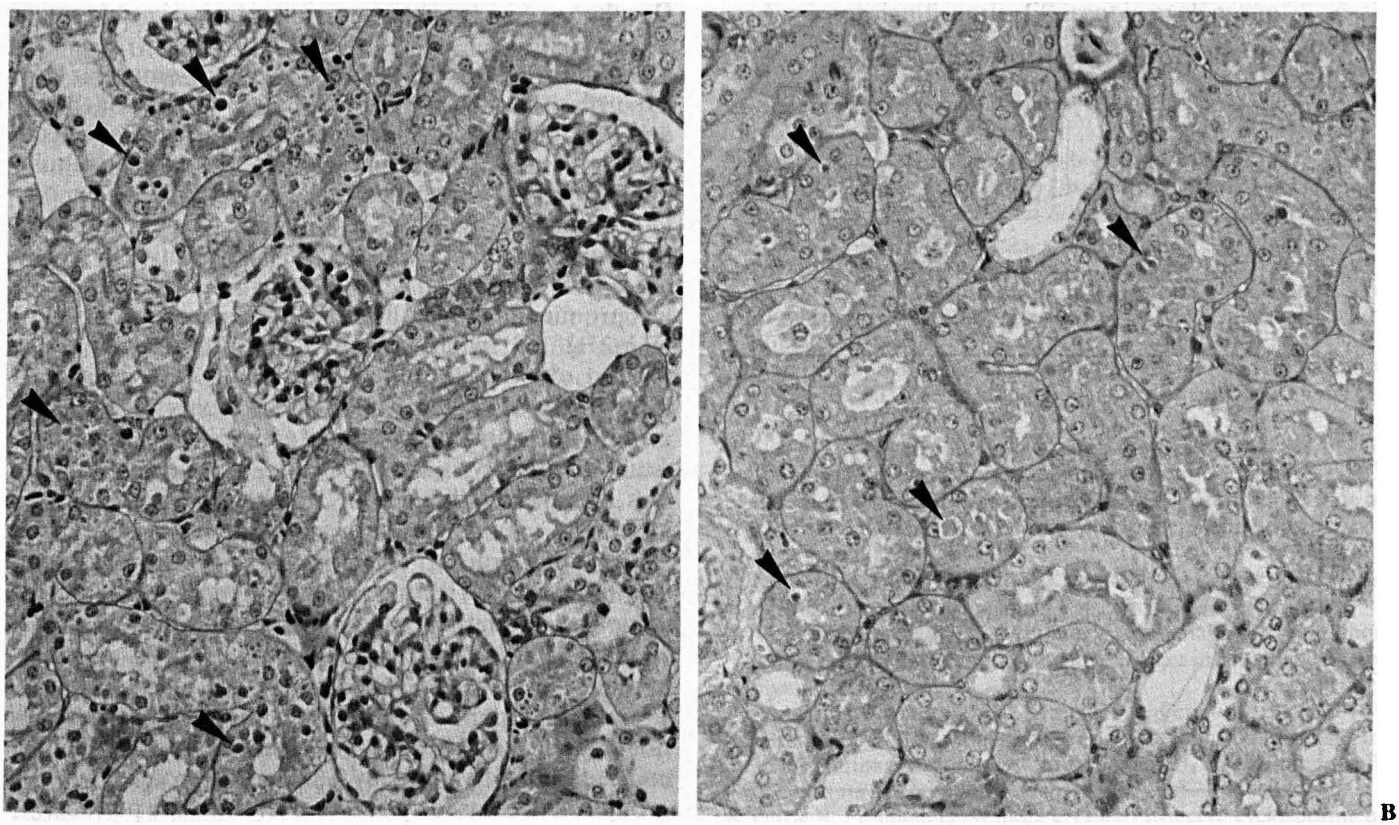
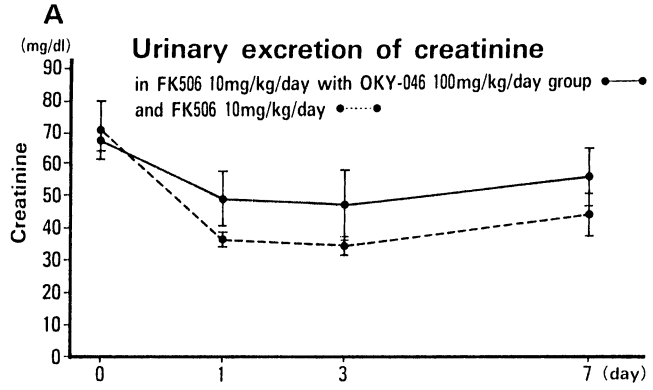


Fig. 2 A, B. PAS-stain positive large granules *(arrowheads)* in the proximal tubules prominent in group 1 (**A**), less prominent in group 2 (**B**) (PAS, original magnification × 50)



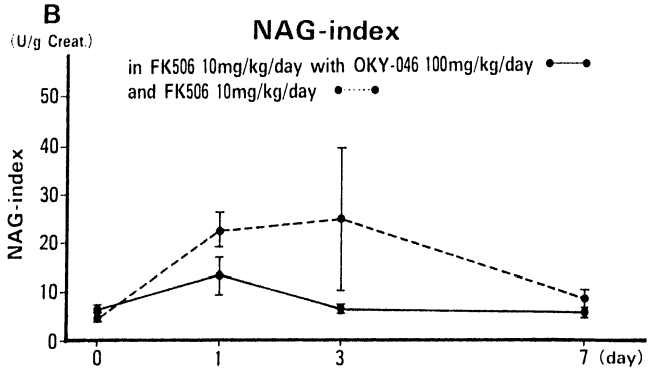
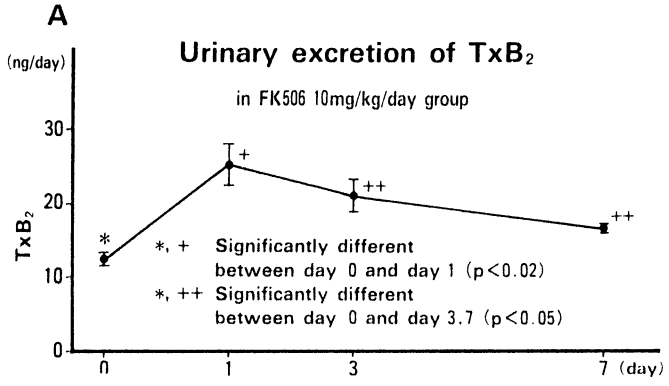


Fig. 3 A, B. Sequential changes of urine creatinine (**A**) and *N*-acetyl-β,D-glucosamini (NAG) index (**B**)



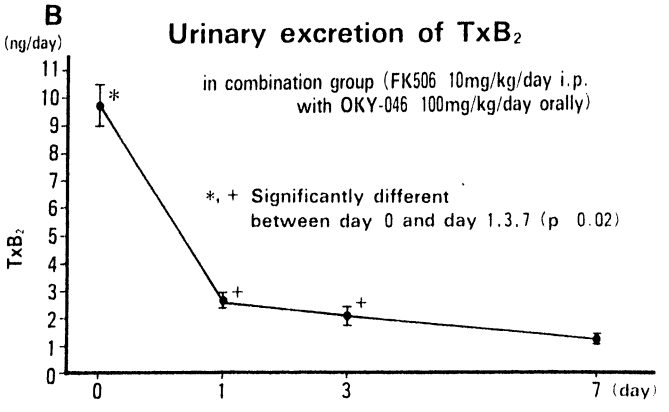


Fig. 4 A, B. Sequential changes sof urinary excretion of thromboxane (TxB_2) in group 1 (**A**) and Group 2 (**B**)

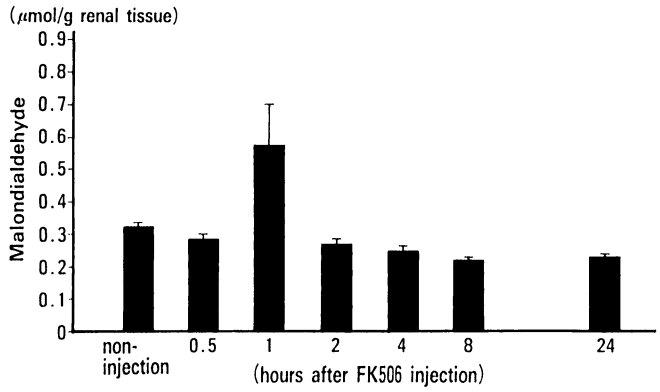


Fig. 5. Malondialdehyde content in renal tissue in groups 4 and 5

urine creatinine level was measured by colorimetry, and the NAG activity was measured by the alkali picrate method. TxB_2 was determined by radioimmunoassay (RIA). The concentration of FK506 in whole blood, i.e., the trough level, was determined by enzyme-linked immunosorbet assay (ELISA). In group 4, the MDA content in the kidneys removed at 0.5, 1, 2, 4, 8, and 24 h after FK506 administration was measured as a marker of lipid peroxidation of the cellular membrane by quantitating thiobarbiturate reactive substances (TRA-RS) and conjugated dienes by the methods of Ohkawa et al. and Suryanarayana and Recknagel, using high-performance liquid chromatography (HPLC). The MDA level was also checked in group 5, the control group.

Statistical analysis. Student's *t*-test was used for the statistical analysis of data for each group.

Results

Under morphological examination, juxtaglomerular (JG) hyperplasia and JG cell transformation as vascular change and PAS-positive large granules in the epithelial cells of the proximal tubules, especially at S1 and S2, as tubular damage were observed in group 1, as described in our previous paper [6] (Fig. 1 A, 2 A). In group 2, after the administration of both FK506 and OKY, PAS-positive large granules in the epithelial cells of the proximal tubules were less prominent and smaller in size compared with group 1 (Fig. 2 B). JG hyperplasia was almost the same as in group 1, although the expansion of the JG cell was slightly less prominent (Fig. 1 B). As for the biochemical data in group 1, the urinary excretion of creatinine declined significantly until day 3 ($P < 0.05$) (Fig. 3 A) and that of TxB_2 was significantly higher at days 1 ($P < 0.02$), 3, and 7 ($P < 0.05$) compared with preadministration values (Fig. 4 A). In group 2, the urinary excretion of TxB_2 was significantly low throughout the experimental period compared with the preadministration value ($P < 0.02$) (Fig. 4 B). Furthermore, both the decrease in the urine creatinine level and the increase in the NAG index during the experimental period were less than in group 1, although not critically so (Fig. 3 B). The urine creatinine level decreased by 37.5 % from day 0 to day 7 in group 1, while in group 2, it decreased by 16.1 % over the same period.

In group 3, histological changes induced by FK506 could not be prevented by the continuous administration

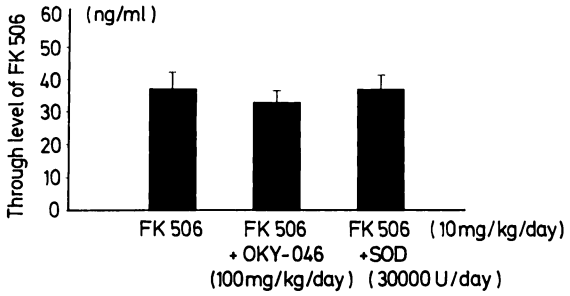


Fig. 6. Concentration of FK506 in whole blood, i.e., trough level, in groups 1, 2, and 3 on day 7 after injection. SOD, superoxide dismutase

of SOD 30000 U/kg daily; however, a relatively slight decrease in PAS-positive granules was observed. Although SOD was not effective, the MDA content in renal tissue removed 1 h after FK506 administration increased in group 4 compared with group 5 (Fig. 5). This rise is compatible with the peak level of FK506 concentration in whole blood (data not given here) when the aforementioned dosage of FK506 is administered intraperitoneally.

The concentration of FK506 in whole blood, i.e., the trough level, in groups 1, 2, and 3 was not significantly higher than in the clinical setting and was not significantly different in each group (Fig. 6).

Discussion

The present study suggests that the morphogenesis of nephrotoxicity induced by a daily intraperitoneal injection of FK506 even for a short period is related to both the change in the production of TxA_2 and the peroxidation of the cellular membrane in the rat kidney. FK506 induces vasoconstriction, especially proximal to the JGA. This vasoconstriction decreases the glomerular blood flow, which in turn causes JG hyperplasia as a compensatory reaction. This hypothesis is compatible with the biochemical data from group 1. The increase in the urinary excretion of TxB_2 even on day 1 suggests vasoconstriction in the renal vessels. Recent work by Benigni et al. [7] indicates that the urinary excretion of TxB_2 reflects the renal cell production of the TxA_2-strong vasoconstrictor, while an increased urinary excretion of 2,3-dinor-TxB_2 reflects TxA_2 synthesis by intrarenal platelets and macrophages. Furthermore, Petric et al. [8] demonstrated that a significant increase in the urinary TxB_2 excretion, along with a decrease in the renal blood flow, creatinine clearance, and urea clearance occurs in Sprague-Dayley rats treated with cyclosporin A. Hence, in our study, the increase in the urinary excretion of TxB_2 associated with FK506 administration in group 1 represents an increased production of TxA_2, specifically in the renal parenchyma. The decrease in the urine creatinine level is compatible with the decrease in the glomerular blood flow, and the increase in the urine creatinine level on day 7 may be a compensatory reaction caused by the decrease in the glomerular blood flow.

Although OKY could completely inhibit the increase in the urinary excretion of TxB_2 induced by FK506, morphological changes, especially in the JGA, could hardly be prevented. Therefore, other mechanisms like the involvement of sympathomimetic effects of FK506 and other vasoconstrictive factors causing the decrease in the glomerular blood flow cannot be ruled out. The rise of MDA in renal tissue 1 h after FK506 injection signifies damage to the cellular membrane, which would encourage the release of vasoconstrictive factors. TxA_2 is one of the strongest vasoconstrictors, and it is important to inhibit the stimulated synthesis of TxA_2 to prevent the vasoconstriction induced by FK506. For further confirmation of the relationship between vasoconstriction and peroxidation of the cellular membrane, experiments with an optimal dosage of SOD and other free radical scavengers and antagonists for vasoconstrictive factors are also necessary. The fact that the decrease in the urine creatinine level in group 2 was relatively less than that in group 1 indicates that the decrease in the number of PAS-positive large granules in the proximal tubules in group 2 might be related to the changes in the urine creatinine level caused by the change in the glomerular blood flow. If the glomerular blood flow decreases, a disturbance in the function of the tubular epithelial cell occurs. Our data on the daily morphological changes induced by FK506 suggests that the tubular damage changes with time and is related to the glomerular blood flow (manuscript in preparation). Hence, the decrease of the tubular damage in group 2 might be attributed to the slightly smaller decrease in the urine creatinine level compared with group 1.

Although the injection dose of FK506 might be thought too high, the trough level of FK506 in groups 1, 2, and 3 was not significantly higher than that in the clinical setting and was not significantly different in each group.

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