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Short-Term and Long-Term FK506 Treatment Alters the Vascular Reactivity of Renal and Mesenteric Vascular Beds

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Abstract. The aims of this study were to investigate the role of endothelin-1 in FK506-induced hypertension and vascular dysfunction of rats treated with the drug for 8 (short-term) or 30 (long-term) days and to measure malondialdehyde levels in the kidneys. Kidney and mesentery of rats were perfused. In the short-term treated groups, there was no significant change in systolic blood pressure. The response to noradrenaline only in renal vascular beds was significantly increased by FK506 and this increase was prevented by Bosentan. FK506 had no significant effect on sodium nitroprusside-induced vasodilation in comparison with solvent in both vascular beds. Bosentan failed to prevent these responses. In the long-term treated groups, at the end of the treatment with FK506, there was a significant increase in blood pressure, but no change in the response to noradrenaline in either kidneys or mesentery. The increase in blood pressure was prevented by bosentan treatment. FK506 increased malondialdehyde levels in the kidneys of the rats from only the long-term treated groups. Bosentan did not change this increase. Our results indicated that endothelin-1 plays a key role in the FK506-induced change in vascular reactivity to noradrenaline in renal vascular beds and drug-induced hypertension in the rats. There was no relationship between oxidative stress and FK506-induced hypertension.

Keywords: bosentan, endothelin-1, isolated perfused kidney and mesentery, malondialdehyde, tacrolimus (FK506)

Introduction

Tacrolimus (FK506) is an immunosuppressant agent that is widely used in transplanted patients. Hypertension is one of the major adverse effects of FK506 treatment. The hypertensive effect of FK506-treatment is observed in about 30% of liver and 40% of heart transplanted patients (1, 2). The mechanism of hypertension remains to be elucidated.

In rats, after 21-day treatment with FK506, hypertension is not induced, but 30 day-treatment causes hypertension (3, 4). FK506-induced hypertension is due to the increase in endothelin-1 synthesis in the vasculature (4). However, the findings related to the release of endothelin-1 due to FK506 are controversial. FK506 induces endothelin-1 release by cultured rat mesangial cells and increases the expression of prepro endothelin-1 mRNA in human umbilical vein endothelial cells (5, 6). In contrast, FK506 does not affect endothelin-1 release in bovine aortic endothelial cells or in cultured renal epithelial cell line (7, 8).

On the other hand, the contribution of FK506-induced vascular reactivity changes to the drug-induced hypertension remains to be evaluated. In our previous experiments, we observed that FK506, given by perfusion for 20 min before the onset of bolus noradrenaline administration, acutely increased noradrenaline responses in rat isolated perfused kidneys (9). Since bosentan prevented these responses, we suggested that endothelin-1 might play a role in the FK506-induced augmentation in noradrenaline responses in the rat kidney (9). Tissue perfusion methods are very suitable techniques for evaluating the tissue-whole vascular bed responses to the drugs applied. Isolated perfused tissues are frequently used to evaluate drug effects and they have all the local control mechanisms without the intervention of central sympathetic and humoral regulation. We pro-

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posed that changes in the vascular reactivity (increased and decreased responsiveness to vasoconstrictors and vasodilators, respectively) of renal and mesenteric beds might play an important role in the regulation of systemic blood pressure. Additionally, we wondered about the long-term effects of FK506 on blood pressure and on the reactivitiy of renal and mesenteric vasculature. For these reasons, we selected these two vascular beds to investigate the reactivity changes induced by giving FK506 intraperitoneally to the rats for 8 and 30 days. To the best of our knowledge, there is no comparably detailed literature about the vascular effects of FK506 on these vascular beds in relation to the development of hypertension. Therefore, our aim was to investigate whether FK506-induced vascular reactivity changes in renal and mesenteric beds are parallel to the drug-induced hypertension and additionally, whether these effects could be prevented by the endothelin ET-1 receptor antagonist bosentan. Furthermore, there is evidence that oxidative damage plays an important role in hypertension (10). It has also been reported that administration of FK506 increases the synthesis of malondialdehyde (end product of lipid peroxidation promoted by oxidative stress) in rat livers (11). Moreover, malondialdehyde levels in liver homogenates have been found to be higher in hypertensive rats (12). In the light of these findings, our secondary goal was to determine whether there is a positive relationship between oxidative stress and FK506-induced hypertension in rats. Thus, we also measured kidney malondialdehyde levels in the rats treated short- or long-term with FK506.

Materials and Methods

Animals

Animals were purchased from Hacettepe University, Animal House. The use of experimental animals and the study protocol were both approved by the Animal Care Committee of Hacettepe University.

Design of experimental groups

Eight experimental groups were designed: Rats were treated for 8 days (short-term treatment) with 1) FK506 (1 mg/kg per day, i.p.), 2) solvent (HCO-60; at equivalent doses, i.p.), 3) saline (0.2 ml/250 g BW per day, i.p.), 4) FK506 plus bosentan (10 mg/kg per day, i.p.) (groups 1 – 4), and the same treatment for 30 days (long-term treatment, groups 5 – 8).

Drugs

The following drugs were used: Bosentan (Hoffman-LaRoche, Basel, Switzerland), FK506 (Fujisawa Pharmaceutical Co., Ltd., Osaka), noradrenaline hydrochloride (Sigma, St. Louis, MO, USA), phenylephrine hydrochloride (Sigma), and sodium nitroprusside (Fluka Chemika, Buchs, Switzerland).

All drugs were dissolved in distilled water, except FK506, which was dissolved in HCO-60 (Polyoxyethylene Hydrogenated Castor Oil 60; Nikko Chemicals Co., Ltd., Osaka).

Isolated organ perfusion methods

Male Wistar rats (230 – 300 g) were anesthetized with urethane (1.25 g/kg, i.p.). After opening the peritoneal cavity, the left renal or mesenteric artery was cannulated via a polyethylene catheter. Then kidney or mesentery was removed and transferred into a warmed plexiglass chamber and perfused continuously with warmed (37°C) and bubbled (95% O_2 and 5% CO_2 gas mixture) Krebs-Henseleit solution with the following composition: 118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, and 10 mM glucose. Perfusion was performed with a peristaltic pump (Eyela MP-32; Rikakikai, Tokyo) delivering a constant flow (8 – 10 ml/min for kidney and 5 – 6 ml/min for mesentery) throughout the experiment (9). Drugs were either constantly perfused or given as a bolus injection delivered into the silicone rubber perfusate tubing, close to the kidney. Renal and mesenteric vascular responses were monitored by a pressure transducer connected to the Biopac MP150 data acquisition system (BIOPAC Systems, Inc., Goleta, CA, USA). The preparations were stable for 2 – 3 and 4 – 5 h for kidneys and mesentery, respectively. Basal perfusion pressures were 84.3 ± 9.5 and 23.3 ± 2.7 mmHg, for the kidneys and mesentery, respectively, at the beginning of the experiment and were 81.9 ± 6.5 and 25.7 ± 3.5 mmHg, respectively, at the end. Sodium nitroprusside was tested when the basal perfusion pressure was raised by phenylephrine at submaximal concentration.

Measurement of vascular reactivity

In all groups, the responses to noradrenaline and sodium nitroprusside were obtained. After an equilibration period of 30 min, subsequent doses of noradrenaline (M) were given by bolus injections and dose-dependent increases in perfusion pressure (vasoconstrictions) were recorded. To obtain the decreases in perfusion pressure (vasodilator responses), perfusion with phenylephrine, at a concentration (3×10^{-6} M for kidney, 10^{-5} M for mesentery) that causes submaximum constriction (60% – 80% of maximum response), was initiated and continued until the end of the experiment. After the phenylephrine-induced vasoconstriction had reached a plateau, subsequent doses of the endothelium-indepen-

dent vasodilator sodium nitroprusside (M) were given by bolus injections and dose-dependent vasodilations were recorded.

Measurement of systolic blood pressure

Systolic blood pressures were measured via the tail-cuff method (13). The tails of the rats were warmed for 20 min at 40°C and a 20-mm cuff attached to a manometer was placed on the proximal part of each tail. When the cuff was then inflated to a pressure exceeding the systolic pressure, the vibrations ceased. With slow deflation of the cuff, the vibrations suddenly reappeared and increased in amplitude as the cuff pressure approached zero. The pressure at which the pulsations made their first reappearance was taken as the systolic endpoint. In the short-term treated groups, systolic blood pressures of the rats were measured every day. In the long-term treated groups, systolic blood pressures of the rats were measured in three-day intervals.

Measurement of plasma FK506 levels

For the measurement of plasma FK506 levels, abdominal aortas were clamped at the point above where the mesenteric artery branches from. After removing the mesenteric or renal vascular beds, 1.5 – 2 ml of blood from the abdominal aortas of the rats was taken into the tubes containing EDTA and then was immediately stored at 4°C. These samples were stored for 24 h before being assayed. Finally, plasma FK506 levels were measured using an automated enzyme immunoassay system on the IMx analyzer (Abbott Laboratories, Abbott Park, IL, USA) (14).

Measurement of renal malondialdehyde levels

The levels of lipid peroxides were measured as the concentration of thiobarbituric acid reactive substances for each rat. The right kidneys were removed from all of the rats in all 8 groups after opening the peritoneal cavity and the samples obtained from each rat were immediately frozen and stored at –20°C for assay of malondialdehyde. Lipid peroxidation in renal tissues was determined by the method of Mihara and Uchiyama (15). Tissues were homogenized in 10 volumes (weight /volume) of cold phosphate buffer (pH 7.4). A total of 0.5 ml homogenate was mixed with 3 ml 1% H_3PO_4. After the addition of 1 ml 0.67% thiobarbituric acid, the mixture was heated in boiling water for 45 min. The color was extracted into *n*-butanol, and the absorption at 532 nm was measured. Using tetramethoxypropane as a standard, tissue lipid peroxide levels were calculated in nanomoles per gram of wet tissue.

Data and statistical analyses

Vascular responses were measured as the increase or decrease in perfusion pressure and expressed as percentage of submaximum response to phenylephrine. The results were expressed as means ± S.E.M. For statistical analysis of vascular responses and blood pressure measurements, repeated-measures ANOVA was used. When the F ratio of the ANOVA reached a critical value of $P<0.05$, Scheffé post hoc test was used to compare first time period (day 0) with other time periods for blood pressure measurements. Student's *t*-test was used for the evaluation of renal MDA levels of the rats. When P was less than 0.05, differences were considered to be statistically significant.

Results

Plasma FK506 levels

FK506 plasma levels in the rats treated with FK506 (1 mg/kg per day) were 4.1 ± 0.41 ng/ml (n = 5) and 4.08 ± 0.14 ng/ml (n = 13) after 8 and 30 days of treatment, respectively.

The effect of FK506 and bosentan on the survival of the rats

In the experiments performed by giving 1 mg/kg doses of FK506 to the rats for 8 days, no deaths were observed. When the same dose was given for 30 days, 20% of the rats died within 15 – 20 days of treatment onset. However, no deaths were observed when bosentan was given together with FK506 treatment for 30 days. No deaths were observed in the rats from short-term and long-term groups treated with solvent or saline.

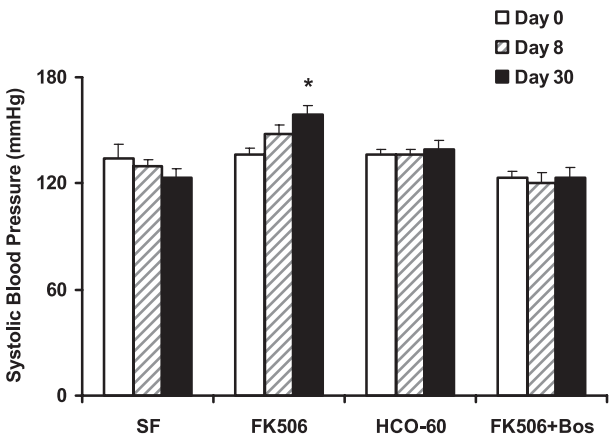

Fig. 1. The effects of 8-day treatment (short-term) and 30-day treatment (long-term) with FK506 (1 mg/kg per day, n = 14), HCO-60 (at equivalent doses, n = 20), saline (SF; 0.2 ml/250 g per day, n = 8; control), and FK506 (1 mg/kg per day) + Bosentan (Bos; 10 mg/kg per day, n = 8) on systolic blood pressure of rats. ANOVA for repeated measures: F 3.669, $P<0.001$. *$P<0.05$ *vs* day 0.

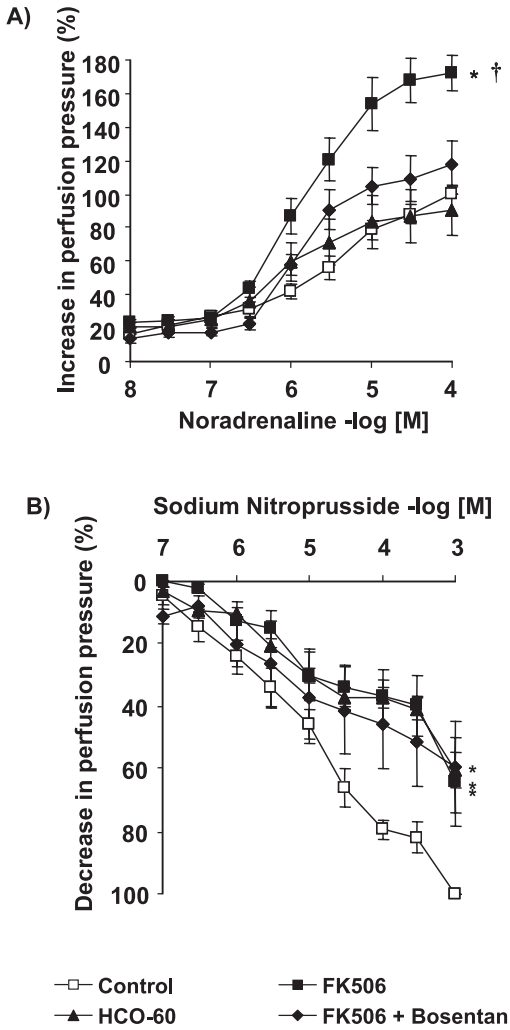

Fig. 2. The effects of 8-day treatment (short-term) with FK506 (1 mg/kg per day, n = 9), HCO-60 (at equivalent dose, n = 6), saline (0.2 ml/250 g per day, n = 6; control), and FK506 (1 mg/kg per day) + Bosentan (10 mg/kg per day, n = 6) on noradrenaline-induced increases in perfusion pressure (A) and with FK506 (1 mg/kg per day, n = 8), HCO-60 (at equivalent dose, n = 5), saline (0.2 ml/250 g per day, n = 6; control), and FK506 (1 mg/kg per day) + Bosentan (10 mg/kg per day, n = 6) on sodium nitroprusside-induced decreases in perfusion pressure (B) in isolated rat perfused kidney. **P*<0.05 *vs* control, †*P*<0.05 *vs* FK506 + Bosentan.

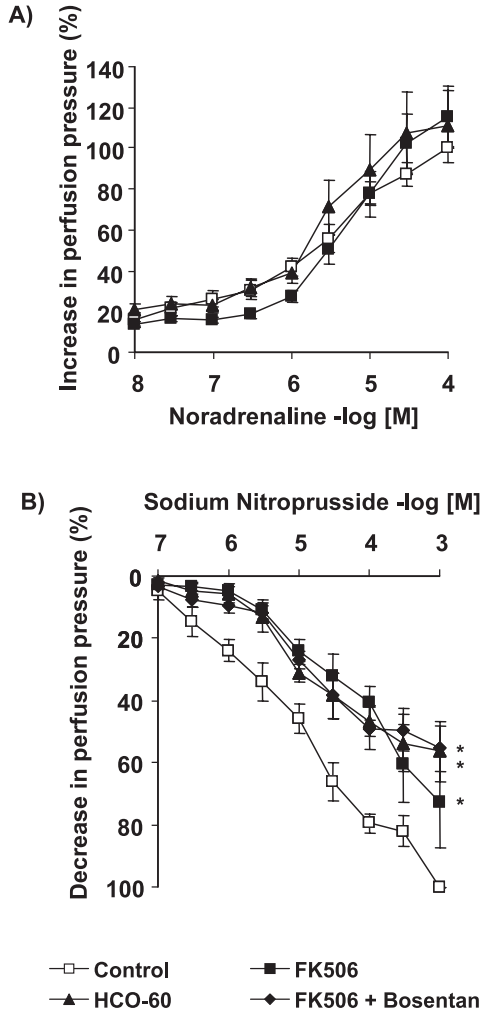

Fig. 3. The effects of 30-day treatment (long-term) with FK506 (1 mg/kg per day, n = 9), HCO-60 (at equivalent dose, n = 13), and saline (0.2 ml/250 g per day, n = 6; control) on noradrenaline-induced increases in perfusion pressure (A) and with FK506 (1 mg/kg per day, n = 10), HCO-60 (at equivalent dose, n = 5), saline (0.2 ml/250 g per day, n = 6; control), and FK506 (1 mg/kg per day) + Bosentan (10 mg/kg per day, n = 6) on sodium nitroprusside-induced decreases in perfusion pressure (B) in rat isolated perfused kidney. **P*<0.05 *vs* control.

The effect of FK506 on systolic blood pressure of rats

Control blood pressures were 134.6 ± 2.3 mmHg. There was no significant change in systolic blood pressure of the rats treated for 8 days with FK506 or solvent. In the rats treated for 30 days with FK506, FK506 increased the systolic blood pressure (Fig. 1) as compared to the 0th day of treatment. The significant increase appeared on the 28th day of treatment. Bosentan (10 mg/kg per day) given together with FK506 (1 mg/kg per day) for 30 days prevented drug-induced hypertension (Fig. 1).

The effects of FK506 on the noradrenaline- and sodium nitroprusside-induced responses in isolated perfused kidney

Short-term treatment: Noradrenaline (10^{-8} – 10^{-4} M) produced dose-dependent constrictions in perfused renal vascular beds. FK506-treatment significantly increased the noradrenaline-induced vasoconstrictions (Fig. 2A). Bosentan (10 mg/kg per day) given together with FK506 prevented the increase in noradrenaline responses (Fig. 2A). There was no significant change in the solvent-treated group (Fig. 2A).

Sodium nitroprusside (10^{-7} – 10^{-3} M) induced dose-

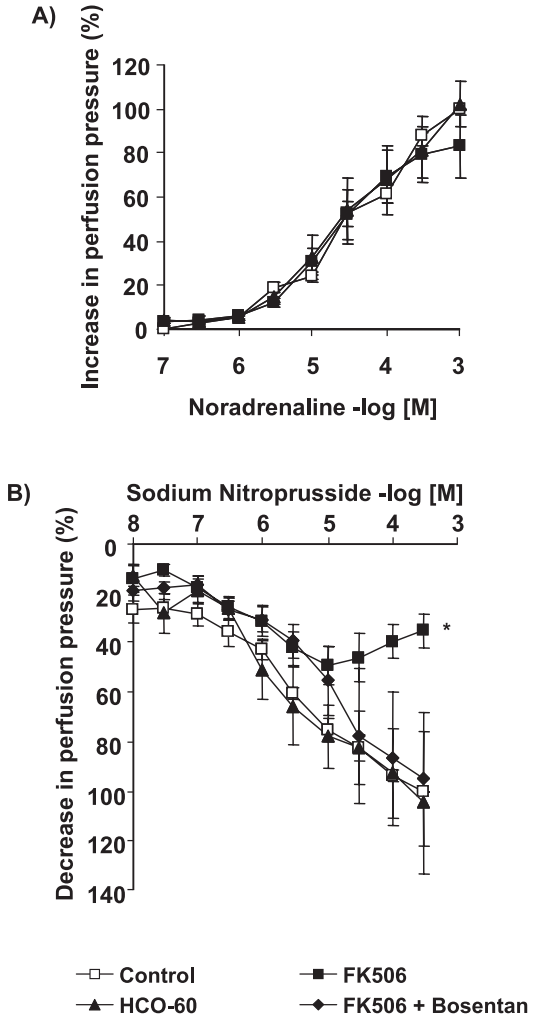

Fig. 4. The effects of 8-day treatment (short-term) with FK506 (1 mg/kg per day, n = 5), HCO-60 (at equivalent dose, n = 8), and saline (0.2 ml/250 g per day, n = 6; control) on noradrenaline-induced increases in perfusion pressure (A) and with FK506 (1 mg/kg per day, n = 6), HCO-60 (at equivalent dose, n = 5), saline (0.2 ml/250 g per day, n = 6; control), and FK506 (1 mg/kg per day) + Bosentan (10 mg/kg per day, n = 6) on sodium nitroprusside-induced decreases in perfusion pressure (B) in rat isolated perfused mesentery. **P*<0.05 *vs* control.

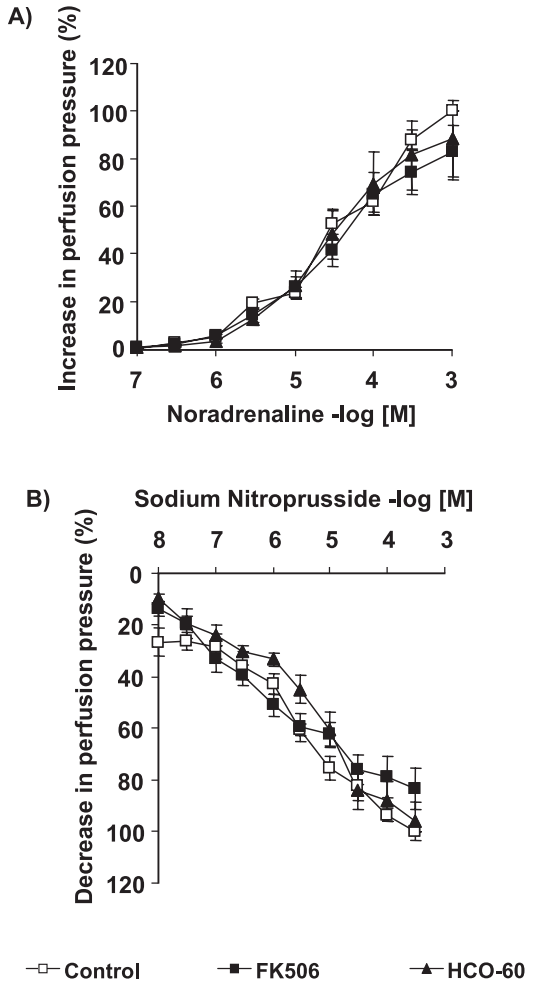

Fig. 5. The effects of 30-day treatment (long-term) with FK506 (1 mg/kg per day, n = 8 – 13), HCO-60 (at equivalent dose, n = 7 – 12), and saline (0.2 ml/250 g per day, n = 6; control) on noradrenaline-induced increases (A) and sodium nitroprusside-induced decreases (B) in perfusion pressure in rat isolated perfused mesentery.

dependent vasodilation in kidney vasculature. Sodium nitroprusside-induced vasodilator responses were decreased by FK506 (Fig. 2B) but also by the solvent (Fig. 2B). FK506 had no significant effect on sodium nitroprusside-induced vasodilator responses in comparison with solvent (Fig. 2B). Bosentan-treatment (10 mg /kg per day) failed to reverse sodium nitroprusside responses (Fig. 2B).

Long-term treatment: Noradrenaline-induced vasoconstrictor responses in renal vascular beds were not significantly different in FK506- and solvent-treated groups in comparison to controls (Fig. 3A). Bosentan given together with FK506 had no effect on the responses (not shown).

Sodium nitroprusside-induced vasodilator responses in renal vascular beds were decreased by FK506 (Fig. 3B) as well as by solvent (Fig. 3B). FK506 had no significant effect on sodium nitroprusside-induced vasodilator responses in comparison with solvent (Fig. 3B). Bosentan (10 mg/kg per day) given together with FK506 had no effect on these responses (Fig. 3B).

The effects of FK506 on the noradrenaline- and sodium nitroprusside-induced responses in isolated perfused mesentery

Short-term treatment: Noradrenaline (10^{-7} – 10^{-3} M) induced dose-dependent vasoconstriction in the isolated perfused mesenteric beds. Noradrenaline-induced vasoconstrictor responses were not significantly different

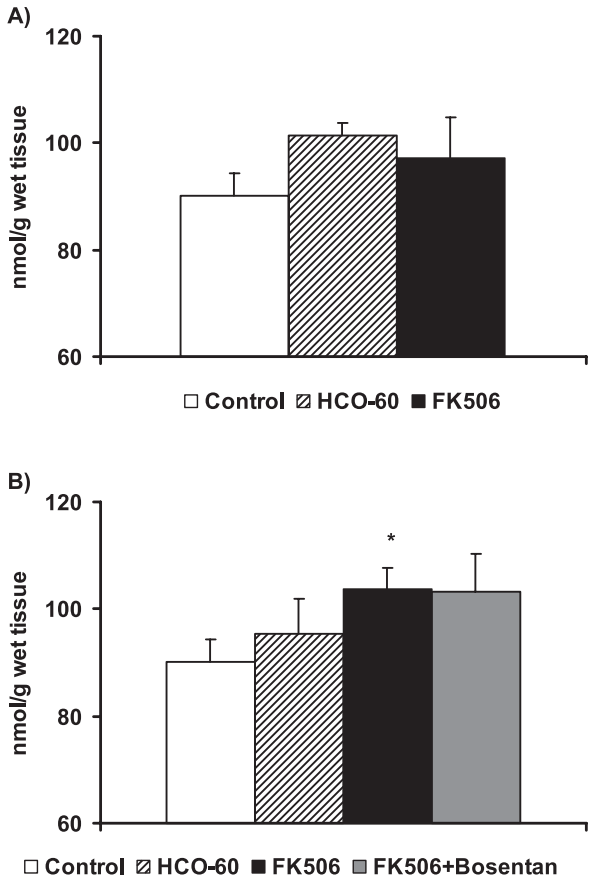

Fig. 6. The effects of 8-day treatment (short-term) with FK506 (1 mg/kg per day, n = 9), HCO-60 (at equivalent dose, n = 6), and saline (0.2 ml/250 g per day, n = 11; control) (A) and 30-day treatment (long-term) with FK506 (1 mg/kg per day, n = 11), HCO-60 (at equivalent dose, n = 9), saline (0.2 ml/250 g per day, n = 11; control), and FK506 (1 mg/kg per day) + Bosentan (10 mg/kg per day, n = 8) (B) on malondialdehyde levels in rat kidney homogenates. **P*<0.05 *vs* control.

in the FK506-treated group (Fig. 4A). There was no significant change in the solvent-treated group (Fig. 4A). Bosentan given together with FK506 had no effect on the responses (not shown).

Sodium nitroprusside ($10^{-8} - 3 \times 10^{-4}$ M) produced dose-dependent vasodilation in the perfused mesentery. Sodium nitroprusside-induced vasodilator responses were significantly decreased by FK506 (Fig. 4B) and bosentan-treatment (10 mg/kg per day) failed to prevent the decrease in these responses (Fig. 4B). There was no significant change in the solvent-treated group (Fig. 4B).

Long-term treatment: Noradrenaline-induced vasoconstrictor responses in mesenteric vascular beds were not significantly different in the FK506- and solvent-treated groups in comparison to controls (Fig. 5A). Bosentan given together with FK506 had no effect on the responses (not shown).

FK506 and the solvent did not affect sodium nitroprusside-induced vasodilator responses (Fig. 5B). Bosentan given together with FK506 had no effect on the responses (not shown).

Malondialdehyde levels in renal homogenates

Short-term treatment with FK506 did not alter malondialdehyde levels in kidneys in comparison to controls (Fig. 6A). Bosentan given together with FK506 had no effect on the responses (not shown). Kidney malondialdehyde levels were significantly higher after 30 days of treatment with FK506 (Fig. 6B). Bosentan given together with FK506 did not prevent this augmentation (Fig. 6B). The solvent of the drug did not alter the malondialdehyde levels in either the short- or long-term treated groups (Fig. 6: A and B).

Discussion

This study demonstrated that FK506 affects renal and mesenteric vascular beds in different ways. Moreover, it showed that different mechanisms play a role in the drug-induced increase in vasoconstriction and the decrease in vasodilator responses. Our study also has shown that endothelin-1 plays a role in the FK506-induced increase in the vasoconstrictor responses to noradrenaline in kidneys, as well as FK506-induced hypertension. Whereas the FK506-induced increase in renal malondialdehyde levels is unlikely to be mediated by endothelin-1, the solvent appeared to be responsible for the inhibitory effect of FK506 on sodium nitroprusside-induced vasodilation in renal vasculature.

It has been demonstrated that splenic lymphocyte proliferative activity is significantly decreased by 1.0 and 2.0 mg FK506/kg per day, indicating that FK506 doses given intraperitoneally are immunosuppressive (16). In preliminary experiments, we used a dose of 2 mg/kg per day, but we had to diminish the dose to 1 mg/kg per day because of the high rate of adverse effects of FK506 (body weight loss, adhesion of intra-abdominal organs, intestinal perforation, and death) observed with higher doses. In our following experiments performed by giving FK506 to the rats in a dose of 1 mg/kg per day, adverse effects appeared less frequently and measured blood levels of FK506 were still comparable to the reported values after intraperitoneal administration (16). Therefore, we used this dose throughout the current experiment. Even with the dose of 1 mg/kg per day for 30 days, a decrease in movement activities, depressive state, loss of appetite, body weight loss, and even deaths were observed in the rats. Bosentan completely improved the general condition of the rats and prevented the death of the animals. Our results

related to the improving effects of bosentan are in agreement with the previous study that reports beneficial effects of the drug on the general condition and survival of mice in a septic shock model (17).

Although a hypertensive effect was not observed after 8 days of treatment with FK506, a significant increase in systolic blood pressure occurred following 30 days of treatment. Our result is in accordance with the finding of Takeda et al. (4). They reported that administration of FK506 (5 mg/kg per day, by gavage) for 4 weeks results in hypertension in rats (4). Our finding also demonstrated that endothelin-1 contributed to the increased blood pressure induced by long-term treatment with FK506, since increased blood pressure returned to the control levels following the addition of bosentan to the treatment. It has been reported that FK506 increases blood pressure via an increase in endothelin-1 production (4).

In our study, noradrenaline-induced vasoconstrictor responses were increased by short-term FK506 treatment, and this increase was prevented by bosentan in the perfused rat kidneys. Therefore, it could be suggested that FK506 caused the release of endothelin-1 in the rat renal vasculature and noradrenaline-induced vasoconstrictor responses were potentiated by endothelin-1. There exist a number of studies that demonstrate controversial results about the release of endothelin-1 by FK506 from various cultured cells (5 – 7). Likewise, there is no consistent evidence on its validity with regard to its relevance to perfusion pressure of renal and mesenteric vascular beds. It has been reported that vascular resistance increased by FK506 is not affected or partially inhibited by the endothelin ET-1 receptor antagonist in isolated rat kidneys (18, 19). We report here, for the first time, that antagonism of endothelin ET-1 receptors by bosentan prevents the FK506-induced potentiation of vasoconstrictor responses to noradrenaline after 8 days of treatment in renal vascular beds. Renal vasculature is very sensitive to vasoconstrictor effects of endothelin-1 (20). Endothelin-1, in even lower concentrations, increases the renal vascular resistance (21). In addition to its vasoconstrictor effect, endothelin-1 potentiates the effects of the sympathetic nervous system (22).

In contrast to its effects on renal vascular beds in our study, noradrenaline-induced vasoconstrictor responses were not potentiated by 8-day treatment with FK506 in mesenteric vasculature. Mesenteric vasculature might be less sensitive to the vasoconstrictor effects of endothelin-1 than renal vasculature. Alternatively, the amount of endothelin-1 released from mesenteric vasculature by FK506-treatment might be less than that released from renal vascular beds. However, it has been demonstrated that 24-h incubation of rat isolated mesenteric arteries with FK506 increases the responses to noradrenaline (3). Conversely, 8-day- and 21-day treatment with FK506 decreases noradrenaline-induced contractile responses (3). These findings are not consistent with our results. This controversy may be due to the different experimental methods (isolated tissue in organ bath *vs* perfused system method) and different levels of the tissue studied (segment of the artery *vs* whole vascular bed).

In our study, sodium nitroprusside-induced vasodilator responses were impaired by 8-day treatment with FK506 in both renal and mesenteric vascular beds. More importantly, solvent, by itself, caused an inhibitory action on sodium nitroprusside-induced dilation similar to that of FK506 in kidneys. Therefore, the inhibitory effect of FK506 on sodium nitroprusside-induced dilation is most likely to be due to the action of its solvent. Likewise, the solvents of another immunosuppressive drug, cyclosporine, namely cremophor EL and Labrafil, which are chemical substances related to HCO-60, have been reported to exhibit similar activities in various vascular preparations (23, 24).

The changes in vascular reactivity of renal and mesenteric beds were not associated with the hypertension that developed. Although hypertension appeared after 30 days of treatment with FK506, there was no increased reactivity to noradrenaline in both vascular beds. An impairment in sodium nitroprusside-induced relaxation continued in the kidneys of rats treated with FK506 for 30 days. However, the last effect of the drug was not its own effect, as discussed above. It could be expected that vascular changes to noradrenaline and sodium nitroprusside would increase more after 30 days of treatment as compared to 8-day administration, in a parallel manner to the increase in blood pressure induced by FK506. An explanation for this disparity might be that even though there is changed reactivity observed in the short-term groups, treatment with the drug was blunted or suppressed by some compensatory mechanisms developed after long-term administration. Alternatively, increased or impaired vascular reactivity caused by FK506 was unlikely to be the sole explanation for its hypertensive side effect. Gardiner et al. (25) have reported that the blood pressure increasing effect of FK506 must be due to an increase in cardiac output. When given by bolus i.v. injection, FK506 causes a pressor effect, which is maximal when there is no significant regional vasoconstriction in conscious rats (25).

There is increasing evidence that both oxidative stress and associated oxidative damage play an important role in various cardiovascular pathologies, including

hypertension (10). Higher malondialdehyde levels in liver homogenates, which reflect the oxidative damage in this tissue, have been found in spontaneously hypertensive rats (SHR) than of Wistar-Kyoto (WKY) rats (12). On the other hand, it has also been reported that administration of FK506 increases the synthesis of malondialdehyde in rat livers (11). Similarly, in our study, we showed that FK506, after 30 days of treatment, augmented malondialdehyde levels significantly in the kidneys of rats. In the light of these findings, it could be assumed that FK506 would cause oxidative damage, which might lead to hypertension. Is there any link between oxidative damage and endothelin-1? There are in vivo findings indicating that increased oxidative stress is associated with activation of the endothelin system, through endothelin ET-1 receptors, in hypertension (26). In our experiments, bosentan prevented the blood pressure increase due to FK506; however, it failed to restore increased malondialdehyde levels by the drug in kidneys. It is not surprising that bosentan has no effect on malondialdehyde levels in kidney, if oxidative stress induced by FK506 affects directly or indirectly endothelial cells to release ET-1. For this reason, the linkage between oxidative damage to the kidneys and hypertension induced by the drug applied for 30 days should be carefully interpreted. More importantly, the oxidative damage reflected by increased malondialdehyde levels in kidneys might be an index for nephrotoxicity, which is another important side effect induced by FK506. Although the exact mechanism of nephrotoxicity is not fully understood, several factors, including the increased release of endothelin-1, have been implicated in its pathogenesis (27). However, in the light of our finding, the possible role for endothelin-1 in nephrotoxicity seems to be unlikely. Oxidative stress plays a key role in endothelial dysfunction, vascular, and end-organ damage by promoting nitric oxide inactivation, lipid peroxidation, DNA damage, and protein modification. The vascular damage promoted by oxidative stress in renal vascular beds may be a reasonable explanation for the lack of increased responsiveness to noradrenaline in the rats treated with FK506 for 30 days, since endothelial dysfunction will also lead to a defective release of endothelin-1.

In conclusion, we demonstrated that endothelin-1 contributed to the FK506-induced hypertension and increased responsiveness due to noradrenaline in the kidneys of rats. The use of bosentan together with FK506 may be recommended to prevent drug-induced hypertension and initial reactivity changes in kidneys, although its usefulness in preventing oxidative damage remains to be clearly understood in this tissue.

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