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The role of endothelin in FK506-induced vascular reactivity changes in rat perfused kidney

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Abstract

Tacrolimus (FK506) is an immunosuppressant agent that is widely used in transplanted patients. The aim of this study was to investigate the role of endothelin in the acute effects of FK506 on the vascular reactivity in perfused isolated rat renal and mesenteric vasculature. Left kidney/mesentery of male Wistar rats (230–300 g) were perfused by a constant flow and perfusion pressure was recorded. The responses to noradrenaline and sodium nitroprusside were obtained both in the absence and presence of FK506 (10^{-7} M) or polyoxyethylene hydrogenated castor oil 60 (HCO-60 and solvent of the drug at equivalent concentrations). FK506 significantly increased the noradrenaline-induced vasoconstrictor responses in renal, but not in mesenteric vascular beds. Bosentan (10^{-5} M), a nonselective endothelin ET-1 receptor antagonist given by perfusion, reversed the increase in noradrenaline responses in the kidney. Sodium nitroprusside-induced vasodilator responses in both renal and mesenteric vascular beds were significantly decreased by FK506. However, in renal vasculature, there was no significant difference between the inhibitory effects of FK506 and HCO-60, although the effect of the solvent was not significantly different from that of the control. While in the mesenteric bed, the solvent significantly inhibited nitroprusside-induced vasodilation, similar to that of FK506. The effect of FK506 on vasodilation in both vascular beds was not reversed by bosentan. Our results indicated that FK506 increased the reactivity of the renal vascular bed to noradrenaline through endothelin ET-1 receptor activation. The mechanism of impaired vasodilation due to FK506 appears to be due to its solvent action and is independent of endothelin release.

Keywords: Tacrolimus (FK506); Vascular reactivity; Endothelin; Isolated perfused kidney and mesentery; Bosentan

1. Introduction

Tacrolimus (FK506), a macrolide antibiotic, has potent immunosupressant effects. FK506 has been used in kidney, liver and heart transplant patients to prevent graft rejection. The major adverse effects of FK506 are hypertension and nephrotoxicity. The mechanism(s) of drug-induced hypertension and nephrotoxicity are as yet unknown. Vascular reactivity alterations in the arteries probably play a role in FK506-induced hypertension.

FK506 has potentiated pressor responses to noradrenaline in rat renal arteries (Schwertfeger et al., 2001). Additionally, it induced a direct and dose-dependent contractile effect, which was only observed in the presence of intact endothelium (Schwertfeger et al., 2001). In contrast, in human interlobar arteries, FK506 failed to induce direct vasoconstriction and did not significantly potentiate constrictor responses to noradrenaline (Schwertfeger et al., 2001). In isolated mesenteric arteries, FK506 increased the response to noradrenaline and decreased the responses to acetylcholine and sodium nitroprusside (De Lima et al., 1999). Although, the acute effects of FK506 on isolated rat renal and mesenteric arteries have been widely studied, FK506-induced acute vascular reactivity changes in the rat renal and mesenteric vascular beds have not.

The relationship between FK506 and endothelin is one of conflict. FK506 causes endothelin release in cultured rat mesangial cells (Goodall et al., 1995), increases expression

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of prepro endothelin-1 mRNA in human umbilical vein endothelial cells (Marsen et al., 2000), and increases urinary endothelin (Textor et al., 1995) and plasma endothelin levels (Slowinski et al., 2002) after transplantation in humans. However, FK506 does not affect endothelin release in bovine aortic endothelial cells (Benigni et al., 1992) nor in cultured renal epithelial cell lines (Nakahama et al., 1991).

The relationship between vascular resistance increase due to FK506 and endothelin is not well understood. Vascular resistance was increased by FK506 administration and endothelin receptor antagonists did not alter this increase in isolated rat kidneys (Chen and Ma, 2001). In contrast, FK506-induced increase in renal vascular resistance was partially attenuated by endothelin receptor antagonist, TAK-044 (Uchida et al., 1998). We thought that it would be interesting to investigate the acute effects of FK506 on the vascular reactivity of renal and mesenteric vasculature, which are both important in regulating blood pressure, and if drug-induced effects would be converted by the endothelin receptor antagonist bosentan.

2. Materials and methods

2.1. Dissection and tissue preparation

The use of experimental animals as well as the study protocol were approved by the Animal Care Committee of Hacettepe University. Male Wistar rats (230–300 g) were anesthetized with urethane (1.25 g/kg; i.p.). After opening the peritoneal cavity, the left kidney and left renal and mesenteric arteries were isolated and cannulated via a polyethylene catheter, then removed, and transferred into a warmed Plexiglass chamber.

2.2. Perfusion

The kidney and mesentery were perfused continuously with warmed (37 °C) and aerated (95% O_2 and 5% CO_2 gas mixture) Krebs–Henseleit solution with the following composition (in mmol/l): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaCO_3$, 25; glucose, 10, by using a peristaltic pump (Eyela MP-32; Rikakikai, Tokyo, Japan) delivering a constant flow (8–10 ml/min for kidney; 5–6 ml/min for mesentery) throughout the experiment. 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 “Biopac MP150 data acquisition system”.

2.3. Measurement of vascular reactivity

In controlled experiments, to obtain vasoconstrictor responses, following an equilibration period of 30 min, subsequent doses of noradrenaline (mol/l) were given by bolus injection and then dose-dependent vasoconstrictions were recorded. To obtain the vasodilator responses, perfusion with phenylephrine at a concentration (3×10^{-6} mol/l for kidney; 10^{-5} mol/l 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-independent vasodilator sodium nitroprusside (mol/l) were given by bolus injection and dose-dependent vasodilations were recorded.

2.4. Drugs used

Bosentan (Roche), FK506 (Eczacibasi and Fujisawa), noradrenaline hydrochloride (Sigma-Aldrich Chemie), phenylephrine hydrochloride (Sigma-Aldrich Chemie), and sodium-nitroprusside (Fluka Chemika) were used. All drugs were prepared daily and dissolved in distilled water, except FK506 and bosentan. FK506 and bosentan were dissolved in polyoxyethylene hydrogenated castor oil 60 (HCO-60; Nikko Chemicals) and Dimethyl Sulfoxide (DMSO), respectively.

2.5. Data and statistical analysis

Vascular responses were measured as the increase or decrease in perfusion pressure and expressed as a percentage of submaximum response to phenylephrine ($3x10^{-6}$ mol/l for kidney; 10^{-5} mol/l for mesentery). The results were expressed as mean ± S.E.M.. For comparisons between groups, analysis of variance for repeated measurements was applied. Differences were considered to be statistically significant when p was less than 0.05.

3. Results

3.1. Vascular responses in the isolated perfused kidney

When applied by bolus injection at different doses (10^{-6}-10^{-4} M), FK506 induced vasoconstriction in some of the perfused kidneys. However, this direct vasoconstriction was neither dose-dependent nor reproducible.

Noradrenaline, applied at concentrations of 10^{-8}–10^{-4} M, caused dose-dependent constrictions in perfused renal vascular beds. FK506 (10^{-7} M), given by perfusion, significantly increased noradrenaline-induced vasoconstrictor responses in comparison with the control group (Fig. 1A). Bosentan (10^{-5} M), a non-selective endothelin ET-1 receptor antagonist given by perfusion, reversed the FK506-induced increase in noradrenaline responses in the kidneys (Fig. 1A). There was no significant change in the kidneys to which HCO-60 (solvent of FK506) was applied (Fig. 1A). Bosentan, (10^{-5} M) when applied alone, did not alter the noradrenaline-induced vasoconstrictor responses (not shown).

Sodium nitroprusside (10^{-7}–10^{-3} M) induced dose-dependent vasodilator responses in renal vascular beds. These vasodilator responses were significantly decreased by FK506 (10^{-7} M; Fig. 1B). There was no significant change in the responses to sodium nitroprusside in solvent-applied kidneys (Fig. 1B). Unexpectedly, the responses to nitroprusside in FK506-applied kidneys were not significantly different from that of the solvent-applied ones. Bosentan (10^{-5} M) did not alter the sodium nitroprusside-induced vasodilator responses when administered alone (not shown) or with FK506 (Fig. 1B).

3.2. Vascular responses in the isolated perfused mesentery

Noradrenaline (10^{-7}–10^{-3} M) caused dose-dependent vasoconstrictor responses in mesenteric vascular beds. In FK506-

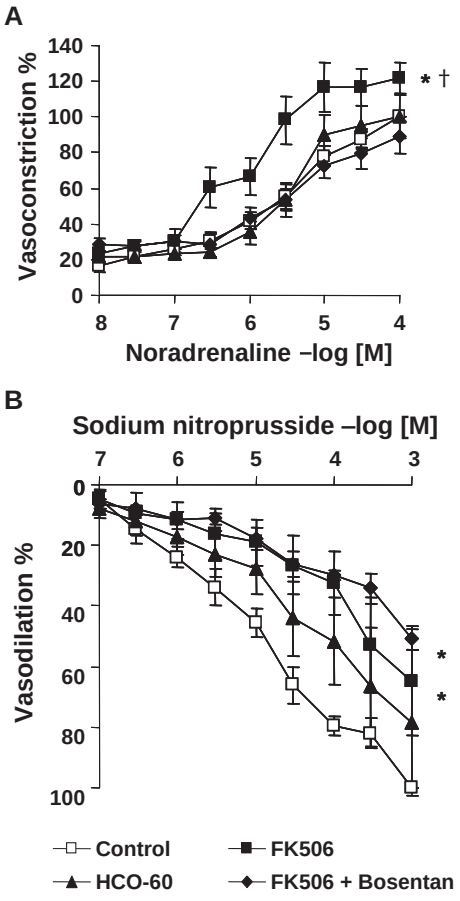

Fig. 1. The effects of FK506 (10^{-7} M; n=5-7), HCO 60 (at equivalent concentration; n=5–6) and FK506 (10^{-7} M)+Bosentan (10^{-5} M; n=4–5) administration on the noradrenaline-induced vasoconstrictor responses (A) and on the sodium nitroprusside-induced vasodilator responses (B) in the rat isolated perfused kidney (* P<0.05 vs. control, † P<0.05 vs. FK506+Bosentan).

administered (10^{-7} M) kidneys, noradrenaline-induced vasoconstrictor responses were not significantly different from those of the control group (Fig. 2A). No significant change was observed in solvent-applied mesenteric beds compared with the control group (Fig. 2A).

Sodium nitroprusside (10^{-8}–3×10^{-4} M) induced dose-dependent vasodilator responses in mesenteric beds, which were significantly decreased by both FK506 (10^{-7} M) and the solvent (Fig. 2B). Bosentan (10^{-5} M) did not reverse the decrease in the sodium nitroprusside-induced vasodilatation due to FK506 (Fig. 2B).

4. Discussion

In this study, we showed that FK506 potentiated a noradrenaline-induced perfusion pressure increase in rat renal vascular beds, but not in the mesenteric vasculature. This is a good example of the varying behavior of different vascular beds to an applied drug. Parallel to our finding, FK506 potentiates noradrenaline-induced pressor responses in isolated rat renal arteries (Schwertfeger et al., 2001).

In the present study, FK506 was applied at the concentration of 10^{-7} M. The rationale for choosing this concentration was to reach a FK506 concentration of 100 ng/ml (approximately 10^{-7} M) in the rat plasma, assuming a plasma volume of 10 ml (Ortola et al., 1987). This concentration of FK506 was comparable to that which has been measured in plasma and tissues after administration of a dose of FK506 that is known to be immunosuppressive (Venkataramanan et al., 1990). However, while this in vitro study involved very short exposures to FK506, clinical treatment with this drug may continue for days or months.

It has been reported that FK506 induces a concentration-dependent direct contractile effect in isolated rat arteries (Schwertfeger et al., 2001), but not in bovine renal and coronary arteries (Epstein et al., 1998). In perfused renal vascular beds, FK506 did not result in reproducible and dose-dependent constrictor activity in our study. Likewise, it has been demonstrated that acute administration of FK506 does not alter renal vascular resistance (Benigni et al., 1992). Since we could not observe a reasonable direct effect

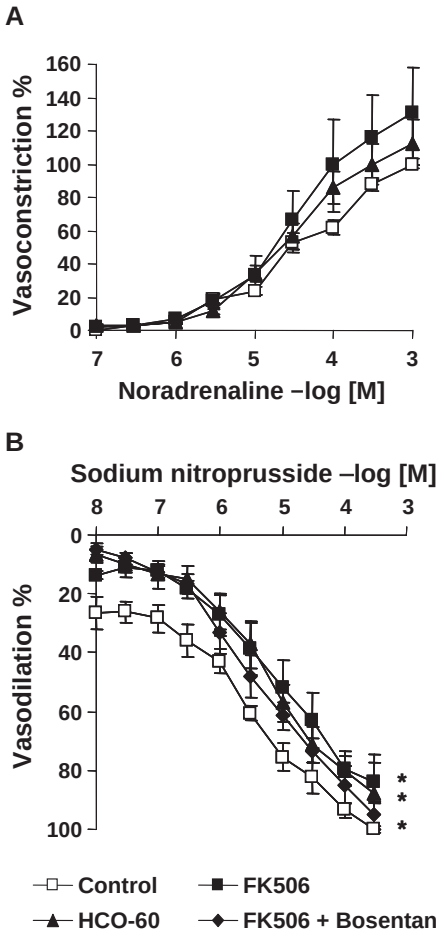

Fig. 2. The effects of FK506 (10^{-7} M; n=7), HCO 60 (at equivalent concentration; n=5–6) and FK506 (10^{-7} M)+Bosentan (10^{-5} M; n=6) administration on the noradrenaline-induced vasoconstrictor responses (A) and on the sodium nitroprusside-induced vasodilator responses (B) in the rat isolated perfused mesentery (* P<0.05 vs. control).

of the drug on renal vasculature, we evaluated its effect on noradrenaline-induced vasoconstriction and on sodium nitroprusside-induced vasodilatation, in essence, its effect on vascular reactivity.

There are conflicting results of endothelin release induced by FK506. In isolated rat-kidneys, vascular resistance that was increased by FK506 was not affected by endothelin receptor antagonist (Chen and Ma, 2001). On the other hand, it has been reported that endothelin receptor antagonists partially attenuate the increased renal vascular resistance of FK506 (Uchida et al., 1998). However, in our study, the potentiation of noradrenaline-induced vasoconstriction by FK506 was totally reversed by bosentan in perfused rat kidneys. This finding suggests that endothelin might play a role in immunosuppressive drug-induced increase in vascular reactivity. To the best of our knowledge, this study is the first to demonstrate the involvement of endothelin, functionally, in the potentiation of vasoconstriction of renal vascular beds, in response to noradrenaline.

Unlike the response in renal vascular beds, noradrenaline-induced vasoconstrictor responses were not affected by FK506 administration in mesenteric vasculature. It might be speculated that FK506 also increased the endothelin release in the mesenteric vascular bed, but mesenteric vasculature was less sensitive to the vasoconstrictor effects of endothelin. Indeed, it was demonstrated that renal and coronary vascular beds were more sensitive to endothelin than splanchnic and hindquarters vascular beds (Miller et al., 1989; Clozel and Clozel, 1989; Gardiner et al., 1990). Renal vessels are highly sensitive to the vasoconstrictor effects of endothelin (Pernow et al., 1988). Endothelin increases renal vascular resistance, even in low concentrations (Simonson and Dunn, 1993). In addition to its direct contractile effect, endothelin, in threshold concentrations, potentiates the effects of the sympathetic nervous system (Wong-Dusting et al., 1990; Yang et al., 1990), although this effect could not be demonstrated in humans (Cockcroft et al., 1991). Alternatively, the quantity of endothelin release as a result of FK506 administration might be more excessive from renal vasculature than from mesenteric vasculature. On the other hand, De Lima et al. (1999) have shown that incubation of FK506 for 24 h increased the noradrenaline-induced vasoconstrictor responses in isolated rat mesenteric arteries. This contradiction may result from the differences in the tissues studied (whole vascular bed vs. isolated arterial segments) and the methods used (perfusion method vs. isolated organ chamber experiments).

Although FK506 did not affect noradrenaline-induced vasoconstriction, it attenuated sodium nitroprusside-induced vasodilatation in mesenteric vasculature. The drug impaired the vasodilator responses to sodium nitroprusside not only in mesenteric beds, but also in renal vasculature. However, the solvent appeared to be responsible for the FK506 effect in both vascular beds, since its action was not statistically different from that of the drug. The decrease in sodium nitroprusside-induced vasodilatation by FK506 was not mediated by endothelin, since in both vascular beds, bosentan did not change the inhibition in sodium nitroprusside responses induced by the drug. Thus, the mechanism of FK506 in decreasing sodium nitroprusside-induced vasodilatation is entirely different from the mechanism of increasing noradrenaline-induced vasoconstriction and is most likely due to its solvent action, which needs further investigation. Here, it has to be emphasized that solvents of immunosuppressive drugs, themselves, may cause direct effects in arteries. For example, the solvents of another immunosuppressive drug, cyclosporine, namely cremophor EL and Labrafil which are structurally similar to HCO-60, increased vascular reactivity in isolated arteries of rats and rabbits (Amorena et al., 1990; Yaris et al., 1992).

In conclusion, our results indicated that bosentan was able to correct the FK506-induced increase in the responsiveness of renal vascular beds to noradrenaline, an adrenergic neurotransmitter. Bosentan may be used in combination with FK506 so as to prevent this drug-induced reaction, which will deteriorate further, resulting in hypertension when the immunosuppressive drug, alone is used. In the light of our findings, it would be of great importance to investigate the effects of the long-term administration of FK506 on the reactivity of renal and mesenteric vascular beds.

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