

Mechanism of FK506-induced renal hypoperfusion and its reversion in rats

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KEY WORDS tacrolimus; perfusion; nephrotoxicity; endothelins; calcium channel blockers; kidney; rats

ABSTRACT

AIM: To investigate the mechanism of renal hypoperfusion induced by tacrolimus (FK506) and to test the related agents acting against the process. **METHODS:** Experiments were performed in 6 groups of isolated perfused rat kidneys (IPRK). The parameters of renal function and the concentration of endothelin in the perfusate and urine were assessed at an interval of 15 min. Four groups of IPRK were perfused with normal saline and varied concentrations of FK506 (10 nmol/L – 10 μmol/L) to set up a control and a hypoperfusion model. The other 2 groups were used as hypoperfusion models to test the actions of endothelin receptor antagonist FR139317 and calcium channel blocker diltiazem.

RESULTS: Hypoperfusion model was established in IPRK by adding FK506 10 μmol/L in the perfusate, with the significant decreases of perfusate flow rate (PFR) and glomerular filtration rate (GFR), the significant increase of perfusion resistance (PR) and the concomitant increase of endothelin in perfusate and urine ($P < 0.01$). When FR139317 was added into the perfusate, only the depressed GFR was improved ($P < 0.05$) while the increased PR was not ($P > 0.05$). However, the addition of diltiazem reversed both the increase of PR and the decrease of GFR completely ($P < 0.01$).

CONCLUSION: Endothelin is likely to play an important role in the pathogenesis of FK506-induced acute renal hypoperfusion. Diltiazem can completely prevent the renal hypoperfusion induced by FK506 in IPRK.

INTRODUCTION

Tacrolimus (FK506), as a newly developed immunosuppressant to abate the rejection of transplanted grafts, is more potent in efficiency than the reputed cyclosporin (CsA)^[1]. Although they are unrelated in molecular structure, their manifestations of nephrotoxicity are extremely alike^[2,3]. The incidence of acute renal failure was 18.3% – 34.6% after liver transplantation in the FK506-treated patients^[4].

It has been shown that the CsA-induced renal hypoperfusion starts through a process of intense vasoconstriction, and the potent vasoconstrictive peptide, endothelin (ET), is involved in the event^[5-10]. ET causes increase of renal vascular resistance and decreases of renal blood flow and glomerular filtration in a dose-dependent manner^[8]. However, it is not clear whether ET also plays a similar role in the nephrotoxicity induced by FK506. The present study was carried out to investigate the mechanism of renal hypoperfusion and test the actions of ET receptor antagonist and calcium channel blocker in the renal hypoperfusion induced by FK506.

MATERIALS AND METHODS

Animals and drugs Male Sprague-Dawley rats, weighing 280 – 350 g, were provided by the Center of Experimental Animals of Xuzhou (Grade II, Certificate No 97032). FK506, FR139317, and diltiazem were purchased from Fujisawa Pharmaceutical (Osaka, Japan).

Preparation of isolated perfused rat kidneys (IPRK) The animals were anesthetized with nembtal (50 mg/kg, ip). After opening the abdominal cavity, the right ureter was catheterized with a polyethylene tube (PE-10), and the right kidney was removed and treated according to the method of Nishitsutsuji-Uwo *et al*^[11]. Briefly, a 14-G needle was inserted via the aorta through the supermesenteric artery into the right renal artery after the administration of heparin 400 U. The kidney was

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removed and flushed with 10 mL of oxygenated and heparinized perfusate, and perfused at 35 °C in the IPRK apparatus (Fig 1). The perfusate was the Krebs-Henseleit solution containing 7.5 % bovine albumin, 8 kinds of amino acid (Sigma), (in mmol/L: methionine 0.5, alanine 2.0, glycine 2.0, serine 2.0, arginine 1.0, proline 2.0, asparagine 3.0, isoleucine 1.0) and 0.01 % creatinine, and gased with 95 % O₂ and 5 % CO₂ at a speed of 400 mL/min. The perfusate flow rate was adjusted to maintain the renal arterial perfusion pressure at 13.3 kPa. The isolated kidney was initially perfused for 30 min to reach a steady condition before experimentation.

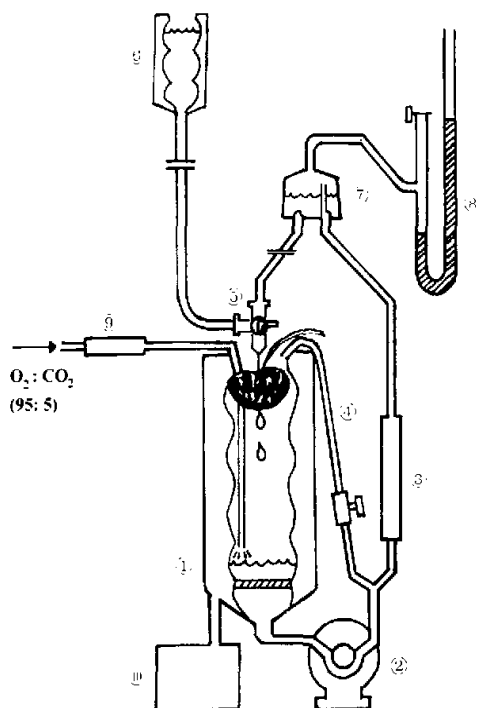


Fig 1. Illustration of IPRK apparatus. 1) Oxygenator column; 2) Pump; 3) Flowmetry; 4) Bypass way; 5) Three-way switch; 6) Pre-perfused perfusate; 7) Gas bubble extinguisher; 8) Mercury sphygmomanometer; 9) Gas moistened; 10) Thermostat.

Experimental protocol Thirty-six kidneys were divided into 6 groups ($n=6$ for each group). After two sets of 15-min samples were collected for base line assessments, normal saline, and FK506 of varied concentrations (10 nmol/L, 1 μ mol/L, 10 μ mol/L) were separately added to the perfusate in 4 of the 6 IPRK groups to choose an adequate dosage for FK506 to induce

hypoperfusion. Three urine samples as well as midpoint perfusate samples were analysed in succession at 15-min intervals. To protect the kidney against the nephrotoxicity of FK506, FR139317 (a kind of ET receptor antagonist), and diltiazem (a calcium channel blocker), together with FK506 of adequate dosage were added to the perfusate in the other 2 IPRK groups to test the efficacy of them respectively.

Determination of nephrologic parameters

The parameters including urine volume, perfusate flow rate (PFR), glomerular filtration rate (GFR), perfusion resistance (PR), and fractional reabsorption of sodium (FRNa) were determined. In some experiments, the perfusate and urine samples were placed in ice-chilled tubes containing edetic acid to measure the content of ET. The sodium concentration was determined by flame photometry. Creatinine levels in urine and perfusate were determined by alkaline picric acid method. ET was measured by specific radioimmunoassay. The data of other parameters were obtained by reading flowmetry, sample weighing or calculation.

Statistics Data were presented as $\bar{x} \pm s$. Paired *t*-test was conducted and a *P* value less than 0.05 was considered to be significant.

RESULTS

Effects of FK506 on renal function in IPRK

FK506 at the concentration of 10 μ mol/L caused considerable decreases in PFR and GFR by the extent of 22.1 % - 35.1 % ($P < 0.01$), and caused an increase in PR by 29.7 % ($P < 0.01$) to 13.6 % ($P < 0.05$). FRNa was slightly decreased but still remained at almost the same level compared with the control ($P > 0.05$, Fig 2).

Changes of ET in the perfusate and urine

The ET concentration in perfusate was increased progressively after the addition of FK506 10 μ mol/L into the perfusate, with similar increases of ET found in the urine ($P < 0.01$, Tab 1). The changes of ET in urine were parallel to those of PFR and GFR. Besides, addition of exogenous ET to the perfusate (62.5 ng/L) resulted in decreases of PFR and GFR and the increase of PR to nearly the same extents as those in the case of FK506 of 10 μ mol/L.

FR139317 and diltiazem abated the FK506-induced hypoperfusion in rat kidneys

The administration of FR139317 along with FK506 brought about remarkable improvement in GFR ($P < 0.05$), but

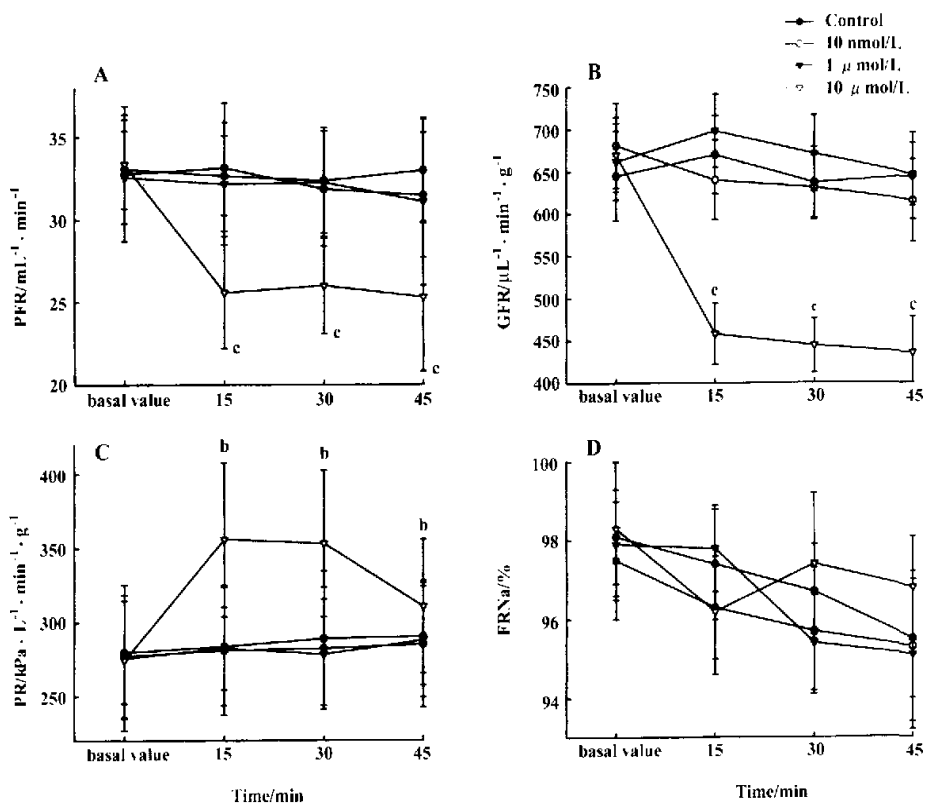


Fig 2. Effects of FK506 (10 nmol/L - 10 μmol/L) on renal function in isolated perfused rat kidney model. A) Perfusion flow rate; B) Glomerular filtration rate; C) Perfusion resistance; D) Fractional reabsorption of sodium. *n* = 6. $\bar{x} \pm s$. ^b*P* < 0.05, ^c*P* < 0.01 vs control.

Tab 1. Changes of endothelin in the perfusate and urine from the IPRK after the addition of FK506 (10 μmol/L) into the perfusate. $\bar{x} \pm s$. ^c*P* < 0.01 vs control.

		ET/ng·L ⁻¹			
		0 min	15 min	30 min	45 min
Control	Perfusate	8 ± 3	9 ± 3	8.0 ± 2.3	12 ± 5
	Urine	11 ± 4	16 ± 5	15 ± 7	12 ± 5
FK506	Perfusate	6.0 ± 2.1	33 ± 12 ^c	38 ± 15 ^c	52 ± 17 ^c
	Urine	12 ± 4	45 ± 16 ^c	49 ± 18 ^c	56 ± 22 ^c

insignificant improvement in PR (*P* > 0.05), as compared with that in the group of FK506 10 μmol/L alone (Fig 3). Diltiazem almost completely reversed the FK506-induced changes in GFR and PR (*P* < 0.01, Fig 3).

DISCUSSION

The present study demonstrated that the perfusion of IPRK with FK506 induced dose-dependent decrease of

PFR and concomitant increase of renal vascular resistance. And owing to a decrease in PFR, GFR was also declined, as noted by other workers^[12]. The study also showed that in the hypoperfusion model, the concentration of ET was significantly increased in the perfusate and urine. These changes of ET were parallel to the changes of PFR and GFR, indicating that ET must be involved in the process of vasoconstriction in the FK506-induced renal hypoperfusion.

FR139317, as a novel specific endothelin receptor antagonist^[10], partially diminished the nephrotoxic effects of FK506, but could not abolish them completely. This can be attributed to the fact that FR139317 only inhibits the vasoconstriction of afferent glomerular arterioles, not that of efferent ones, and even causes the latter to constrict^[13,14]. This may explain why FR139317 improved the GFR without improving the PR in the present study. Previous studies also showed that CsA enabled the increase of mRNA expression of the ET_B receptor in the medulla of rat kidney, but not the ET_A

receptor. This would perhaps account for the present findings about FR139317, which is known to be specific to the ET_A receptor^(15,16). Other vasoconstrictors synthesized in the kidney, such as angiotensin II and sympathomimetic substances, may also be the reasons^(17,18) why FR139317 can not completely reverse the toxic effects of FK506 on kidney.

can not completely block the process of vasoconstriction induced by FK506, diltiazem is able to do it. The IPRK is a good model for investigating the extensive changes in renal hemodynamics induced by FK506.

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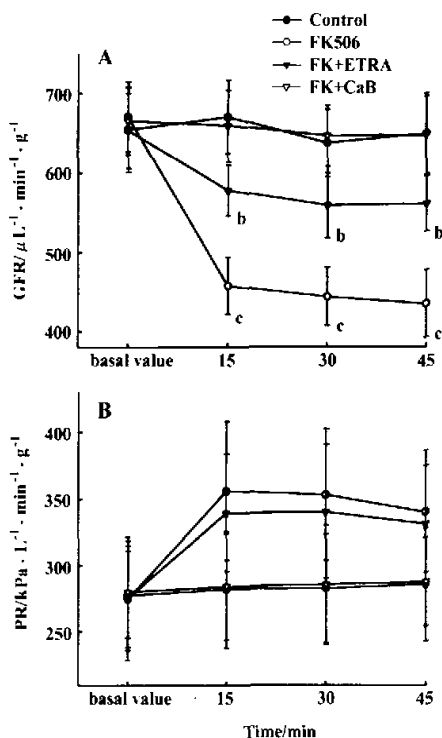


Fig 3. Reversion of FK506-induced renal hypoperfusion with FR139317 10 μmol/L or diltiazem 10 μmol/L. A) Glomerular filtration rate; B) Perfusion resistance. n = 6. $\bar{x} \pm s$. * $P < 0.05$, ** $P < 0.01$ vs FK506 group.

Diltiazem, a calcium channel blocker, completely antagonized the nephrotoxic effects of FK506, because it is effective in reversing the ET-mediated vasoconstriction via binding the membrane receptor with the consequent stimulation of phosphatidylinositol response and the generation of transmembrane signals, including intracellular Ca²⁺ mobilization, activation of protein kinase C and influx of Ca²⁺ through Ca²⁺ channels^(19,20). The calcium channel blocker thus functions to prevent the FK506-induced nephrotoxicity.

The present results support that FK506 causes renal vasoconstriction and the consequent renal hypoperfusion, which is possibly mediated by ET. Though FR139317

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关键词 它克雷默; 灌流; 肾毒性; 内皮素类; 钙通道阻滞药; 肾; 大鼠

目的: 研究它克雷默(FK506)诱发肾低灌注的机制并筛选相应拮抗药物。 **方法:** 利用离体肾灌流模型, 实验分为6组。灌流过程中每15 min, 对肾功能指标进行测定, 同时测定了灌流液和尿中内皮素的浓度。其中4组用生理盐水及不同浓度的FK506进行灌流, 建立对照和低灌流模型, 另外2组用于测定内皮素受体拮抗剂FR139317和钙通道阻滞剂地尔硫卓对肾低灌注的作用。 **结果:** 灌流液中加入10 $\mu\text{mol/L}$ 的FK506诱发肾脏低灌注, 肾灌流量、肾小球滤过率明显下降, 肾灌注阻力明显升高, 灌流液和尿液中内皮素水平升高。用FR139317和地尔硫卓分别与FK506一起灌流, 地尔硫卓完全纠正FK506所引起的肾小球滤过率下降和灌注阻力的升高, FR139317部分纠正了肾小球滤过率改变, 但对灌注阻力变化的影响不明显。 **结论:** 在FK506诱发的急性肾低灌注中, 内皮素可能起重要作用, 地尔硫卓能够完全拮抗FK506引起的肾脏低灌注。

FK506 诱发的大鼠肾低灌注机制及其逆转

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