□ 지상발표 □

# Preconditioning of Donors with Tacrolimus Improved Organ Quality and Function After Kidney Transplantation

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## BACKGROUND

Ischemic-reperfusion (IR) injury remains an important clinical consideration in kidney transplantation because it may act to trigger or amplify host allo-response and influence on early graft function , the acute rejection occurrence and late chronic change[1, 2]. So protective maneuvers of donor kidney at that time of transplantation should clearly benefit the fate of the organ.

Some evidence demonstrating the efficacy of ischemic or hyperthermia preconditioning to prevent IR injury of kidney is available from animal study[3-7]. But the challenging premise of intentionally subjecting patients and their organ to transient ischemia or hyperthermia has acted a formidable psychological and ethical impediment to the widespread clinical application of organ preconditioning[8]. Recently, pharmacologically induced preconditioning as a more palatable method seem to confer similar organ protection in renal IR injury model in rats. Organ preconditioning renders an organ more tolerant to subsequent sustained ischemia-reperfusion[9]. Although the mechanism of ischemic or pharmacologic preconditioning are unclear, potential mediator include heat shock protein, nitric oxide, adenosine and endothelin. Pretreatment with tacrolimus has been demonstrated to be renoprotective against a IR injury by the induction of heat shock protein 70 (HSP70)[6-11].

Preconditioning study against IR injury in animal, there are some difference between renal IR injury model and renal transplantation model at the immunological and physiological aspects. Renal injury and physiologic change in parenchyme and vessels by immunologic insults and renal denervation in transplanation model could be delayed recovery from IR injury. The some humoral factors in circulation and protecting substances in tissue induced by IR in IR injury model affect recovery of tissue from injury. But humoral factors do not affect in transplantation model because it is not transferable to recipient[12-15].

## HYPOTHESIS

Recently we reported that renal cholesterol levels rise within 18 hours and persist for 2 weeks after treatment of tacrolimus[16]. These increments serve to protect the kidney from subsequent ischemia reperfusion injury. We hypothesized that preconditioning of donors with tacrolimus would prevent renal dysfunction and examined the onset and duration of the protective effect in a rat kidney transplantation model.

## EXPERIMENTAL GROUPS

F344 rat donor kidneys were perfused with UW solution and exposed to 2h of cold ischemia and orthotopic kidney transplant (Tx) was performed[17] in syngeneic F344 rats with a warm ischemia time of 20 min.

1. Control animals with right uni-nephrectomy (Con)

2. Kidney transplantation from donors treated with saline (Tx)

3. Kidney transplantation from donors treated with tacrolimus 1 mg/kg 24 hour before transplantation (Tx+FKacu)

4. Kidney transplantation from donors treated with tacrolimus 1 mg/kg 7 days before transplantation (Tx+FKdelay).

## MATERIALS AND METHODS

Drug. Tacrolimus (FK) for injection was dissolved in 0.9% NaCl solution to a final concentration of 1 mg/mL. Tacrolimus was administered intraperitoneally at a dose of 1 mg/kg.

Functional studies. GFR was measured 3 and 7 days after Tx by $C^{14}$ inulin clearance (mL/min/100 g body weight). Plasma creatinine was also determined.

Histological scores. The kidneys were perfused with cold saline. At least 30 fields at 200X structural injury, namely tubular dilation, vacuolization, atrophy and interstitial inflammation was assessed using the following semi-quantitative scoring system utilizing digital image analysis : Tubular injury : 0=normal; 0.5=<5% injured; 1=5-20%; 1.5=21-35%; 2=36-50%; 2.5=51-65%; 3=>65%.

Statistical analysis. Results are presented as mean±SE (n=6-7), and all statistical analyses were calculated with SPSS for Macintosh version 6.1 (SPSS Inc. Chicago, IL). Comparisons between groups were done by analysis of variance (Kruskal-Wallis test, followed by Tukey test). The level of statistical significance was chosen as $p<0.05$.

## RESULTS

1. GFR : GFR of the Tx group was strikingly reduced by more than 90% compared to the 1K Control group at day 3 and slowly recovered up to 50% of the control animals at day 7. Pretreatment of donors with FK 24 hours before Tx significantly protected renal function at 3 days after Tx and GFR quickly returned to the normal levels at day 7 (Fig. 1).

2. Plasma creatinine : FK pretreatment group animals had slightly increased plasma creatinine levels but they were not significantly different from the control creatinine levels at both day 3

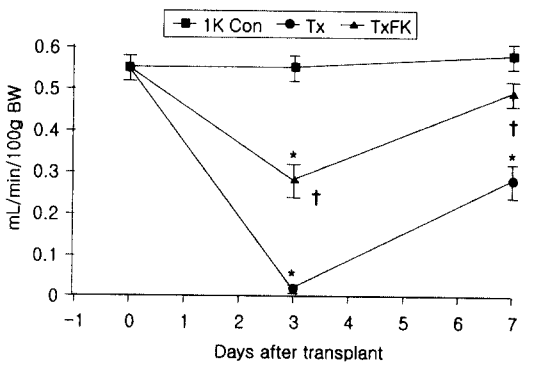


*Fig. 1. GFR in each group. *p<0.05 vs 1K Con, †p<0.05 vs Tx.*

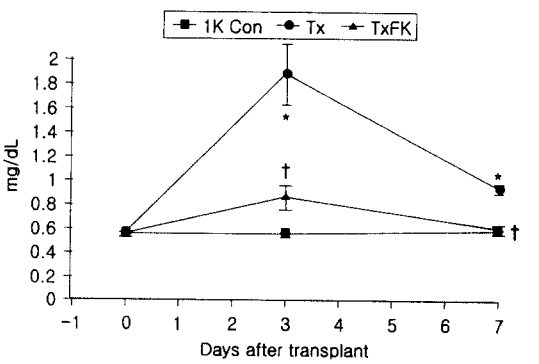


*Fig. 2. Plasma creatinine levels in each group. *p<0.05 vs 1K Con, †p<0.05 vs Tx.*

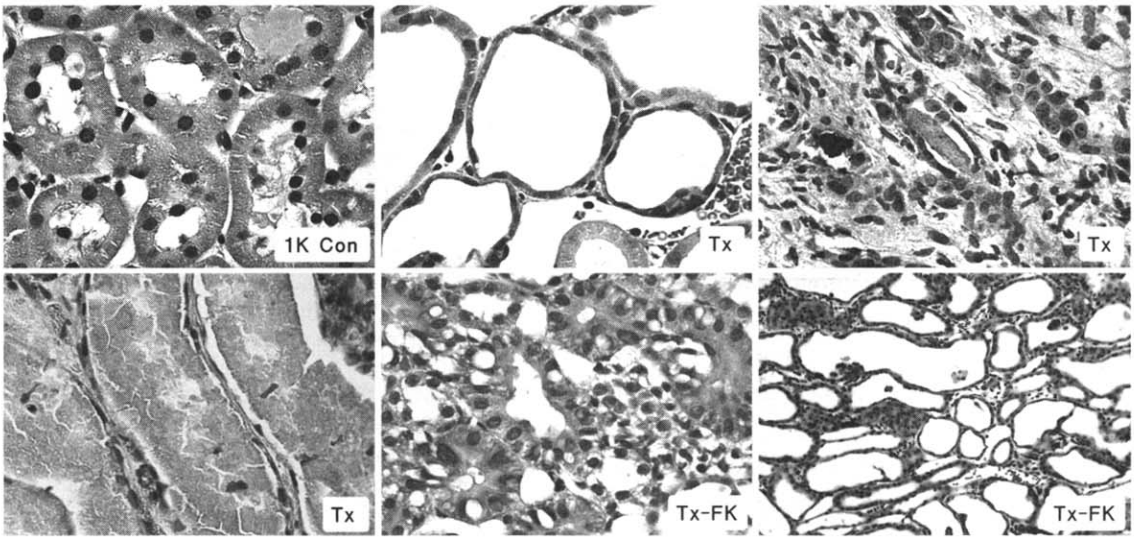


***Fig. 3.*** *Histology in each group.*

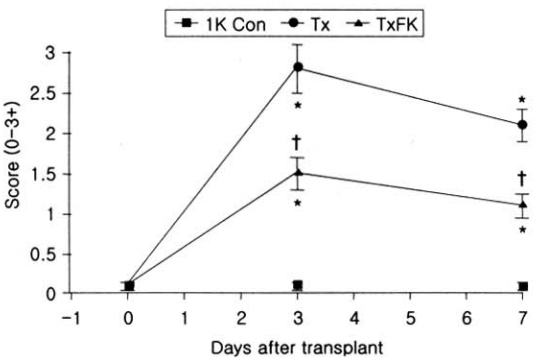


***Fig. 4.*** *Histological scores in each group. *p<0.05 vs 1K Con, †p<0.05 vs Tx.*

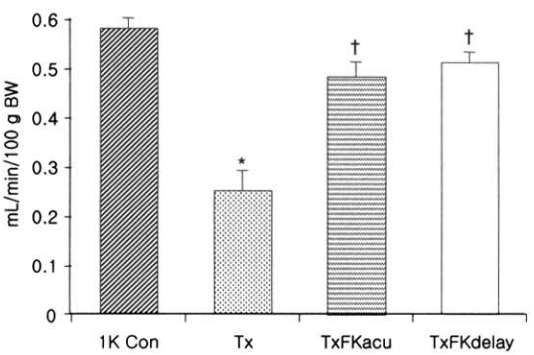


***Fig. 5.*** *GFR in each group on 7 days after transplant. *p<0.05 vs 1K Con, †p<0.05 vs Tx.*

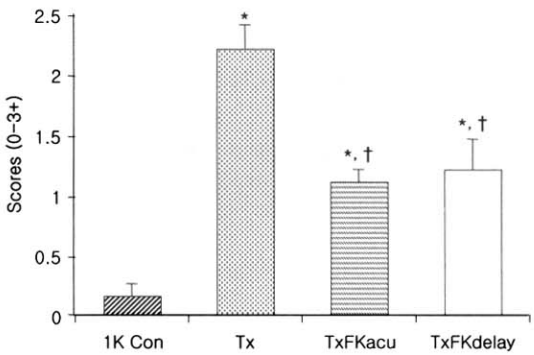


***Fig. 6.*** *Histological scores in each group on 7 days after transplant. *p<0.05 vs 1K Con, †p<0.05 vs Tx.*

and 7. In sharp contrast, plasma creatinine levels of Tx animals were increased by 200% at day 3 and remained significantly high even 7 days after Tx (Fig. 2).

3. Histology : The KTx group kidneys displayed significant tubular injury, dilatation, interstitial inflammation and tubular necrosis at both day 3 and 7. The histological changes in the Tx-FK group were significantly less severe than in the Tx group (Fig. 3, 4).

4. GFR and histology of the TxFKacu and TxFKdelay group : Pretreatment of donor animals

with only one shot FK either 24 hours or 7 days before Tx significantly protected renal function and structure 7 days after Tx (Fig. 5, 6).

## SUMMARY AND CONCLUSION

Both acute and delayed preconditioning with FK significantly improved graft function and I/R structural injury associated with Tx. These data demonstrate protective effect of donor preconditioning with FK on kidney graft function and structure. This effect is transferable from donors to recipients after Tx and can be sustained for at least 7 days. Although the underlying mechanism remains to be elucidated, a renal stress response including cholesterol accumulation may be involved.

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