FK778 ameliorates post-transplant expression of fibrogenic growth factors and development of chronic rejection changes in rat kidney allografts*

Jukka M. Rintala1, Johanna Savikko1,2, Sini E. Rintala1 and Eva von Willebrand1

1Transplantation Laboratory, University of Helsinki and Helsinki University Central Hospital, Helsinki and 2Department of Surgery, Päijät-Häme Central Hospital, Lahti, Finland

Abstract

Background. Acute rejection is the major risk factor for the development of subsequent chronic allograft nephropathy (CAN), which is the primary reason for late allograft loss in kidney transplantation. Platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-β) are the main mitogens mediating mesenchymal cell proliferation. Their early post-transplant induction may start cascades leading to the development of CAN. An immunosuppressive drug, FK778, inhibits de novo pyrimidine biosynthesis and several receptor tyrosine kinases (RTKs). Here we investigated its effects on acute and chronic rejection as well as post-transplant PDGF and TGF-β expression in combination therapy with calcineurin inhibitors (CNIs).

Methods. Kidney transplantations were performed from DA to WF rats. Syngenic DA-DA grafts were used as controls. Allografts were immunosuppressed with a combination of FK778 (10 mg/kg/day p.o.) and CsA (1.5 mg/kg/day s.c.) or tacrolimus (Tac) (1.5 mg/kg/day p.o.). Grafts were harvested 5 and 90 days after transplantation for histology or immunohistochemistry (PDGF-A, PDGF-B, PDGFR-α, PDGFR-β, TGF-β, TGF-βR). The dose response of FK778 on acute rejection was studied with monotherapy of 5, 10 and 20 mg/kg/day. Chronic changes were scored according to the Chronic Allograft Damage Index (CADI).

Results. FK778 ameliorated the early post-transplant inflammatory response dose dependently. Additive effects were seen with FK778 and CNIs. Significantly lower CADI scores were seen in combination therapy of FK778 and CNIs compared with CNI monotherapies. FK778 also significantly reduced both early and late PDGF and TGF-β expression when combined with CNIs.

Conclusions. These results indicate that FK778 could prevent the development of CAN and be a promising therapy also in clinical kidney transplantation.

Keywords: acute rejection; chronic allograft nephropathy; FK778; PDGF; TGF-β

Introduction

Acute rejection remains the single most important risk factor for the development of subsequent chronic allograft nephropathy (CAN) [1,2]. CAN is characterized by interstitial fibrosis, inflammation, allograft arteriosclerosis and tubular and glomerular lesions [3,4]. Repeated episodes of acute rejection, even subclinical ones, correlate with the progression of histological changes in protocol biopsies of renal transplant patients [5]. Although the exact mechanisms leading to CAN are largely unknown, acute rejection could cause the primary injury leading to the induction of reparative processes. These reparative mechanisms are partly controlled by growth factors and they result in fibrosis and mesenchymal cell proliferation.

Leflunomide (LFM) has potent immunomodulatory and anti-inflammatory effects but its long half-life has restricted its use as an immunosuppressant. FK778 is an analogue of LFM and therefore it could be a more useful choice as a drug for solid organ transplantation [6,7]. Like LFM, FK778 inhibits T- and B-cell proliferation and functions interfering in de novo pyrimidine biosynthesis by inhibiting the dihydro-orotate dehydrogenase (DHODH) [6]. Additional immunosuppressive effects of LFM and FK778 are achieved by inhibition of several growth factor receptor tyrosine kinases (RTKs). LFM inhibits RTKs such as epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) in vitro [8,9]. It also inhibits PDGFR RTK activity and strongly inhibits the growth of PDGFR...
FK778ameliorates post-transplant expression of fibrogenic growth factors overexpressing C6 glioma in vivo [10]. In addition, FK778 is vasculoprotective and inhibits neointima formation by way of a mechanism that is independent of DHODH inhibitory activity and therefore is likely mediated through the inhibition of growth factor RTKs [11]. It also reduces endothelial adhesion molecule upregulation and attenuates the lymphocyte–endothelium interaction both in vitro and in vivo [12, 13]. Recently, FK778 monotherapy has also been shown to inhibit the progression of CAN in a Fisher 344 and Lewis experimental rat renal transplantation model [14].

Early post-transplant induction of fibrogenic growth factors such as TGF-β and PDGF may be an important step in a cascade subsequently leading to CAN. TGF-β and PDGF have synergistic effects in increasing the number of renal interstitial fibroblasts that may have a crucial role in increasing fibrosis and cytokine levels.

In the present study, we first investigated the effect of FK778 on acute rejection in both monotherapy and combination therapy with calcineurin inhibitors cyclosporin and tacrolimus. In addition, we analysed the early graft infiltration of CD4+ and CD8+ lymphocytes and ED3+–activated macrophages. We then proceeded to investigate the effect of FK778 on the development of CAN in combination therapy with CNIs. We also studied the effect of these drugs on PDGF and TGF-β ligand and receptor expression at both early and late phases after transplantation.

**Materials and methods**

**Animals**

Specific, pathogen-free, inbred male WF (RT1u) and DA (RT1a) rats (Scanbur, Sollentuna, Sweden) weighing 300–350 g were used for transplantations. They received regular rat food and tap water ad libitum, and were maintained on a 12-h light/dark cycle. The animals received human care in compliance with the Guide for the Care and Use of Laboratory Animal Resources published by the National Institutes of Health and Office of Animal Care and Use (National Research Council, Washington DC, National Academy Press, 1996).

**Kidney transplantations**

Transplantations were performed from the DA to the WF rat strain using a modified microsurgical technique of Fisher and Lee [15]. The donor kidney was transplanted heterotopically to the recipient’s abdominal aorta and inferior vena cava using end-to-side aortic and vena caval anastomosis under intraperitoneal chloralhydrate anaesthesia (240 mg/kg). Uretral anastomosis was performed end-to-end close to the renal pelvis. The right native kidney was removed during transplantation. Left nephrectomy was performed at the seventh postoperative day for animals operated for studying CAN. In animals operated for studying acute rejection, the left kidney was left in situ because the grafts were allowed to recover from early dysfunction. Buprenorphinum (Temgesic; Reckitt & Colman, Hull, UK) was used for postoperative pain relief.

**Experimental design and medication**

The experimental design is summarized in Table 1. Syngenic transplantations were performed from DA to DA rats and allogenic transplantations from DA to WF rats. No immunosuppressive treatment was given to recipients of syngenic grafts. Four different allograft groups (n = 3–5) were formed to investigate FK778 in combination with CNIs in rat renal allograft rejection. Allografts were treated either with cyclosporine (CsA, Sandimmun, Novartis, Basel, Switzerland) 1.5 mg/kg/day s.c. or tacrolimus (Tac, Fujisawa, Osaka, Japan) 1.5 mg/kg/day p.o. monotherapy or with a combination of FK778 (10 mg/kg/day) and CsA (1.5 mg/kg/day s.c.) or Tac (1.5 mg/kg/day p.o.). In addition, the dose response of FK778 in acute rejection was studied with 5, 10 and 20 mg/kg/day doses. Grafts were harvested 5 days after transplantation to study acute rejection and 90 days after transplantation to study CAN.

**Drugs**

CsA was dissolved in intralipid (Kabi Vitrum, Stockholm, Sweden) to a final concentration of 1.5 mg/ml and administrated subcutaneously. Tac was dissolved in distilled water to a final concentration of 1.5 mg/ml and administrated per oral. FK778 (Fujisawa, Osaka, Japan) was dissolved in 1% carboxy methyl cellulose to final concentrations of 5 and 10 mg/ml and administrated per oral. All drugs were given once a day.

**Histopathology**

The kidney grafts were bisected horizontally and fixed in 4% paraformaldehyde. The specimens were cut into 2-µm-thick sections and stained with Mayer’s haematoxylin-eosin.
(HE), Masson’s trichrome, diastase-periodic acid-schiff (D-PAS), methenamine silver PAS and Unna-Pappenheim (UP) stains.

Quantification of histopathology

Histopathological analysis was done in a blind review. To investigate acute rejection, the intensity of perivascular and diffuse interstitial inflammation of the samples was scored from 0 to 3 as follows: 0, no inflammation; 1, faint inflammation; 2, moderate inflammation and 3, intense inflammation. Chronic changes were scored according to the chronic allograft damage index (CADI). The CADI value is a sum of six parameters scored from 0 to 3 including interstitial inflammation and fibrosis, tubular atrophy, glomerular mesangial matrix increase, glomerular sclerosis and arterial intimal proliferation. The CADI grading of fibrosis and tubular atrophy from 1 to 3 are analogic with the BANFF-score (Banff 2005, category 5 grades I, II and III) [16].

Immunohistochemistry

For immunohistochemistry, the renal samples were incubated in 4% paraformaldehyde for 24 h and then routinely fixed for paraffine blocks. Four-micrometre-thick sections were cut in series on glass slides. Before staining, the samples were deparaffinized. For epitope retrieval, the slides were cut in series on glass slides. Before staining, the samples were deparaffinized. For epitope retrieval, the slides were heated in a microwave oven for 20 min in sodium citrate buffer (pH 6.0) and then allowed to cool down in room temperature (RT) for 20 min. To demonstrate the expression and localization of PDGF-AA and -BB and their receptors PDGFR-α and -β as well as TGF-β1 and TGF-βR1, the samples were immunostained using a Vectorstain Elite ABC Kit (Vector Laboratories Inc., Burlingame, CA, USA). For immunostaining, the specimens were blocked with 1% normal goat serum/phosphate-buffered saline (PBS), pH 7.40, and then incubated with primary antibodies at optimal dilution at RT for 30 min. The primary antibodies were diluted in a 1% bovine serum albumine/PBS solution. After washing in PBS, endogenous peroxidase activity was blocked with a 20-min incubation with a 1% hydrogen peroxidase (30%)/PBS solution. With intervening washes in PBS, the specimens were incubated with biotinylated goat anti-rabbit absorbed antibodies in the PBS buffer at RT for 30 min; avidin-biotinylated horseradish complex in the PBS buffer at RT for 30 min and the reaction was revealed by NovaRed (Vector Laboratories) used according to the manufacturer’s instructions, yielding a red reaction product. The slides were counterstained with Mayer’s haemalum and permanently mounted after incubation in alcohol series and xylene.

Polyclonal rabbit IgG antibodies to PDGF-AA (2 µg/ml, sc-128, Santa Cruz Biotechnology Inc, CA, USA), PDGF-BB (5 µg/ml Abcam, Cambridge, UK), PDGFR-α (10 µg/ml, Lab Vision Corp, Fremont, CA, USA), PDGFR-β (2 µg/ml, Santa Cruz Biotechnology), TGF-β (2 µg/ml, Santa Cruz Biotechnology) and TGF-βR1 (2 µg/ml, Santa Cruz Biotechnology) were purchased from commercial suppliers.

To demonstrate the infiltration of activated macrophages and T cells, a three-layer indirect immunoperoxidase technique was used [17]. The primary monoclonal mouse antibodies used were ED3 (Serotec Ltd, Oxford, UK), rat CD4 and rat CD8 (BD PharMingen, San Diego, CA, USA). Peroxidase-conjugated rabbit anti-mouse IgG (DAKO A/S, Copenhagen, Denmark) and peroxidase-conjugated goat anti-rabbit IgG (Caltag Laboratories, Burlington, CA, USA) were used sequentially.

Quantification of immunohistochemistry

Immunohistochemical analysis was done in a blind review. The intensity of the staining of the samples was scored from 0 to 3 as follows: 0, no visible staining; 1, cells with faint staining; 2, moderate intensity with multifocal staining and 3, intense diffuse staining. The morphology of positively stained cells was also analysed. Cells stained positively for CD4, CD8 and ED3 were counted in three visual fields from the renal cortex at ×400 magnification, and the mean number of positive cells per field of vision was calculated.

Statistical analysis

The results are expressed as mean ± SE, and P < 0.05 was accepted as significant. The significance between groups was determined by parametric analysis of variance and Least Significant Difference test, ANOVA (SPSS version 10; SPSS, Chicago, IL, USA).

Results

Clinical course

No recipients were lost before the end of the follow-up period. In animals operated to study acute rejection, no signs of renal failure were seen. FK778 combined with CsA in most time points failed to decrease the creatinine levels compared with CsA monotherapy (Figure 1). With FK778–Tac combination compared to Tac monotherapy there was a trend towards lower creatinine levels although no statistical difference between these groups was seen.

Acute rejection and early post-transplant cell infiltration

In syngenic grafts almost no inflammation was seen 5 days after transplantation (Figure 3a). In contrast, moderate perivascular and diffuse interstitial inflammation was seen in rats treated with CNI monotherapy. FK778 combined with CNIs ameliorated both perivascular and diffuse interstitial inflammation when compared to CNI monotherapies (Figure 3a). With Tac this additive immunosuppressive effect was somewhat stronger than with CsA.

FK778 monotherapy ameliorated dose dependently early post-transplant inflammation as shown in Figure 2a. This same dose-dependent trend was also seen in infiltration of both ED3+ activated macrophages and CD8+ lymphocytes although no statistical difference between these groups was seen (Figure 2b). The effect of FK778 monotherapy on CD4+ lymphocyte infiltration was not dose dependent.

The highest density of CD4+ cells in renal cortex was seen in the CsA monotherapy group (Figure 3b). Also in
the Tac monotherapy group, a moderate number of CD4+ cells were observed. FK778 combined with CNIs somewhat ameliorated the infiltration of CD4+ lymphocytes although no significant reduction was seen. The number of ED3+ activated macrophages in renal interstitium was markedly higher in the CsA monotherapy group compared to the Tac monotherapy group (Figure 3b). FK778 decreased the amount of infiltrating CD4+ and CD8+ cells when combined with CNIs although the effect on CD4+ lymphocytes was insignificant. FK778 significantly ameliorated also the infiltration of activated macrophages when combined with CNIs (Figure 3b).

**Chronic allograft nephropathy**

No histological signs of CAN were seen in syngenic grafts 90 days after transplantation, whereas moderate chronic changes were seen in allografts treated with CsA or Tac monotherapy (Figure 4a and b). These included increased fibrosis, inflammation and intimal proliferation. Almost no tubular atrophy or glomerular sclerosis was seen in any of the study groups. In the syngenic group, almost no glomerular mesangial matrix increase was seen whereas a mild-to-moderate glomerular mesangial matrix increase was recorded in all allograft groups. However, the difference in the glomerular mesangial matrix increase between allograft study groups remained small. The CADI scores in CsA and Tac monotherapy groups were 7.5 ± 0.7 (mean ± SE) and 6.8 ± 1.7, respectively; in the combination therapy group FK778 ± CsA, the CADI score was 2.7 ± 0.6 and in the FK778 ± Tac group 2.3 ± 0.7 (Figure 4a). FK778 significantly ameliorated inflammatory, fibrosis and intimal proliferation seen in CNI monotherapy-treated allografts (Figure 4b).

**Immunohistochemistry**

**PDGF expression.** In syngenic controls, PDGF-A, -B and PDGFR-α and PDGFR-β expression was almost nonexistent both 5 and 90 days after transplantation (Figure 5a and b, respectively). FK778 monotherapy decreased the expression of PDGF ligands and receptors in a dose-dependent manner (Figure 2c).

In allografts treated with CNI monotherapy moderate PDGF ligand and receptor expression was seen 5 days after transplantation (Figure 5a). The difference in PDGF ligand and receptor expression between CsA and Tac monotherapy groups remained relatively small. This PDGF ligand and receptor expression was localized mainly in capillary endothelial cells and infiltrating leucocytes (Figure 5c). FK778 significantly decreased PDGF expression when combined with CNIs compared to CNI monotherapies (Figure 5a).

In chronically-rejecting allografts, the PDGF expression pattern differed somewhat from acutely rejecting allografts and remained more scattered. PDGF-A was localized mainly in inflammatory cells and renal arteries whereas PDGF-B was seen mainly in inflammatory cells and in glomerules. The expression of both PDGFR-α and -β was observed mainly in inflammatory cells, and mild PDGFR-β expression was also seen in arteries. In the CsA monotherapy group, PDGF ligand and receptor expression was moderate 90 days after transplantation (Figure 5b). In the Tac monotherapy group PDGF-A, PDGFR-α and PDGFR-β expression was moderate whereas PDGF-B expression remained mild (Figure 5b). FK778 significantly ameliorated PDGF ligand and receptor expression also in chronic allografts when combined with CNIs (Figure 5b).

**TGF-β expression.** There was no noteworthy TGF-β ligand or receptor expression in syngenic grafts at 5 and 90 days after transplantation (Figure 5a and b). FK778 monotherapy decreased the expression of TGF-β and TGF-βR dose dependently (Figure 2c).

In acutely rejecting allografts, moderate TGF-β and TGF-βR expression was observed and TGF-β and TGF-βR were mainly expressed in renal capillaries and infiltrating leucocytes (Figure 5a). Combination therapy of FK778 and CsA or Tac was effective in decreasing the TGF-β and TGF-βR expression when compared with CNI monotherapy (Figure 5a).

In chronically rejecting allografts, the expression pattern was quite similar to what was seen in acutely rejecting allografts. However, also mild glomerular expression was seen 90 days after transplantation. Moderate TGF-β and
Fig. 2. FK778 monotherapy reduced the early inflammatory response and fibrogenic growth factor expression seen 5 days after transplantation as shown here (mean ± SE). (A) FK778 monotherapy dose dependently ameliorated the early post-transplant inflammatory response. (B) Also graft infiltration of CD8+ lymphocytes and ED3+ activated macrophages was reduced in a dose-dependent manner. However, this dose-dependent effect was not seen on infiltration of CD4+ lymphocytes. (C) The effect of FK778 monotherapy on reducing early post-transplant expression of PDGF and TGF-β ligands and receptors in infiltrating leucocytes was also dose dependent. *P < 0.05 (combination therapy of FK778 and CsA or Tac versus CNI monotherapy).

Discussion

In this study we demonstrate that FK778 effectively inhibits the development of acute rejection and early post-transplant induction of fibrogenic growth factors PDGF-A, PDGF-B and TGF-β. We also demonstrate that FK778 combined with calcineurin inhibitors ameliorates the development of chronic changes typically associated with CAN. In addition, combination therapy with FK778 and CNIs decreases both early and late post-transplant expression of PDGF and TGF-β ligands and receptors when compared to CNI monotherapy.

FK778 monotherapy ameliorated dose dependently early graft infiltration of CD8+ lymphocytes and ED3+ activated macrophages. In addition, FK778 monotherapy reduced both diffuse interstitial and perivascular...
FK778 ameliorates post-transplant expression of fibrogenic growth factors

Fig. 3. Combination therapy of FK778 and CNIs ameliorated the early inflammatory response and fibrogenic growth factor expression seen 5 days after transplantation compared to CNI monotherapies (mean ± SE shown). (A) Moderate perivascular and diffuse interstitial inflammation was seen in rats treated with both CNI monotherapies. FK778 combined with CNIs ameliorated both perivascular and diffuse interstitial inflammation when compared to CNI monotherapies. (B) Combination therapies effectively ameliorated also early post-transplant graft infiltration of CD8+ lymphocytes and ED3+ activated macrophages when compared with CNI monotherapies. Also infiltration of CD4+ lymphocytes was somewhat reduced with combination therapy; however, this effect was insignificant. *P < 0.05 (combination therapy of FK778 and CsA or Tac versus CNI monotherapy). Moderate inflammation was seen 5 days after transplantation in rats treated with CsA (C) and Tac (E) monotherapy. Combination therapy of FK778 and CsA (D) and combination therapy of FK778 and Tac (F) showed nearly normal histology without inflammation. Haematoxylin-eosin staining, magnification ×200.
Fig. 4. Combination therapy of FK778 and CNIs reduced the development of chronic rejection changes 90 days after transplantation compared to CNI monotherapies. (A) Moderate chronic rejection changes were seen in allografts treated with CsA or Tac monotherapy when analysed by CADI score. FK778 significantly decreased CADI score when combined with CNIs. (B) FK778 combination therapies significantly ameliorated inflammation, fibrosis as well as intimal proliferation seen in CNI monotherapy-treated allografts. Increased chronic inflammation was seen in the CsA monotherapy group (C) 90 days after transplantation while combination therapy with FK778 and CsA (D) significantly ameliorated inflammation. Increased interstitial fibrosis in the Tac monotherapy group (E) was seen 90 days after transplantation whereas almost no fibrosis was seen in rats treated with combination therapy of FK778 and Tac (F). *P < 0.05 (combination therapy of FK778 and CsA or Tac versus CNI monotherapy), mean + SE shown. (C) and (D) PAS-staining, magnification ×400, (E) and (F) Masson’s trichrome staining, magnification ×100.
FK778 ameliorates post-transplant expression of fibrogenic growth factors

Fig. 5. Combination therapy of FK778 and CNIs decreased the post-transplant expression of fibrogenic growth factors compared to CNI monotherapies. The expression of PDGF and TGF-β ligands and receptors in infiltrating leucocytes at Day 5 post-transplant (A) and at Day 90 post-transplant (B). Combination therapy with FK778 and CNIs effectively inhibited the early post-transplant induction of fibrogenic growth factors PDGF-A, PDGF-B and TGF-β and their receptors 5 days after transplantation (C). Magnification ×400. *P < 0.05 (combination therapy of FK778 and CsA or Tac versus CNI monotherapy).
inflammation in a dose-dependent manner. The difference
between doses of 10 and 20 mg/kg/day was only modest.
Minimal drug doses are favoured for combination therapy
in clinical kidney transplantation to avoid any side effects
of individual drugs. For this reason we selected FK778 at the
dose of 10 mg/kg/day for combination therapy with CNIs.

Acute rejection remains the single most important risk
factor for the development of subsequent CAN which is
the major cause of late kidney allograft loss [1,2]. Mono-
cytic infiltrations are typical for acute rejection and inva-
sion of tubular epithelium by lymphoid cells is a defining
pathological feature [18]. Also graft infiltration by macrophages has been shown to promote the development of
chronic changes in allografts [19,20]. According to our
data, FK778 showed additive effects with CNIs on the early
post-transplant inflammatory response. Almost no diffuse
interstitial or perivascular inflammation was seen in grafts
treated with the combination of FK778 and calcineurin in-
hibitors at Day 5 after transplantation. Combination ther-
apy effectively ameliorated also early post-transplant graft
infiltration of CD8+ lymphocytes as well as ED3+ acti-
vated macrophages when compared with CNI monother-
apy. Taken together, these results indicate that FK778 is
a potent immunosuppressive drug for acute rejection. It
has also additive effects with both CsA and Tac on acute
rejection.

Both TGF-β and PDGF are likely to participate in early
induction of molecular changes that may subsequently lead
to interstitial fibrosis and other histological features of
CAN. Early post-transplant inhibition of these fibrogenic
growth factors could be an elegant way to prevent harmful
cascades at their primary points before cytokine expres-
sion strengthens the immune response. Here we demon-
strate that FK778 effectively decreases both early and late
post-transplant expression of TGF-β and PDGF ligands and
receptors in renal allografts when combined with CsA and
Tac. FK778 inhibits several growth factor RTKs and there-
fore offers a different immunosuppressive mechanism which
could supplement the effect of CNIs. Drug combinations
are also routinely used in clinical transplantation to avoid
the side-effects of CNI monotherapy such as nephrotoxi-
city. According to our results FK778 could be a valuable
option for combination therapy with calcineurin inhibitors.

One of the main pathological features of CAN is in-
terstitial fibrosis that is partly caused by activated fi-
broblasts producing excess amounts of extracellular ma-
trix. Under normal physiological conditions renal tissue
fibroblasts are relatively quiescent and scattered but they
proliferate in pathological circumstances. More intersti-
tial fibroblasts are also derived from tubular epithelial
cells by epithelial-mesenchymal transformation and from
bone-marrow-derived circulating fibroblasts [21]. Here we
demonstrate that the combination therapy of FK778 and
CNIs significantly decreases the development of interstitial
fibrosis in kidney allografts. FK778 also ameliorated other
histological changes that are typically seen in CAN such as
chronic inflammation and intimal proliferation when com-
bined with CsA and Tac. Decreased post-transplant expres-
sion of fibrogenic growth factors, e.g., of PDGF and TGF-β
may be an important factor mediating this effect of FK778
in the development of interstitial fibrosis.

In conclusion, our results demonstrate that FK778 is a
potent immunosuppressive drug that has at least an addi-
tive effect with calcineurin inhibitors. It attenuates both
perivascular and diffuse interstitial inflammation as well
as the early post-transplant infiltration of CD8+ lymphocytes
and ED3+ activated macrophages. It also prevents chronic
changes typically associated with CAN including fibrosis
and intimal proliferation. In addition, FK778 ameliorates
the expression of PDGF and TGF-β which both are impor-
tant factors mediating pathological cascades subsequently
leading to chronic allograft nephropathy and graft loss.

Chronic rejection remains the most important challenge
in clinical transplantation. According to our data FK778
could be a promising agent to prevent chronic rejection in
clinical transplantation. Unfortunately the clinical studies
with FK778 were stopped when no significant benefit on
acute rejection was seen after 1-year follow-up although
the side-effect profile was milder with FK778 compared
to other immunosuppressive agents. Taken together our re-
results indicate that FK778 could be a potent intervention for
chronic rejection also in clinical kidney transplantation.

Acknowledgement. This work was supported in part by Finska
L¨akares¨allskapet and Research and Science Foundation of Farmos.

Conflict of interest statement. None declared.

References
1. Meier-Kriesche HU, Ojo AO, Hanson JA et al. Increased impact of acute rejection on chronic allograft failure in recent era. Transplan-
tation 2000; 70: 1098–1100
13
the Banff working classification of kidney transplant pathology. Kid-
ney Int 1993; 44: 411–422
5. Shishido S, Asanuma H, Nakai H et al. The impact of repeated subclin-
ic acute rejection on the progression of chronic allograft nephropathy.
6. Fitzsimmons WE, First MR. FK778, a synthetic mononitrilamide. Toso-
ne Med J 2004; 45: 1132–1135
7. Vanrenterghem Y, van Hooff JP, Klinger M et al. The effects of FK778 in combination with tacrolimus and steroids: a phase II multi-
center study in renal transplant patients. Transplantation 2004; 78: 9–
14
8. Bartlett RR, Dimitrijevic M, Mattar T et al. Leflunomide (HWA 486), a novel immunomodulating compound for the treatment of autoim-
mune disorders and reactions leading to transplantation rejection. Agents Ac-
ctions 1991; 32: 10–21
9. Shawver LK, Schwartz DP, Mann E et al. Inhibition of platelet-
derived growth factor-mediated signal transduction and tumor growth
by N-[4-(trifluoromethyl)-phenyl]-5-methylisoxazole-4-carboxamide.
10. Xu X, Shen J, Mall JW et al. In vitro and in vivo antitumor activity of
a novel immunomodulatory drug, leflunomide: mechanisms of action.
Biochem Pharmacol 1999; 58: 1405–1413
11. Savikko J, Von Willebrand E, Hayry P. Leflunomide analogue FK778 is
vasculoprotective independent of its immunosuppressive effect: po-
tential applications for restenosis and chronic rejection. Transplanta-
tion 2003; 76: 455–458, discussion 471–453