Early short-term imatinib treatment is sufficient to prevent the development of chronic allograft nephropathy

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Abstract

Background. Chronic allograft nephropathy (CAN), now defined as interstitial fibrosis and tubular atrophy not otherwise specified, is a near universal finding in kidney grafts by the end of the first decade posttransplantation. Platelet-derived growth factor (PDGF) is a major mitogen mediating mesenchymal cell proliferation in CAN. Here, we investigated whether early short-term PDGF inhibition with imatinib could prevent CAN.

Methods. Kidney transplantations were performed from Dark-Agouti (DA) to Wistar-Furth (WF) rats and syngenic controls were done between DA rats. Allografts were immunosuppressed with cyclosporine. One group was also treated with imatinib for the first 30 days after transplantation. Serum creatinine levels were measured once a week. Grafts were harvested 90 days after transplantation.

Results. In control allografts, moderate to intense chronic changes were seen, whereas in syngenic grafts, no changes were seen. The early imatinib treatment prevented the development of CAN significantly compared to control allografts. Only few histological changes were seen. Fibrogenic growth factor ligand and receptor induction as well as inflammatory cell response was significantly inhibited by imatinib. Creatinine values of imatinib-treated allografts were also significantly lower compared to controls.

Conclusions. We show that short-term imatinib treatment is sufficient to prevent CAN significantly, indicating that early PDGF induction has an important role in the pathogenesis of CAN. Here, we provide preclinical work that will need to be confirmed in patients with CAN.

Keywords: chronic allograft nephropathy; imatinib; kidney transplantation; platelet-derived growth factor

Introduction

Despite the dramatic impact of modern immunosuppression and anti-infective prophylaxis on reducing acute graft loss, there has been little impact on long-term graft loss. Chronic allograft nephropathy (CAN), now defined as interstitial fibrosis and tubular atrophy not otherwise specified, still remains one of the most important challenges in clinical kidney transplantation. It is an irreversible fibrotizing process leading eventually to the loss of the graft. The development of chronic allograft dysfunction is a multifactorial process including both immunological and nonimmunologic factors [1, 2]. There have been a number of approaches to reduce the impact of CAN, mostly centered around the avoidance of calcineurin inhibitors. However, there is no sufficient treatment available for preventing CAN. Late identification of CAN in individual patients means that strategies for intervening to prevent chronic allograft dysfunction and subsequent grafts loss tend to be insufficient [3].

Acute rejection is the single most important risk factor for the subsequent development of chronic allograft rejection [4, 5]. Repeated episodes of acute rejection, even subclinical ones, correlate with the progression of histological changes in renal allograft protocol biopsies [6]. Although the exact mechanisms leading to CAN are not well known, acute rejection could cause the primary injury, leading to the induction of reparative mechanisms. Posttransplant induction of fibrogenic growth factors is most likely a significant step in a cascade, leading to interstitial fibrosis and inflammation and other features of CAN. Platelet-derived growth factor (PDGF) is one of the most ubiquitous peptide regulatory growth factors in human body. To date four different PDGF ligands are known and they can form five different isoforms after dimerization. There are two types of PDGF receptors, alpha and beta, which also dimerize after ligand binding [7]. Overexpression of PDGF ligands and receptors has been shown to be associated with acute and chronic renal allograft rejection [8–11].

Early posttransplant inhibition of PDGF could be an elegant way to prevent harmful preparative processes at their primary points before cytokine expression strengthens the immune response in renal allograft rejection. Imatinib, an orally administered receptor tyrosine kinase inhibitor, is well known as the first small molecule inhibitor of tyrosine kinases to be licensed for cancer treatment. Imatinib is a selective PDGF receptor tyrosine kinase inhibitor cross-reacting with vAbl-Bcr and c-Kit [12]. Imatinib has been shown to inhibit both PDGF-α and -β receptors. In clinical oncology, this compound has been shown to be well tolerated.
Early short-term imatinib treatment

and curative in the treatment of various malignant diseases [13, 14].

We have previously demonstrated that blocking the PDGF signalling with imatinib prevents the development of CAN in an experimental rat kidney transplantation almost totally [15]. In our recent study, nearly normal histological findings were seen in imatinib-treated allografts 90 days after transplantation when imatinib was administered daily during the entire follow-up, whereas massive chronic changes were seen in control allografts. Imatinib also restored the kidney graft function. Creatinine values of imatinib-treated animals were markedly lower compared to control animals; they were at the same level as in animals with syngenic kidney transplants.

According to our previous data, imatinib should be used daily continuously to prevent CAN in clinical kidney transplantation. This would be very expensive and potential long-term side effects would decrease patient compliance and safety. To make clinical use more competent, shorter administration time would be feasible. This in mind, the aim of our present study was to investigate the role of PDGF shortly after transplantation and whether early short-term imatinib treatment could prevent the development of CAN.

Materials and methods

Animals and kidney transplantations

Specific pathogen-free inbred male WF (RT1<sup>a</sup>) and DA (RT1<sup>a</sup>) rats (Harlan, Horst, the Netherlands) weighing 300–350 g were used for transplantations. Transplantations were performed as described earlier [11].

Experimental design and medication

Syngenic transplantations were performed from DA to DA rats and allografts from DA to WF rats. Nontreated syngenic grafts (Group I) were used as controls. Allografts (Groups II and III) were immunosuppressed with cyclosporine A (CsA) 1.5 mg/kg/day subcutaneously (s.c.). Group III allografts were treated in addition to CsA with imatinib 10 mg/kg/day p.o. for the first 30 days after transplantation, after which imatinib was discontinued. The imatinib dose was chosen based on our previous studies with imatinib in rat aorta denudation and rat kidney transplantation models [15, 16]. Grafts (n = 7 in each study group) were harvested 90 days after transplantation.

CsA (Sandimmun; Novartis, Basel, Switzerland) was dissolved in Intralipid (KabiVitrum, Stockholm, Sweden) to a final concentration of 1.5 mg/kg and administered s.c. once a day. Imatinib (Gleevec; Novartis, Basel, Switzerland) was dissolved in aqua to a final concentration of 10 mg/kg and administered p.o. once a day.

Serum creatinine and CsA levels were measured using radioimmunoassay (Sandimmun-Kit; Novartis).

Histopathology

The kidney grafts were bisected horizontally and fixed in 4% paraformaldehyde for 24 h and then routinely fixed for paraffine blocks. Four-micrometer thick sections were cut in series on glass slides. Before staining, 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 at room temperature for 20 min. To demonstrate the expression and localization of PDGF-AA, PDGF-BB, PDGFR-α, PDGFR-β, TGF-β and TGF-βRI, the samples were immunostained using an EnVision™ G/2 System/AP, Rabbit/Mouse (Permanent Red)-kit (Dako systems, Denmark) according to the manufacturer’s instructions. After staining, the slides were counterstained with Mayer’s hemalum 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), PDGF-BB (5 μg/mL; Abcam, Cambridge, UK), PDGFR-α (10 μg/mL; Lab Vision Corp., Fremont, CA), PDGFR-β (2 μg/mL; Santa Cruz Biotechnology Inc. Santa Cruz, CA), TGF-β (2 μg/mL; Santa Cruz Biotechnology Inc.) and TGF-βRI (2 μg/mL; Santa Cruz Biotechnology Inc.) were purchased from commercial suppliers.

To demonstrate the infiltration of activated macrophages and T cells, a three-layer indirect immunoperoxidase technique was used [19]. The primary monoclonal mouse antibodies used were ED3 (Serotec Ltd, Oxford, UK), rat CD4 and rat CD8 (BD Pharmingen). Peroxidase-conjugated rabbit anti-mouse Ig (DAKO A/S, Denmark) and peroxidase-conjugated goat anti-rabbit IgG (Caltag Laboratories, Burlington, CA) were used sequentially.

Quantification of immunohistochemistry

Immunohistochemical analysis was done in a blind review. The intensity of the growth factor ligand and receptor 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.

CD4, CD8 and ED3 positively stained cells were counted in three visual fields from the renal cortex at magnification ×400, and the mean number of positive cells per field of vision was calculated.

Statistical analysis

The results are expressed as mean ± SE (n = 7 in each study group), and a probability of <0.05 was accepted as significant. The significance between groups was determined by parametric analysis of variance (SPSS version 10; SPSS, Chicago, IL).
Results

Clinical course

No recipients were lost before the end of the follow-up period, and both CsA and imatinib were well tolerated. No adverse effects were seen in imatinib-treated animals. Creatinine levels of imatinib-treated allografts were significantly lower through the follow-up time compared to the CsA-treated control allografts (P < 0.05) (Figure 1).

Histopathology

No signs of CAN were seen in syngenic grafts, CADI 0.4 ± 0.2 (mean ± SE). In CsA-treated control allografts, moderate to intense chronic changes were seen, CADI 6.1 ± 0.5. Short-term imatinib treatment ameliorated the development of CAN highly significantly compared to control allografts (Figure 2). Only few chronic histological changes were seen, CADI 2.3 ± 0.6. CADI value of imatinib-treated allografts was markedly lower compared to control allografts (P < 0.001). Especially, interstitial inflammation and fibrosis as well as arterial intimal proliferation and glomerular mesangial matrix increase were significantly decreased in imatinib-treated allografts compared to control allografts. Almost no tubular atrophy or glomerular sclerosis was detected in any of the grafts studied.

Immunohistochemistry

Infiltration of inflammatory cell into grafts was detected using CD4, CD8 and ED3 antibodies and demonstrated in Figure 3. In syngenic control grafts, almost no inflammatory cells were seen. In CsA-treated control allografts, moderate to intense infiltrations of inflammatory cells were seen. These infiltrations consisted of both CD4- and CD8-positive T cells as well as activated macrophages. Imatinib treatment significantly decreased the infiltration of CD4- and CD8-positive T cells and activated macrophages (P < 0.001) compared to CsA-treated control allografts.

Expression of growth factor ligand and receptor is demonstrated in Figure 4. The expression remained nearly nonexistent in syngenic control grafts. Induction of PDGF-A and -B ligands and receptor-β was significantly inhibited in imatinib-treated allografts compared to CsA-treated control allografts (P < 0.05). Expression of both PDGF-A and -B ligands was decreased especially in interstitial leukocytes and tubules. PDGF-B expression was also significantly inhibited in arterial smooth muscle cells in imatinib-treated allografts compared to control allografts. Expression of PDGF-α and -β receptors was decreased markedly in graft infiltrating inflammatory cells and arterial smooth muscle cells. PDGF-β receptor induction was also inhibited in tubules.

Fig. 2. Histological findings of the study. Short-term imatinib treatment decreased significantly chronic changes compared to control allografts 90 days after transplantation. CADI score findings are summarized in (A). Especially, inflammation, fibrosis, mesangial matrix increase and intimal proliferation were prevented by imatinib (B). The histologic changes of renal allografts at the end of study (C). Fibrosis and glomerular changes were scored after Masson staining, magnification ×100 and magnification ×200, respectively. Inflammation and arterial changes were scored after periodic acid-Schiff staining, magnification ×100 and magnification ×400, respectively.
In CsA-treated control allografts, moderate to intense TGF-β ligand and receptor expression were detected in inflammatory cells and tubules. Imatinib treatment inhibited significantly the induction of TGF-β and TGF-β receptor expression both in infiltrations of inflammatory cells as well as in tubules (P < 0.05).

**Discussion**

CAN, now defined as interstitial fibrosis and tubular atrophy not otherwise specified, still remains an unsolved problem in clinical kidney transplantation. It is highly prevalent, with moderate to severe CAN present in 24.7% of recipients at 1 year posttransplant and in 89.8% of recipients by 10 years posttransplant [20]. Although registry data can be used to define some risk factors, the pathophysiology of CAN is poorly understood. We have previously demonstrated that early posttransplant induction of fibrogenic growth factors are likely to participate in induction of molecular changes subsequently leading to the development of CAN [10, 21]. We could also show that inhibition of PDGF receptor signaling with imatinib prevented chronic histological changes in transplant and restored the kidney graft function through the long-term follow-up when administered daily during the entire follow-up time [15].

Our current findings show that even early short-term imatinib treatment is sufficient to prevent the development of chronic allograft rejection and restores the long-term graft function. This suggests that the early induction of
Fig. 4. The expression and localization of fibrogenic growth factors at the end of the study. Imatinib treatment ameliorated significantly the expression of PDGF ligands (A), PDGF receptors (B) as well as TGF-β ligand and receptor expression (C) compared to control allografts. Immunohistochemical stainings of growth factor ligand expression at the end of the study shown with magnification ×200 (D).
PDGF is an important mechanism in the pathophysiology of CAN. Together with PDGF, TGF-β is suggested to be a major mitogen mediating mesangial cell proliferation in chronic kidney diseases [22, 23]. It has been demonstrated that imatinib inhibits also TGF-β signalling via a non-Smad TGF-β pathway by blocking c-abl activation [24]. This may explain part of the beneficial effect of imatinib also in our model. In the present study, expression of TGF-β ligand and receptor was also investigated in our model.

In our study, imatinib prevented both interstitial fibrosis and inflammation as well as chronic glomerular and arterial changes. At the end of the study, the expression of PDGF and TGF-β was decreased compared to control allografts showing that there was no fibrogenic activity in the imatinib-treated allografts. Also, PDGF and TGF-β receptor induction was inhibited by short-term imatinib treatment. In addition, the infiltration of inflammatory cells was decreased in imatinib-treated allografts compared to control allografts. This suggests that the early short-term inhibition of harmful reparative processes is an effective intervention to restore the graft function and to prevent graft loss due to CAN.

The important finding of the study was not only the nearly normal histological findings but also the good function of the imatinib-treated allografts. The creatinine values of the imatinib-treated allografts were significantly lower compared to those of control allografts shortly after transplantation.

Based already on our previous data, imatinib could be a potential intervention in clinical kidney transplantation. However, the life-long daily administration of imatinib in transplant patients would be very expensive. In addition, potential side effects of long-term use are not yet known, although imatinib has become standard treatment for several malignant diseases and has been shown to be well tolerated in clinical oncology. According to our current data, life-long imatinib treatment may not be necessary for preventing CAN. This makes imatinib more competent option for intervening CAN in clinical kidney transplantation.

In recent years, more indications for imatinib have arisen. Imatinib has been demonstrated to widen the window for anticoagulate treatment in ischaemic stroke [25, 26]. Interestingly, imatinib has also been demonstrated to have beneficial activity in sclerotic chronic graft-versus-host disease after allogeneic stem cell transplantation [27, 28]. This suggests that the indications for imatinib treatment are likely to broaden also to other than malignant diseases in future. Although there have been some cases of renal failure after imatinib treatment [29–31], it has been shown recently that no dose modifications are required for patients with mild or moderate renal dysfunction, but in patients with chronic renal failure, there is some need for caution owing to a lack of data [32, 33]. Interestingly, there is a case report demonstrating that imatinib has been successfully used after kidney transplantation to treat chronic myeloid leukemia [34]. In that case, graft biopsy showed no signs of chronic histological changes supporting our experimental data. In addition, the renoprotective and therapeutic effect of imatinib has been demonstrated in various other animal models of kidney diseases [24, 35–37].

In conclusion, our results demonstrate that early PDGF induction has an important role in the pathogenesis of chronic renal allograft rejection. Based on our findings, short-term imatinib treatment could be a potential intervention in preventing chronic allograft dysfunction in clinical kidney transplantation.

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Conflict of interest statement. None declared.

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