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Thymoglobulin and ischemia reperfusion injury in kidney and liver transplantation

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Abstract

Since the beginning of organ transplantation, graft preservation has been one of the most important concerns. Ischemia reperfusion injury (IRI), which plays an important role in the quality and function of the graft, is a major cause for increased length of hospitalization and decreased long term graft survival.

Among numerous attempts which have been made to minimize graft damage associated with IRI, the use of Thymoglobulin (TG) seems to offer potential benefits. TG is a polyclonal antibody which blocks multiple antigens related to IRI, in addition to its better known T cell depleting effects. This review will focus on the use of TG in preventing IRI in kidney transplantation (KTx) and liver transplantation (LTx).

Different studies in experimental and clinical transplantation have shown that TG protects renal and liver grafts from IRI. Improvement in early graft function and decreased delayed graft function (DGF) rates are some of the clinical benefits of TG. Additionally, it is used in patients with hepatorenal syndrome to support the recovery of kidney function after LTx, by allowing reduced exposure to nephrotoxic calcineurin inhibitors as well as improving liver graft function by minimizing IRI. TG can reduce acute rejection rates in kidney and liver transplant recipients, decrease the length of hospital stay, and hence reduce transplantation costs. TG can play an important role in expanding the donor pool in both KTx and LTx by improving long-term graft and patient survival rates which increases the possibility of using marginal donors. Although controversial, the development of post-transplant lymphoproliferative disorder is a potential side effect of TG. No single optimal immunosuppressive regimen has given consistent results in decreasing the graft damage following IRI; however, TG usage in KTx and LTx appears to have some benefits in reducing IRI.

Keywords: thymoglobulin; ischemia reperfusion injury; kidney transplantation; liver transplantation

Introduction

Preservation of graft functions has been one of the most important concerns since the beginning of organ transplantation. Due to the nature of the solid organ transplant procedure, it is not possible to transplant an organ without ischemia and microcirculatory disturbance, which consequently causes reperfusion injury and functional impairment [1]. Ischemia reperfusion injury (IRI) is associated with an increased rate of acute rejection, primary non-function of the graft, delayed graft function (DGF) or initial poor graft function (IPGF), and also late graft failure leading to graft loss [2–4]. Additionally, it has been shown that DGF in KTx, defined as the requirement for dialysis within the first week after transplantation [5], has a significant impact on long term outcome [6]. The incidence of DGF due to IRI has been reported in approximately 25% of the kidneys obtained from deceased donors [7]. Therefore IRI contributes significantly to increasing length of hospital stay and decreasing long term graft survival. Currently in kidney transplantation, the annual number of newly registered transplants approximately equals the number of renal grafts that fail [8]. Thus, finding a solution to overcome the problems associated with IRI would greatly increase the number of grafts for transplantation by reducing the demand for

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re-transplants. Various strategies have been described to prevent IRI in solid organ transplantation. The aim of this paper is to give an overview of the clinical evaluation of Thymoglobulin (TG) with the emphasis on its potential role in decreasing IRI in kidney transplantation (KTx) and liver transplantation (LTx).

The mechanism of ischemia reperfusion injury

Two basic mechanisms play an important role in IRI: (a) systemic imbalance of oxidative stress/antioxidant status and (b) restoration of metabolic processes which trigger the immune/inflammatory responses.

It seems that reactive oxygen species (ROS) initiate and induce the adaptive alloimmune response (acute rejection) predominantly through activation of antigen-presenting cells. Furthermore, the ROS-induced injury contributes to the development of alloatherosclerosis of donor organ vessels (chronic rejection) through endothelial injury-induced proliferation of smooth muscle cells [9]. Loss of oxygen supply during the ischemic period and subsequent reperfusion of the graft trigger the loss of osmotic equilibrium and increased permeability of cellular membranes, which leads to cell necrosis and decreased overall organ function [3,9–13]. Furthermore, the formation of reactive oxygen species that cause direct oxidative damage to nucleic acids, proteins, and lipids plays an important role in aggravating cell and tissue damage [11,14,15]. Tissue hypoxia is only one of the factors contributing to cellular damage related to ischemia-reperfusion during organ transplantation. Reperfusion also triggers the expression of inflammatory cytokines and adhesion molecules that increases the rate of apoptosis in the reperfused tissue [3,16].

The role of white blood cells, which are closely related to the development of inflammatory damage in ischemia-reperfusion, has been demonstrated in various studies [7,17,18]. Preservation and revascularization which occur early in the transplantation process initiate a cascade of molecular and cellular events which trigger the release of proinflammatory mediators and attract various cell types which infiltrate the tissues [10,17]. Leukocytes have been considered to be responsible for many pathophysiologic changes during IRI [19–25]. They may exacerbate tissue hypoxia by plugging capillaries [7,25,26] and mediate direct cytotoxicity by producing oxygen radicals [27] and proteolytic enzymes [28]. The alteration of vascular resistance during ischemia-reperfusion is another important role of these mediators causing IRI [18]. In fact, activation of components of the inflammatory response exacerbates the damage already caused by the oxidative radicals [29]. Therefore, already existing ischemia-induced damage is further exacerbated by cytotoxic cells and effects on adhesion molecules [7,30].

Attempts to decrease ischemia reperfusion injury

Numerous attempts have been made to minimize graft damage associated with IRI, but so far no single optimal approach has given reliable and consistent results [25,31-33]. The following options have been evaluated and may potentially reduce IRI probability. Cold ischemia time is a determinant for the severity of IRI and prolonged cold ischemia time is associated with a higher probability of IRI [16]. Therefore, using cold preservation solutions which contain antioxidative compounds may optimize the preservation of the transplanted organs [34–36]. Attempts to reduce the likelihood and/or severity of IRI should be focused on three main facts: 1) restoration of microvascular blood fluidity, 2) inhibition of the effects of oxygen radicals, and 3) blockade of activation/adhesion of inflammatory cells and mediators [7,37,38]. Using vasodilators to increase nitric oxide synthesis and availability may reduce the likelihood of IRI [39]. Furthermore, avoiding vasoconstrictive effect of calcineurin inhibitors (CNI) early post-transplant may diminish IRI [40]. In addition, isovolemic hemodilution and prevention of edema formation by hypertonic or hyperoncotic solutions have been shown to have some benefits against IRI [41–44]. The application of oxygen radical scavengers, such as superoxide dismutase, was also found to significantly reduce IRI [35]. Increasing the presence of growth factors that can enhance local repair responses (such as hepatocyte growth factor) is another possibility for decreasing the risk of IRI [7].

In addition, the post ischemic inflammatory response could be successfully inhibited by leukocyte depletion or functional inactivation [7,25,45,46]. Moreover, inactivation of T and B-cells has been described as prerequisite to achieve some protection against IRI [47]. In this way, post transplant leukocyte adhesion to microvascular endothelium (rolling and sticking) can be specifically blocked by monoclonal or polyclonal antibodies directed against specific adhesion molecules and subsequently reduces IRI in transplanted organs [48–52]. Among the various polyclonal antibodies evaluated, Thymoglobulin (TG), a purified IgG fraction of sera from rabbits against human thymocytes, is the only polyclonal agent that has been extensively evaluated [2,53–56].

Thymoglobulin

Polyclonal antibodies were first developed over a century ago, when their anti-inflammatory effect was first described by Metchnikoff and colleagues [57]. Their immunosuppressive potential was demonstrated in 1951 [58]. TG, a rabbit derived polyclonal antibody, has been used since 1984 in different fields including in organ transplantation for the prevention and treatment of acute rejection, treatment of aplastic anemia, also prevention and treatment of graft versus host disease in hematopoietic stem cell transplantation [59–61].

The *mechanism of TG* in IRI has been thought to result primarily from a direct effect on blocking the cell-to-cell interactions [3] and reducing the degree of leukocyte rolling and adhering along capillary endothelial surfaces [56]. This effect is due to down modulation of adhesion molecules and specific receptors which are responsible for these interactions (LFA-1, VLA-4, CCR5, and CCR7) [53]. TG can also indirectly reduce inflammatory mediators and inhibit leukocyte-chemotaxis or chemokine receptor expression [3, 56, 62]. For that reason, inhibition of leukocyte homing and trafficking to the graft by binding to chemokine receptors is another way by which TG affects IRI [63]. Additionally, TG reduces the number of peripheral lymphocytes from the circulating pool by inducing T-cell depletion through complement-related lysis or activation associated apoptosis [60]. Moreover, it causes anergy and functional impairment of non-depleted lymphocytes and prevents migration of memory T-cells [53, 60]. Lopez *et al*. showed that the therapeutic effect of TG is not only due to T-cell depletion, but also due to generation of regulatory T-cell [54]. As a polyclonal agent, directed against molecules participating in IRI, it can minimize the IRI related problems in the grafted organ and subsequently preventing DGF [2, 6].

Few experimental studies have been published to show benefits of TG in reducing IRI. Preville *et al*. performed an experimental study in a non-human primate model to investigate the extent of T-cell depletion in lymphoid tissue after TG usage. The purpose of this study was to establish a better concept of the mechanisms of action of TG and to determine the appropriate dosage of TG in different applications. Using skin grafts and heart transplantation models, TG treatment induced a dose-dependent lymphocytopenia and T-cell depletion in spleen and lymph nodes due to T-cell apoptosis [60].

Beiras-Fernandez *et al*. performed another study on two different groups of primates (Cynomolgus monkeys); one group was treated with TG and the other one without TG. The study was designed to evaluate the effect of TG on the prevention of apoptosis in reperfused limb after ischemia and also to monitor its ability to increase lymphocyte apoptosis. There was a significant decrease of apoptotic cells in skeletal muscle, connective tissue, and endothelial cells in the TG treated animals after 60 minutes of warm ischemia. Additionally, white blood cell (WBC) infiltration in muscles was reduced while the apoptosis of WBCs was increased. Furthermore, mononuclear cells in peripheral blood, expression of adhesion molecules, and tissue damage were significantly decreased in the TG treated animals. The authors concluded that TG not only increased the rate of apoptosis in WBCs, but also protected the reperfused tissue against IRI [3].

The role of TG in reducing IRI in KTx

In clinical transplantation, Brennan and colleagues were among the first group of investigators who studied the benefits of TG induction therapy in decreasing IRI in a randomized double-blinded study, comparing TG and Atgam (equine anti-thymocyte globulin) in kidney transplant recipients. The main goal of the study was to compare the efficacy and safety of TG and Atgam. They concluded that a brief (7-day) induction therapy with TG significantly decreased the incidence of acute rejection and caused less severe rejection and a better event-free survival than Atgam (94% *vs* 63%). Furthermore, less cytomegalovirus infection and fewer serious adverse events were seen in TG-treated patients in comparison to Atgam. The authors postulated these results were due to a more profound and durable lymphopenia [62]. Following Brennan's research; Matas *et al*. performed a pilot study to test the possibility of using TG in living donor kidney transplant recipients to rapidly discontinue steroids in order to overcome post-operative complications of prolonged steroid therapy. This is important especially because late post-transplant steroid withdrawal is associated with an increased risk of acute rejection. In 51 patients intra-operative TG induction therapy allowed rapid steroid withdrawal without significant differences in 6- and 12-month patient and graft survival [64].

Agha *et al*. performed a study to see if short course TG induction, which is accompanied by less drug-related complications in KTx, is as effective as a long course therapy. Their prospective non-randomized trial demonstrated that a 3-day course of TG induction was as effective as a 7-day course treatment without any significant differences in acute rejection (5% *vs* 4%), graft survival (95% *vs* 98%), and patient survival [65]. In order to determine the safety and efficacy of intermittent TG induction therapy, Peddi *et al*. used a prospective protocol to administer TG intermittently based on peripheral blood $CD3^+$ lymphocyte counts in high-risk cadaver transplant recipients (such as repeat transplant recipients, prolonged cold ischemia time, prolonged donor hypotension). They found that intermittent TG therapy is safe and is associated with low acute rejection rate. Additionally, in comparison to traditional daily TG administration this approach resulted in a significant reduction of the total cumulative dose and costs [66].

Shortly after, a prospective randomized clinical trial showed the superiority of intra-operative TG administration compared to post-operative administration. Goggins *et al*. specifically investigated the effect of TG on DGF by reducing IRI in recipients of cadaver kidney transplants. Two groups of patients, one treated by intra-operative TG and another one by post-operative TG administration were studied. This study showed that intra-operative administration of TG resulted in a better reduction in the incidence of DGF compared to post-operative administration (14.8% *vs* 35.5%). They also observed that intra-operative administration of TG improved first month post-transplant early allograft function, and a decrease in post-operative creatinine levels at day 10 (2.4 *vs* 4.3 mg/dl) with significantly reduced acute

rejection rates (3.7% *vs* 16%) without increasing the chance of viral or opportunistic infections. Furthermore, the length of post-operative hospital stay was decreased in patients treated by intra-operative TG administration (7.5 *vs* 11 days). The authors concluded that intra-operative TG administration is beneficial regarding DGF and length of hospitalization [2].

In a further study by Knight *et al*. TG induction was shown to be effective in recipients with an increased risk for acute rejection after KTx (African Americans, re-transplant recipients, and recipients with a panel-reactive antibody greater than 50%). In their study they substituted TG for Basiliximab (interleukin-2 receptor monoclonal antibody), as induction therapy, along with Sirolimus. The result of this study showed that the chance of acute rejection in high immune responders was 26% in the Basiliximab compared to 3% in the TG-treated group. They concluded that the strategy of treatment should be a combination of Basiliximab with Sirolimus for low-immunologic risk recipients and TG with Sirolimus for high-immunologic risk recipients [67].

Due to the critical role of DGF in the outcome of KTx, Cravedi *et al*. carried out a retrospective study among the kidney transplant recipients with DGF who received a cyclosporine-based regimen or a cyclosporine sparing regimen based on early treatment with TG. The TG induction with a cyclosporine sparing regimen resulted in a decrease in the duration of anuria and faster recovery of DGF (11 ± 5.6 *vs* 19.6 ± 8.9 days), a decrease rate of acute rejection during DGF (0% *vs* 24%) and at 2 years (17% *vs* 35%), a shorter hospital stay (17.4 ± 4.3 *vs* 27.4 ± 10.4 days), and consequently lower treatment costs (hospitalization, dialysis and drugs) than a cyclosporine based regimen. They concluded that in recipients with DGF, early TG treatment with delayed cyclosporine administration accelerated kidney function recovery and significantly decreased the rate of acute rejection leading to shorter hospitalization time and reduced treatment cost [68].

Despite the low rate of DGF in living donor KTx, compared to the cadaveric one, Hardinger *et al*. studied the potential advantages versus the safety of TG in living donor KTx with respect of DGF. In a long term follow up of recipients who underwent KTx followed by different TG treatment protocols they compared their patients' outcomes with patients in the national registry who did not receive TG. The TG treated patients did not experience any DGF but the incidence of DGF was reported to be 5–10% in patients who did not receive TG. Moreover, treatment with TG resulted in a lower one year acute rejection rate (2% *vs* 21%) and a higher 5-year graft survival rate (82% *vs* 79%). Importantly, the use of TG was associated with a low rate of post-transplant lymphoproliferative disorder (PTLD) (0.5%), CMV infection (5%), and malignancy (3%). This study presented for the first time benefits of TG in improving clinical outcomes and reducing the complications in living donor KTx [69].

Treatment with TG was studied in patients with auto-immune renal diseases who need KTx; Mezrich *et al*. compared Alemtuzumab (humanized anti–CD52 monoclonal antibody) versus TG induction therapy in 239 patients. In patients with autoimmune glomerular diseases, treated with TG, the recurrence rate was significantly lower (1.5% *vs* 8.3%). Also, patients with SLE exhibited a trend towards decreased recurrence when treated with TG. However, they postulated this TG-related advantage may be only limited to patients with glomerular diseases of autoimmune etiology [70].

The role of Thymoglobulin in reducing IRI in liver transplantation

Until recently, only a few studies focused on the potential role of TG in decreasing IRI in LTx. Tchervenkov *et al*. performed a retrospective analysis to show the benefits of TG induction in a cyclosporine-based immunosuppressive protocol. Their study showed that induction therapy with TG in liver transplanted patients resulted in a decrease in acute rejection rates (7% *vs* 50%), an increase of rejection free episodes (median: 51 *vs* 11.5 days), a decrease in the rate of steroid resistant rejection (by 50%), and a reduction of the re-transplantation frequency (19% *vs* 29%). Although acute rejection and re-transplantation rate were rather high in the control group, the patient survival rate was the same in both groups. They concluded that in selected patients with severe kidney disease who underwent LTx, treatment with TG allowed delay initiation of cyclosporine which resulted in better recovery of the kidney function without increasing the risk of rejection of the transplanted liver [71].

To study the impact of TG on renal function after LTx, the same research group carried out another retrospective study on 298 patients in 2004. This study focused on the administration of TG, with delayed initiation of nephrotoxic CNI therapy. The results of this study revealed that TG induction therapy with delayed CNI initiation resulted in an increase of rejection-free rate at 1 year (72% *vs* 50%), a decrease in acute rejection episodes (28% *vs* 59%), an increase of rejection free graft survival at 1 year (51% *vs* 39%), and a decrease of serum creatinine levels in the first 3 days and at 6 months after LTx. They concluded that TG initiation therapy allowed delayed CNI initiation without compromising graft and patient survival; additionally, preventing early rejection even among patients with baseline kidney dysfunction [72].

Furthermore, a randomized controlled trial in cadaveric liver transplant recipients by Bogetti *et al*. demonstrated advantages of intra-operative usage of TG in ameliorating IPGF and its potential to decrease IRI in LTx. Evaluation of histological changes in the liver donor tissue were achieved by wedge biopsies before and after graft reperfusion in a sample of 22 patients. In these patients; a 100% graft survival at 3 months, no incidence of graft primary

non-function, no need for re-transplantation, and a significant decrease in total bilirubin (2.1 ± 0.23 *vs* 4.5 ± 3.7 mg/dl) were found. Also the post-transplant ALT level at day 1 (262 ± 55 *vs* 942 ± 258 mg/dl) and length of hospital stay were significantly decreased (8 ± 3 *vs* 13 ± 8 days). They concluded that TG induction therapy allows for more marginal liver grafts to be used with an acceptable clinical outcome, due to minimization of IRI and improvement in liver function [4].

The side effects of Thymoglobulin

As reported, TG is not specific for T-cells and contains antibodies directed against different blood cell types (T-cells > B-cells and natural killer (NK) cells > monocytes and neutrophils > platelets > erythrocytes) [60,61]. Due to the presence of cross-reacting antibodies directed against non-lymphoid cells, hemolytic anemia, thrombosis, thrombocytopenia, and neutropenia besides fever and serum sickness can occur [60,61,71]. A higher incidence of leukopenia with TG has been reported when it was used to reverse rejection [73]. As with other antibody preparations, cytokine release syndrome (fever, chills, tachycardia, and hypotension) and serum sickness can occur and the overall incidence of adverse events and infections appears similar to other T-cell depleting antibody agents. However, there is evidence that the rate of CMV infection may be lower by using TG, especially if CMV prophylaxis is used [74]. Some authors reported prophylactic hydrocortisone and heparin administration before peripheral TG induction can decrease the risk of thrombosis.

In addition, Opelz *et al.* showed patients receiving TG had a significant increased risk of developing lymphoma probably caused by inhibition of T-cell control, which allows the uninhibited proliferation of B-cells [75]. However, some authors claim that TG usage in lower conventional doses will not increase the risk of PTLD [2, 69]. A recent study showed among various immunosuppressive agents only OKT3 (one year Hazard Ratio 4.51) and high-dose steroid therapy (one year Hazard Ratio 3.37) were associated with the risk of PTLD. Meanwhile, treatment with lower doses of polyclonal antibodies such as TG was not accompanied with subsequent development of PTLD [76]. Although controversial, the development of PTLD is another possible side effect of TG usage [77].

Conclusion

In summary, protection of renal and liver grafts by decreasing IRI seems to be one of the main advantages of TG usage [4,75]. Improving early graft function, decreasing the rate of DGF in KTx and LTx as well as decreasing the chance of subsequent graft failure are among the other clinical benefits of TG [2,3]. Moreover, TG reduced acute rejection in both kidney and liver transplant recipients and decreased the length of hospital stay and costs for transplantation [2,4]. Additionally, in patients with hepatorenal syndrome, TG and delayed CNI initiation allow the recovery of kidney function after LTx [72,78].

Not only by improving long-term graft and patient survival rate [79], but also by increasing the possibility of using marginal donors, TG might play an important role in expansion of the donor pool. It also decreased the mortality rate among the transplanted patients [75]. One suggested protocol for TG administration in KTx could be an initiation therapy with TG (1 to 1.5 mg/kg) intraoperatively which continued postoperatively for at least 3 doses (not more than six doses). TG should be continued in patients with DGF for a maximum of six total doses (1 mg/kg every other day after the first 3 doses) [2]. In LTx, the TG administration could be 1.5 mg/kg per dose during the anhepatic phase and two doses every other day postoperatively [29]. Although TG usage in KTx and LTx seems to offer some benefits in reducing IRI, no single optimal immunosuppressive regimen has given consistent results in decreasing the graft damage so far. More experimental studies and randomized clinical trials are needed to give transplant specialists valuable insight about the control of IRI, using TG, in the field of transplantation.

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