Transplant Immunology 28 (2013) 203–205

Contents lists available at SciVerse ScienceDirect

Transplant Immunology

journal homepage: www.elsevier.com/locate/trim

Evaluation of histological damage of solid organs after donor preconditioning with thymoglobulin in an experimental rat model

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ARTICLE INFO

Article history:
Received 26 January 2013
Accepted 8 April 2013

Keywords:
Transplantation
Donor preconditioning
Animal model
Thymoglobulin

ABSTRACT

Rabbit anti-rat thymoglobulin (rATG) administered to donors with brain death (BD) may improve organs quality. We explored the effects of rATG administered to BD donors in the histology of heart, lungs and small bowel in a rat experimental model. Animals were randomly assigned to 3 groups: V ($n = 5$) no BD, 2 h ventilation; BD ($n = 5$) BD and 2 h ventilation; BD and rATG: BD, 2 h ventilation, rATG (10 mg/kg) after BD diagnosis. Histopathological damage scores were based on neutrophil infiltration, airway epithelial cell damage, interstitial edema, hyaline membrane formation, and pulmonary hemorrhage (lungs); neutrophil infiltration and interstitial edema (heart); Park score (bowel). Lung damage was significantly lower in BD + rATG group: V 5 ± 1.6; BD 11.25 ± 0.5, BD + rATG 6.5 ± 1.9 ($p < 0.01$). Heart: V 2.0 ± 0.81; BD 4.75 ± 1.25 and BD + rATG 3.5 ± 1.7 ($p > 0.05$). Small bowel: BD 2.25 ± 0.96 vs. BD + rATG 1.00 ± 1.15 (n.s.). Histological damage amelioration in lung and attenuation tendency in heart and small bowel encourages research of cytoprotective strategies to improve organ viability.

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1. Introduction

Brain dead donors are the first source of organs for transplantation [1], but these organs suffer from lower success rates than grafts from living donors, possibly because of the pathophysiological changes caused by BD [2]. To improve organs quality and therefore transplant outcomes, different approaches such as treatment of recipients, interventions on preservation solutions, or donor preconditioning are normally adopted [1]. BD increases the parasympathetic tone and the subsequent endogenous catecholamine release stimulates the sympathetic tone, resulting in vasoconstriction, secondary tissue ischemia, changes in the production of ATP and oxygen-free radicals. The cytosolic calcium production and the nitric oxide synthase concentration augment; then the subsequent hypotensive stage, with diminished sympathetic activity, can reduce oxygen availability to the tissues [3,4]. Immunological responses activate endothelial cells and pro-inflammatory genes [5], and this inflammatory process generates pathological signs of cell death [6]. As BD has a specific cytokine profile, a distinctive approach is required to preserve organs from brain dead donors [7]. Other studies have shown that donors pretreated with steroids had a decreased pro-inflammatory cytokine expression in tissues and serum [8]. In a rat model, preconditioning donors with steroids reduced the IRI rate in kidney recipients [5,9].

Antithymocyte immunoglobulin (ATG) (Thymoglobulin®, Genzyme, Cambridge, MA, USA), generally administered to transplant recipients, is a purified fraction of IgG from the sera of rabbits immunized against human thymocytes. ATG antibodies affect the binding and/or expression of ligands such as intercellular adhesion molecule 1, and surface molecules such as lymphocyte function-associated antigen 1, which intervene in the leukocyte-endothelium interaction. Leukocyte homing and trafficking to the graft is inhibited by antiCCR7, antiCXCR4, and antiCCR5 antibodies in ATG that bind to chemokine receptors [10]. We explored whether the administration of rabbit anti-rat thymoglobulin (rATG) to brain dead donors ameliorated histopathological damage in heart, lungs and small bowel in an animal experimental model with brain death (BD).

2. Materials and methods

Genzyme Corporation (Cambridge, MA, USA) provided rATG, analogous to commercial ATG, Thymoglobulin®, Genzyme. Fifty New Zealand White rabbits were immunized with thymocytes of four strains of rats (Sprague–Dawley, F344 Fisher, Lewis and Long Evans). The thymocyte suspensions were prepared with thymi from donor rats. Rabbits were immunized twice, two weeks apart, and terminally bled two weeks after the second immunization. The rabbit IgG from the serum was pooled and

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http://dx.doi.org/10.1016/j.trim.2013.04.002

purified with a process similar to Thymoglobulin®. Control rabbit IgG was similarly purified from whole normal rabbit serum. Fifteen Sprague–Dawley male rats (300 ± 30 g, University of Buenos Aires, Argentina) were submitted to controlled environmental conditions, with access to water and standard laboratory chow *ad libitum*. Experiments were performed in accordance to the guidelines of the National Institutes of Health (NIH publication No. 28 revised 1996). Rats were randomly divided into three groups: Group V (n = 5) no BD and mechanically ventilated for 2 h; Group BD (n = 5) BD and ventilated for 2 h. Group BD and rATG (n = 5) BD, ventilated for 2 h. After BD diagnosis, rATG was administered intravenously (10 mg/kg, dose suggested by manufacturer). Detailed description of anesthesia, ventilation, induction of BD was presented previously [9]. After 120 minutes of BD [11,9], blood samples were collected, the animals were sacrificed, and samples of heart, lungs and small bowel were collected for histopathological analysis.

Lung damage was evaluated on the basis of neutrophil infiltration, airway epithelial cell damage, interstitial edema, hyaline membrane formation, and pulmonary hemorrhage. Each criterion was scored on a semi-quantitative scale of 0–4 based on the severity of change. An overall histological score was calculated by totaling the scores for criteria 1 to 5. For heart graft damage, a similar score system was used, based on neutrophil infiltration and interstitial edema. The damage of the small bowel was evaluated with the Park score [12]. Continuous data were expressed as mean ± standard deviation. Differences between groups or conditions were analyzed by variance analysis (ANOVA) and when it was significant, the source of difference was located by multiple comparisons with *post hoc* Student–Newman–Keuls test. Non parametric variables were analyzed with Krustal–Wallis non parametric test of ANOVA. A *P* value of <0.05 was considered statistically significant.

3. Results

The lung histological damage score was significantly lower in the BD group treated with thymoglobulin compared to the BD untreated group: V 5 ± 1.6; BD 11.25 ± 0.5 and BD + rATG 6.5 ± 1.9 ($p < 0.001$ for V vs. BD and $p < 0.01$ for BD + rATG vs. BD) (Fig. 1).

In relation to cardiac injury, the scores were as follows: V 2.0 ± 0.81; BD 4.75 ± 1.25 and BD + rATG 3.5 ± 1.7 ($p < 0.05$ for V vs. BD and $p > 0.05$ for BD + rATG vs. BD). The heart histological damage score was lower, though not statistically significant, in the treated group compared to the untreated group. In the small bowel samples the differences in the park score were not statistically significant, however the score of the treated group was lower than the score of the untreated group: Park score (means): BD 2.25 ± 0.96 vs. BD + rATG 1.00 ± 1.15 (n.s.).

4. Discussion

The statistically significant differences found in our study suggest that rATG administered to donors could improve lungs affected by BD process. The pathophysiology of the organs to be transplanted is significantly altered by BD, resulting in inflammation, injury and, eventually, poor post-transplant function and graft survival [13]. This can be explained by the endothelial injury, leukocyte infiltration, and tubular epithelial cell damage [9] as a result of this inflammatory process [14,15]. As a result, BD is associated with compromised kidney graft survival and cardiac function, increased rates of apoptosis and necrosis in cardiomyocytes, and the presence of proinflammatory cytokines and adhesion molecules in lungs [15].

The immunosuppressive efficacy of rATG has been attributed to T cell depletion, the expression modulation of lymphocyte surface antigens and the activation of transcription factors interfering with immune cell processes such as cytokine production, chemotaxis, endocytosis, stimulation, and proliferation [10]. Based on a previous study in which rATG was administered to donors [9], it may be hypothesized that the decreased ATN in the rATG group is a result of increased *in situ* overexpression of IL-10, inhibiting pro-inflammatory cytokines such as TNF-α. Lopez et al. [14] showed that low, no depleting doses of rATG to peripheral blood mononuclear cells could expand human CD4+ CD25+ FoxP3+ regulatory T cells with suppressive properties *in vitro*. ATG can generate changes in different lymphocyte cell subsets in blood and regulatory T cells expansion.

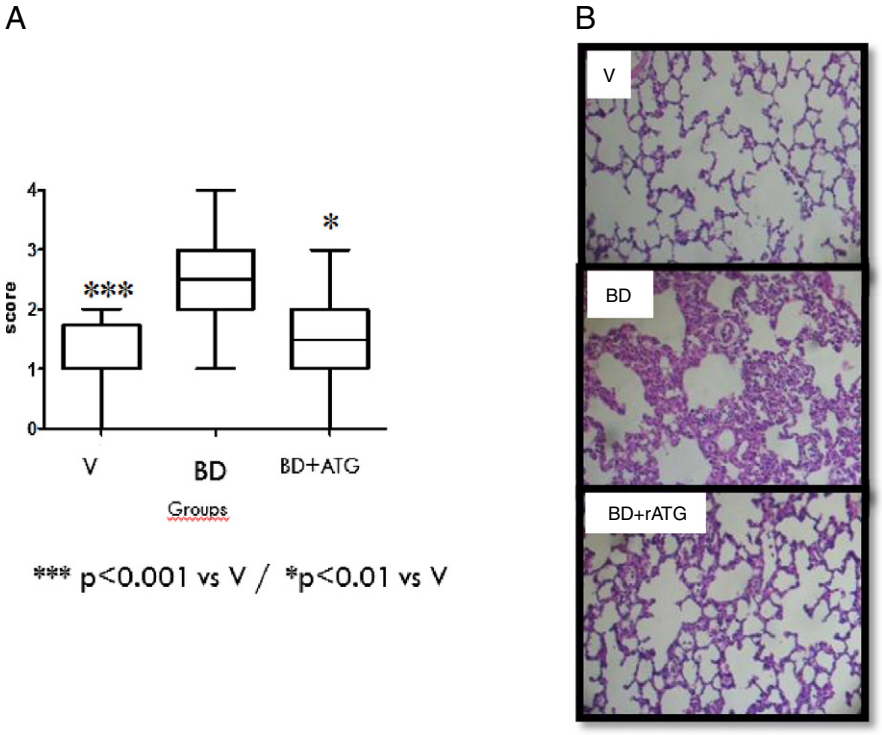

Fig. 1. Histological damage of lung tissue. Lung tissue damage. A. Score based on neutrophil infiltration, airway epithelial cell damage, interstitial edema, hyaline membrane formation, and pulmonary hemorrhage. Each criterion was scored on a semi-quantitative scale of 0–4 based on the severity of change. An overall histological score was calculated by totaling the scores for criteria 1 to 5. Damage score in groups, *p < 0.01, ***p < 0.001. B. H&E staining. Magnification 20X. Abbreviations: Group V no brain death and mechanically ventilated for 2 h; Group BD brain death and ventilated for 2 h. Group BD + rATG brain death, ventilated for 2 h, and after BD diagnosis, rATG was administered intravenously.

The observation time of 2 h has been used in other studies [9,11], but it would be beneficial to design models with longer observation times. Also, further studies should explore the mechanistic of the observed results and include transplantation to complete the process.

5. Conclusions

A comprehensive understanding of the BD process is necessary to improve intervention strategies. Moreover, when a drug is systemically administered, it is critical to comprehend its action on every organ. We found statistically significant attenuation of histological damage in lung as well as a tendency of attenuation in heart graft and small bowel when rATG was administered to donors. These original findings may contribute to continue further research on cytoprotective strategies to improve organ viability.

Conflict of interests

The authors declare no conflict of interests. Although the drug used in the study was kindly provided by Genzyme Corp the authors declare no financial or personal conflict of interests influencing this study.

Acknowledgment

The drug was provided by Genzyme Corporation, Cambridge, MA, USA.

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