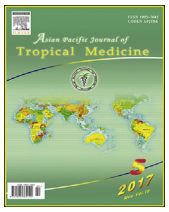
HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm



Original research http://dx.doi.org/10.1016/j.apjtm.2017.05.004

Preoperative application of combination of portal venous injection of donor spleen cells and intraperitoneal injection of rapamycin prolongs the survival of cardiac allografts in mice

Wen-lin Gong[1,#], Chuang Sha[2,#], Gang Du[1], Zhong-gui Shan[3], Zhong-quan Qi[3], Su-fang Zhou[1✉], Nuo Yang[1,4✉], Yong-xiang Zhao[1,4✉]

[1]*National Center for International Research of Biological Targeting Diagnosis and Therapy, Guangxi Key Laboratory of Biological Targeting Diagnosis and Therapy Research, Collaborative Innovation Center for Targeting Tumor Diagnosis and Therapy, Guangxi Medical University, Nanning, Guangxi, 530021, China*

[2]*Department of Cardiac Surgery, Zhongshan Hospital, Xiamen University, Xiamen, China*

[3]*Organ Transplantation Institute, Xiamen University, Xiamen, China*

[4]*Department of Cardio-Thoracic Surgery, The First Affiliated Hospital of Guang Xi Medical University, Nanning 530021, China*

ARTICLE INFO

Article history:
Received 2 Mar 2017
Received in revised form 4 Apr 2017
Accepted 30 Apr 2017
Available online 17 May 2017

Keywords:
Heart transplantation tolerance
Portal venous injection of donor spleen cells
Rapamycin

ABSTRACT

Objective: To investigate the effects of preoperative portal venous injection of donor spleen cells (PVIDSC) and intraperitoneal injection of rapamycin in the acute rejection of cardiac allograft in mice and the underlying mechanisms.

Methods: Homogenous female B6 mice and BALB/c mice were used as recipients and donors of heart transplantation. These mice were randomly divided into different groups and received PVIDSC alone, rapamycin alone, or PVIDSC and rapamycin combined therapy. In addition, the underlying mechanism was studied by measuring a number of cytokines.

Results: Preoperative combination of PVIDSC and intraperitoneal injection of rapamycin significantly prolonged the survival of heterotopic cardiac allograft in mice, but had no effects on the survival time of cardiac allografts in mice pre-sensitized by skin grafting. Preoperative combination of PVIDSC and intraperitoneal injection of rapamycin increased the expression of IL-10 and Foxp3 and reduced the expression of INF-γ. Short-term preoperative administration of rapamycin promotes the expression of $CD4^+CD25^+Foxp3^+$ regulator T cells. However, preoperative using alone of rapamycin, or combination of PVIDSC and rapamycin had no effects on the inhibition of proliferation of memory T cells.

Conclusions: Preoperative application of combination of PVIDSC and rapamycin significantly prolonged the survival time of cardiac allografts in mice but not in mice pre-sensitized by skin grafting. This may be explained by the fact that combination of PVIDSC and rapamycin inhibited the cellular immune response and induced the expression of IL-10 from Tr1 cells and $CD4^+CD25^+FoxP3^+$ regulatory T cells.

First author: Wen-lin Gong, National Center for International Research of Biological Targeting Diagnosis and Therapy, Guangxi Key Laboratory of Biological Targeting Diagnosis and Therapy Research, Collaborative Innovation Center for Targeting Tumor Diagnosis and Therapy, Guangxi Medical University, Nanning, Guangxi, 530021, China.

✉Corresponding authors: Yong-xiang Zhao, Ph.D, Professor, National Center for International Research of Biological Targeting Diagnosis and Therapy, Guangxi Medical University, Shuangyong Road 22, Nanning 530021, China.

Tel: +86 771 5317 061

Fax: +86 771 5317 061

Nuo Yang, Ph.D, National Center for International Research of Biological Targeting Diagnosis and Therapy, Guangxi Medical University, Shuangyong Road 22, Nanning 530021, China.

Tel: +86 771 5356708

Fax: +86 13978891981

Su-fang Zhou, Ph.D. Professor, National Center for International Research of Biological Targeting Diagnosis and Therapy, Guangxi Medical University, Shuangyong Road 22, Nanning 530021, China.

Tel: +86 771 5317 061

Fax: +86 771 5317 061

Peer review under responsibility of Hainan Medical University.

Foundation project: This work was supported, in part, by grants from National Science and Technology Major Project – national major new drug creation (No. 2015GKS-462); National Natural Science Foundation of key projects (No. 81430055); National "Chang Jiang Scholars and Innovative Team Development Program" Innovation Team Rolling Support Project (No. IRT_15R13); Guangxi Science Research and Technology Development Project (No.Gui Ke He 1599005-2-10); Inter-provincial cooperation projects (No.Gui Ke He 14251001).

[#] These authors contributed equally to this study.

1. Introduction

In the 1950s, Dr. Joseph Murray conducted kidney transplant between a pair of twin brothers, which represents a milestone of the human organ transplant [1]. For over 50 years, great success has been achieved in the field of organ transplantation including successful transplantation of kidney, liver, and heart. Organ transplantation has become the most effective treatments to save lives of patients with organ failure. The application of immunosuppressants has greatly increased the success rate of organ transplantation and improved the survival time of transplanted organs. However, long-term use of large dose of immunosuppressants increases the chance of opportunistic infections and tumors, which seriously affected the long-term effects of organ transplant and quality of life of recipients [2]. Therefore, identification and application of immunosuppressants of low toxicity are highlighted in the field of organ transplantation.

Rapamycin is a novel macrolide immunosuppressant. By binding to different cytokine receptors, rapamycin inactivates signal transduction to arrest T cells and other cells at the G1 phase. In this study, rapamycin was selected to study its immunosuppressive effects in cardiac transplantation because it inhibits the repulsion against spleen cells from donors and inhibits graft-versus-host disease (GVHD). In addition, rapamycin can also promote the production of regulator T cells [3], which thereby induces immune tolerance.

In recent years, it has been reported that spleen cells play an important role in the induction of immune tolerance [4]. Studies demonstrated that importing donor spleen cells induced donor-specific immune tolerance and prolonged graft survival time [5,6]. Injection of donor spleen cells via portal venous may be more efficient than via peripheral vein to induce immune tolerance. In this study, we evaluated the effects of preoperative application of PVIDSC and intraperitoneal injection of rapamycin in the acute rejection of cardiac allograft in mice and skin grafts pre-sensitized mice, and to find an efficient way inducing immune tolerance using small amount of immunosuppressant.

2. Materials and methods

2.1. Animals

Homogenous female B6 (H-2^b) mice and BALB/c (H-2^d) mice were used as recipients and donors of heart transplantation, respectively, in this study. All mice (8–12 weeks old and 19–22 g) were purchased from the Shanghai SLAC Laboratory Animal Co., Ltd., Chinese Academy of Sciences.

Twenty-four female BALB/c mice were randomly divided into four corresponding groups (D1, D2, D3, and D4) as heart donors. Twenty-four female B6 mice were randomly divided into four groups (R1, R2, R3, and R4) as recipients. Twenty-four female B6 mice pre-sensitized by skin grafting were randomly divided into four groups (Rsg1, Rsg2, Rsg3, and Rsg4) as recipients and used as control. No preoperative treatments were given to the R1 and Rsg1 group animals. The R2 and Rsg2 group animals received PVIDSC on the 7th day prior to heart transplantation. The R3 and Rsg3 group animals received intraperitoneal injection of rapamycin (1.5 mg/kg/d) for seven days (from the 7th day prior to heart transplantation to the operation day). The R4 and Rsg4 group animals received a combinative treatment of PVIDSC and rapamycin as mentioned above.

Four to six weeks prior to heart transplant, B6 mice in the Rsg1, Rsg2, Rsg3, and Rsg4 groups received skin graft of full-thickness of back skin from BALB/c donor mice [7]. The skin graft is circular with a diameter of about 1.0 cm.

2.2. Preoperative treatments

The spleens of BALB/c donor mice were removed and lysed using red blood cell lysates to produce single cell suspension. The spleen cells from donor animal were injected to B6 recipient mice (0.3 mL; 1×10^7 cells/animal) on the 7th day prior heart transplant via portal vein. Intraperitoneal injection of rapamycin for seven days (from the 7th day prior to heart transplantation to the operation day) was conducted.

Rapamycin (BBI Co., Canada) was dissolved in ethanol and diluted with sodium carboxymethylcellulose and stored at 4 °C. Rapamycin was intraperitoneally injected to B6 recipient mice (1.5 mg/kg).

2.3. Cervical heterotopic heart transplantation

BALB/c mice were used as donors and B6 mice were used as recipients. Cervical heterotopic heart transplantation was conducted according to previous report [8]. Cardiac allograft was observed daily for pulse to evaluate its function. Exclusion was defined as no pulse is observed.

2.4. Histopathological examination of cardiac allografts

The bottom of cardiac allografts was dissected on the 7th day after heart transplantation for histopathological examination. The autopsy was fixed with 10% formaldehyde and embedded paraffin for hematoxylin and eosin staining and examined under light microscopy. Organ rejection grading was evaluated according to the ISHLT reference standard [9,10] and compared between groups.

2.5. Flow cytometry

Monoclonal antibodies include PE-cy5-CD4 (clone GK1.5), PE-cy5-CD8 (clone 53–6.7), FITC-CD44 (clone IM7) and controls were purchased from Biolegend Corporation (USA). BALB/c donor mice were sacrificed by cervical disarticulation and spleens were harvested. Single cell suspensions were prepared by grinding the tissues with the plunger of a 5 mL disposable syringe and were then suspended in RPMI1640 medium. Splenocytes were treated with a hemolysis buffer (17 mM Tris–HCl and 140 mM NH_4Cl, pH 7.2) to remove RBCs [11]. Erythrocytes were lysised using erythrocyte lysate and the suspension went through 400 mesh strainer. The cells were then washed twice using PBS. After adjusting cell number and volume, fluorescence monoclonal antibodies were added and incubated in dark at room temperature for 30 min. Then, cells were washed twice before flow cytometry assay. Flow cytometry was conducted in Beckman EpicsXL (Beckman, USA). The flow cytometry data were analyzed using the FlowJo software.