The Effect of Asarinin on Toll-Like Pathway in Rats After Cardiac Allograft Implantation

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ABSTRACT

Objective. The objective of this study was to study the mechanism of the anti-rejection effect of Asarinin in rats that underwent cardiac allograft implantation.

Methods. Hearts from Wistar rats were transplanted into the abdominal cavity of Sprague Dawley rats (SD rats) 64 SD rats received either cyclosporin A (CsA), Asarinin, or demi-dose of cyclosporine A and Asarinin through oral administration. On the seventh day post-transplantation, the expression of Toll-like receptor 4 (TLR4), chemokine (C-X-C motif) receptor 3 (CXCR3) in myocardium, and the level of interleukin (IL)-12 in the peripheral blood were analyzed 7 days after transplantation.

Results. The survival time in 3 groups (CsA group, Asarinin group, and semi-dose CsA group) prolonged ($P < .01$), the microscope myocardial histopathology in 3 groups (CsA group, Asarinin group and semi-dose CsA group) relieved, the expression of TLR4 and CXCR3 in 3 groups was significantly decreased ($P < .01$) when compared with the control group. The level of IL-12 decreased remarkably ($P < .05$) in the 3 groups when compared with the control group.

Conclusions. The combined data suggested that Asarinin decreased peripheral blood concentration of IL-12 and inhibited the expression of TLR4 and CXCR3, which means Asarinin may have a role on TLR4 pathway and produced prolongation of allograft heart survival.

IMMUNOSUPPRESSIVE therapy is necessary after organ transplantation to inhibit rejection [1]. But immunosuppressants currently available are not satisfactory with their toxicity and side effects in the recipients. Chinese medicinal herbs, which are known for great medicinal value and mild side effects, may be a good new source of immunosuppressive agents. Asarinin, a less toxic compound extracted from a Chinese medicinal herb, has been proven to play an important role in immunosuppression [2] in our former research. In this report, the mechanism of Asarinin was studied for the first time.

METHODS

The chemical formula of Asarinin is shown as Fig 1. In this study, Asarinin is a kind of monomer; its molecular structure is $C_{20}H_{18}O_6$ and its molecular weight is 354.35.

Animals and Experimental Design

Sixty-four male Wistar rats (body weight, 150–200 g) and 64 Sprague-Dawley (SD) rats (body weight, 200–250 g) were used in this study, which was approved by the Experimental Animal Center of the Second Affiliated Hospital of Harbin Medical University. The rats were kept on a 12-hour light-dark cycle with free to access water and food.

The hearts from Wistar rats were transplanted into the abdominal cavity of SD rats following the procedure as described by Ono et al [3]. Briefly, the aorta of the donor and the abdominal aorta of the recipient was end-to side anastomosis; the pulmonary artery of donor and inferior vena cava of receptor was end-to-side anastomosis. We judged the donor survival by recording the heart beating and performing an electrocardiogram (ECG) of the donor everyday.

L.Z. and X.L. contributed equally to this work.

This study was supported by the National Nature Science Foundation of China (81102236/H1006) and the Post-doctor foundation of Heilongjiang province government (LRB03–129).

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0041-1345/15
http://dx.doi.org/10.1016/j.transproceed.2014.12.019

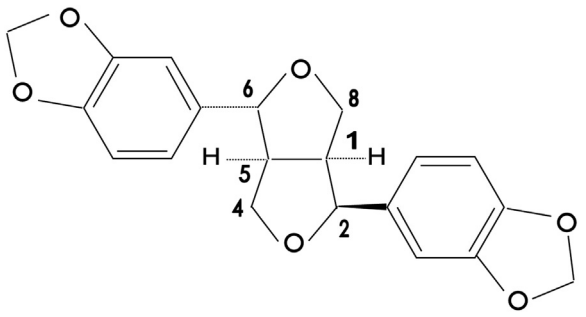

Fig 1. In this study, Asarinin is a kind of monomer. Its molecular structure is $C_{20}H_{18}O_6$, and its molecular weight is 354.35.

All transplanted recipient SD rats were divided randomly into 4 groups: Group A (control group, n = 16), treated with placebo (physiological saline); Group B (cyclosporin A [CsA] group, n = 16), treated with 5 mg/kg CsA; Group C (Asarinin group, n = 16), treated with 25 mg/kg Asarinin; and Group D (semi-dose CsA group, n = 16), treated with 2.5 mg/kg CsA and 25 mg/kg Asarinin. All treatment drugs and placebo were orally administrated into the stomach everyday from 1 day before transplantation until 7 days after transplantation. Half of them were used to observe survival time. The grafts were harvested for histological study and 1 mL blood was collected to analyze cytokine level on the 7th day post-transplantation. All sections were finished in 1 month and were done by 2 authors.

Determination of Interleukin-12 in Peripheral Blood

The levels of interleukin (IL)-12 in the peripheral blood were measured using a specific immunochemistry technique. The procedure was carried out according to the direction of the immunochemistry kits. (The kits were supplied by Baitaike Biotechnology Engineering Limited Company of Beijing, Beijing, China).

Reverse Transcription–Polymerase Chain Reaction Analysis of Toll-Like Receptor 4

Primer was supplied by Baitaike Biotechnology Engineering Limited Company of Beijing. Total RNA was isolated from the myocardium of SD rats, according to the manufacturer's instructions. The respective primer sequences used for reverse transcription–polymerase chain reaction (RT-PCR) of mouse were as follows:

TLR4 5′-ACTCTGCGCCTAAAACCCATT-3′
5′-TTGAGGTTAGAAGCCTCGTGC-3′

The RT is 42°C, 50 minutes; 70°C, 10 minutes; and 4°C, 5 minutes. Two-step real-time PCR: (1) 95°C, 1 circle, 2 minutes; and (2) β-actin: 57°C; Toll-like receptor 4 (TLR4) 61.4°C, 30–40 circles, 60 seconds. The messenger RNA (mRNA) amount of the target gene was normalized relative to β-actin using Option monitor software (Baitaike Biotechnology Engineering Limited Company of Beijing).

Western Blot for Chemokine (C-X-C Motif) Receptor 3 Detection

The chemokine (C-X-C motif) receptor 3 (CXCR3) protein was detected with Western blotting: protein sample preparation, detection of protein, electrophoresis, transfer, blocking, detection of proteins, immune reaction, and picture analysis. (The kits were supplied by Baitaike Biotechnology Engineering Limited Company of Beijing).

Table 1. Survival Time After Heart Transplantation Was Prolonged in Groups B, C, and D Compared With Group A ($P < .05$; x ± s; n = 8)

Group	Immunosuppression	Dose/mg·kg^{-1}·d^{-1}	Survival Time (d)
A	None		8.4 ± 0.9
B	CsA	5	30.5 ± 8.3
C	Asarinin	25	16.5 ± 4.3
D	CsA + Asarinin	2.5 + 25	26.1 ± 5.2

Statistical Analysis

All data were presented as mean ± standard deviation. Statistical significance was analyzed using the 2-tailed paired Student *t* test and identified when $P < .05$.

RESULTS

Survival Time

The survival time was prolonged in Groups B, C, and D compared with Group A ($P < .05$; Table 1).

Cardiac Transplant Rejection Grading (International Society for Heart and Lung Transplantation)

Group A of pathological grade level was IIIA∼IV; Group B was significantly better for IA∼II; Group C grade level was IB∼IIIA; Group D, which mixed the drug groups, was IA∼II. Microscope myocardial histopathology damage was greatly relieved in Groups B, C, and D compared with Group A (Table 2).

Expression of TLR4 in Myocardium

The expression of TLR4 in the CsA group, Asarinin group, and semi-dose CsA group greatly decreased ($P < .01$) when compared with the control group. The expression of TLR4 in the Asarinin group and semi-dose CsA group was significantly higher than that in the CsA group ($P < .05$; Fig 2).

Expression of CXCR3 in Myocardium

The expression of CXCR3 in the CsA group, Asarinin group, and semi-dose CsA group was reduced ($P < .05$) when compared with the control group. There was no statistical difference on expression of CXCR3 between the Asarinin group, CsA group, and semi-dose CsA group ($P > .05$; Fig 3).

Table 2. Cardiac Transplant Rejection Grading (International Society for Heart and Lung Transplantation; n = 8)

Group	1	2	3	4	5	6	7	8
A	IV	IV	IIIB	IIIB	IV	IV	IIIA	IV
B	IA	IB	II	IB	IB	IA	II	II
C	II	IIIA	II	IB	II	IB	IIIA	IIIA
D	IB	IA	II	II	IB	II	II	IA

Note. Group A of pathological grade level was IIIA∼IV; Group B was significantly better for IA∼II; Group C grade level was IB∼IIIA; Group D, the mixed drug group, was IA∼II. Microscope myocardial histopathology damage was greatly relieved in Groups B, C, and D compared with Group A.