Revista Argentina de Clínica Psicológica
2020, Vol. XXIX, N°5, 1299-1303
DOI: 10.24205/03276716.2020.1126

Effect of Atorvastatin on Expression of Inflammatory Cytokines and Osteopontin in Renal Transplant Rats

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Abstract

Purpose: To investigate the effect of atorvastatin on the expression of inflammatory cytokines and osteopontin in renal transplant rats. Methods: 40 adult male SD rats of inbred line were purchased as renal transplant donors and 40 adult male SD rats of inbred line as renal transplant recipients. The SD rats were randomly divided into study group and control group. The control group received conventional triple therapy. The study group was treated with atorvastatin in combination based on control group. The correlated clinical indexes, expression levels of osteopontin and inflammatory cytokines of rats after operation were compared between the two groups.

Results: There was no significant difference in the levels of TC and TG of rats after operation between the two groups ($P > 0.05$), but the levels of SCr, BUN and 24h urine protein in the study group were significantly lower than those in the control group ($P < 0.05$); the expression levels of osteopontin in the study group were significantly lower than those in the control group at different time points after operation ($P < 0.05$); the expression levels of IL-2, IL-6, IL-17 and TNF-α inflammatory cytokines in the study group were significantly lower than those in the control group ($P < 0.05$); the expression level of IL-10 was significantly higher than that of the control group ($P < 0.05$).

Conclusion: Atorvastatin can effectively reduce the expression of inflammatory cytokines and osteopontin in rats after renal transplant, which is beneficial to reduce the interstitial inflammation after renal transplant and the rejection of cells after renal transplant.

Keywords: Atorvastatin; Renal transplant; Inflammatory cytokines; Osteopontin

Renal transplant, as an effective treatment for patients with end-stage renal disease, has a positive impact on prolonging patient survival [1-2]. Rejection after renal transplant is the main factor affecting the survival of renal transplant patients, mainly including ultra-acute rejection, accelerated rejection and chronic rejection, etc., which may result in the loss of renal function after transplant. It has been found that rejection after renal transplant is mainly mediated by T cells and their antibodies, and regulatory T cells play an important role in the regulation of immune response [3]. Osteopontin (OPN), a secreted phosphorylated glycoprotein, has attracted much attention of researchers because of its immune response in the body, and has been found to play a role in rejection after renal transplant [4]. Based on this, 40 adult male SD rats of inbred line were purchased from Laboratory Animal Center of Chinese Academy of Sciences as rental transplant donors and 40 adult male SD rats of inbred line were taken as renal transplant recipients. SD rats were randomly divided into study group and control group after operation. The control group received conventional triple therapy. The study group was combined with atorvastatin treatment on the basis of control group, to analyze the effect of atorvastatin on the expression of inflammatory cytokines and osteopontin in renal transplant rats, it is expected to provide guidance for early prevention of rejection after renal transplant, which is reported as follows.

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1. Data and Method

1.1 General data: 40 adult male SD rats of inbred line were purchased from Laboratory Animal Center of Chinese Academy of Sciences as renal transplant donors, with body weight of 240g-320g and 40 adult male SD rats of inbred line were selected as renal transplant recipients, with body weight of 240g-330g.

1.2 Methods

1.2.1 Surgical method

(1) The renal transplant donor rats were anesthetized by intraperitoneal injection of 1% pentobarbital sodium at a dosage of 3ml/100g. After the corneal reaction and pain reflex of rats disappeared basically and the limbs were floppy, the rats were fixed in supine position.(2) Skin preparation After the hair of the thoracic abdomen of the rats was removed, the exposed skin was disinfected, and aseptic drapes were routinely placed; (3) The median abdomen of the rats was selected as the incision, the bilateral kidneys were separated through the abdominal cavity and the posterior peritoneum, and the abdominal aorta and other branches were ligated respectively;(4) A lateral incision was made in the abdominal aortic wall at the distal end of rats, an epidural catheter was placed in for fixation, the proximal abdominal aorta was ligated and perfused, and the proximal inferior vena cava was ligated at the same time, the distal inferior vena cava was cut to expel the perfusion fluid; (5) After successful perfusion, both kidneys of rats were taken and placed in 4℃ organ maintenance solution for preservation. Take another renal transplant recipient rat, the anesthesia method is the same as above, after routine skin preparation and hair removal disinfection, take the lower abdomen to make oblique incision, separate femoral artery and vein, perform end-to-side anastomosis with donor vein, reconstruct blood circulation and urinary tract, remove the autologous bilateral kidneys of SD rat, use catgut to coincide the transplanted kidney with the recipient, and suture the incision layer by layer.

1.2.2 Intervention grouping

40 SD rats were randomly divided into the study group and the control group. The control group was treated with routine triple therapy (cyclosporine A + mycophenolate ester + glucocorticoid). The study group was treated with atorvastatin on the basis of the control group. The injection dose was 30 mg/kg·d. All the rats were fed and observed under the same conditions after renal transplant.

1.2.3 Test methods

(1) Clinical indicator test: The rats were sacrificed at the 12th week after renal transplant. After collecting renal transplant samples, 3 ml of inferior vena cava was sampled by puncture. It was placed in anticoagulant tube for being centrifuged for 5 min, and the rotational speed was set at 3000 r/min. The serum was separated and the related clinical indicators were detected by automatic biochemistry analyzer. The pathological images of rats' transplanted kidney and normal kidney tissues were shown in Figure 1 and Figure 2; (2) Osteopontin test: The kidney tissues of rats were fixed with 4% formaldehyde for routine paraffin embedding with immunohistochemical method and 4 μm sections were made. After section and dewaxing, peroxidase blocking solution was instilled and incubated at room temperature for 10 min. Then it was placed in citrate buffer solution, heated to boiling with microwave, and allowed to stand for 10 min. After repeated 3 times, instill the serum of non-immune animals, incubate at room temperature for 5 min, instill diluted mouse anti-rat osteopontin antibody primary antibody and macrophage antibody, allow it to stand at 4 ℃ for 8 h, instill goat anti-rat immunoglobulin G secondary antibody, incubate at room temperature for 10 min, instill streptavidin-peroxidase solution, incubate again for 10 min, instill diaminobiphenyl solution, observe under the microscope for 10 min, add hematoxylin and then routinely dehydrate and seal, use image analysis software to observe the positive expression rate of osteopontin under ×200 visual field, as shown in Figure 1 and Figure 2.(3) Inflammatory cytokine test: The kits for interleukin-2 (IL-2), IL-10, L-15, IL-17 and IL-18 were purchased from Shanghai Abcam Company and tested by enzyme-linked immunosorbent assay (ELISA) according to the kit operation.

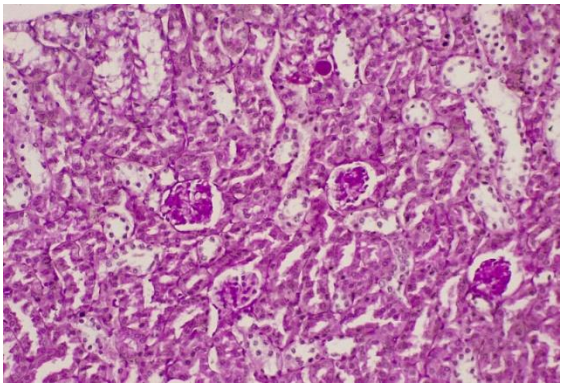

Figure 1. **Histopathology of Renal Transplant in Mice**

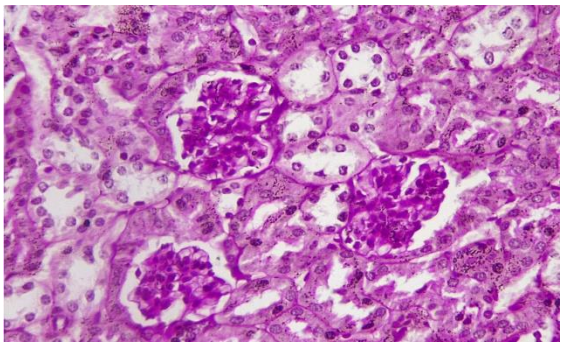

Figure 2. **Histopathology of Normal Kidney in Mice**

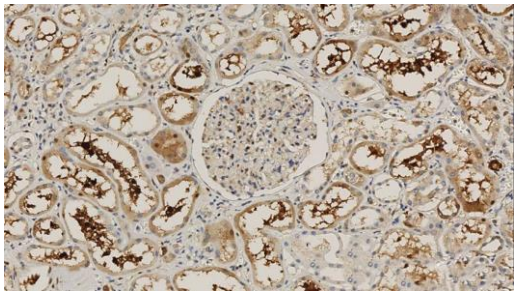

Figure 3. **Expression of Osteopontin in Renal Transplant of Rats in Experiment Group**

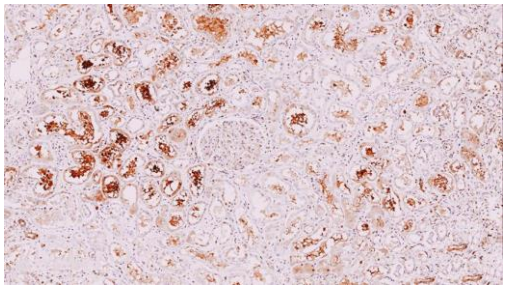

Figure 4. **Expression of Osteopontin in Renal Transplant of Rats in Control Group**

1.3 Observation indexes

(1) The clinical indexes of rats in the two groups were compared 7d after operation, including total cholesterol (TC), triglyceride (TG), serum creatinine (SCr), blood urea nitrogen (BUN) and 24h urine volume; (2) The osteopontin expression levels of rats in the two groups were compared 7d, 14d and 28d after operation; (3) The levels of inflammatory cytokines if rats in the two groups were compared 7d after operation, including IL-2, IL-10, IL-15, IL-17 and IL-18.

1.4 Statistical analysis

The SPSS19.0 statistical software was used for analysis, t-test was used for measurement data, and $P < 0.05$, which means that the difference is statistically significant.

2. Results

2.1 Comparison of the clinical indexes of the two groups

The clinical indexes of the two groups were compared. The results showed that there was no significant difference in TC and TG levels between the two groups ($P > 0.05$). However, the quantities of SCr, BUN and 24h urine protein in the two groups were significantly lower than that of the control group ($P < 0.05$), as shown in Table 1.

Table 1. **Comparison of Clinical Indexes of Rats after Operation between Two Groups ($\bar{x}$ ±s)**

Group	n	TC（mmol/L）	TG（mmol/L）	SCr（μmol/L）	BUN（mmol/L）	24h urine protein quantification (mg/d)
Study group	20	2.83±0.52	0.17±0.01	63.28±5.84	8.51±3.36	38.12±3.39
Control group	20	2.95±0.73	0.18±0.03	113.34±7.91	15.31±2.17	49.55±5.62
t		1.033	0.536	15.512	4.136	10.883
P		0.378	0.629	＜0.001	0.026	0.002

2.2 Changes of osteopontin expression of rats after operation in two groups the expression level of osteopontin of rats after operation at different time in the study group was significantly lower than that in the control group ($P < 0.05$), as shown in Table 2.

Table 2. **Change of Osteopontin Expression of Rats after Operation in Two Groups ($\bar{x}$ ± s, %)**

Group	n	7d after operation	14d after operation	28d after operation
Study group	20	35.15±3.35	30.26±3.21	28.37±2.74
Control group	20	73.81±5.16	68.42±5.48	65.53±4.92
t		13.261	12.844	13.819
P		＜0.001	0.001	＜0.001

2.3 Change of inflammatory cytokines expression level of rats after operation in two groups the inflammatory cytokines expression levels of IL-2, IL-6, IL-17 and TNF-α if ratsP after operation in the study group were significantly lower than those in the control group (< 0.05). The expression level of IL-10 was significantly higher than that in the control group ($P < 0.05$), as shown in Table 3.

Table 3. **Comparison of Inflammatory Cytokines between Two Groups before and after Treatment ($\bar{x}$ ±s)**

Group	n	IL-2（pg/mL）	IL-6（pg/mL）	IL-10（pg/mL）	IL-17（pg/mL）	TNF-α（ng/mL）
Study group	20	183.36±	24.32±3.35	415.83±	389.92±	1.03±0.19
Control group	20	335.51±	30.26±4.11	236.67±	203.35±	2.29±0.33
t		15.814	4.153	20.063	18.841	3.671
P		＜0.001	0.025	＜0.001	＜0.001	0.035

3. Discussion

Renal transplant is the best treatment for end-stage renal disease in clinical practice currently. With the wide application of renal transplant in clinic in recent years, the rejection after renal transplant has attracted a great deal of attention from researchers. How to reduce the acute rejection after renal transplant has become the main research direction in this field [5-6]. Osteopontin is an immunomodulatory factor that has been found to play a proinflammatory role in the body [7-8]. Other studies have found a relatively high level of osteopontin expression in patients with acute renal injury and a significant association with glomerular filtration rate by detecting the level of osteopontin expression in the patient's peripheral blood [9]. Statins, as a commonly used type of lipid-regulating drugs in clinic, can regulate endogenous cholesterol production by inhibiting 3-hydroxy-methyl-glutaryl-CoA reductase [10]. Atorvastatin is one of the most widely used statin drugs in clinical practice. It can reduce low-density cholesterol in peripheral blood and reduce blood lipids [11-12]. It has also been found that atorvastatin not only inhibits cholesterol synthesis, but also plays a positive role in anti-inflammatory and anti-oxidant activities [13]. In recent years, it has been found that atorvastatin plays an active role in the inhibition of inflammatory response in patients after renal transplant, which can reduce the damage caused by renal transplant on body and reduce the cellular rejection in body after renal transplant [14]. However, relatively little research has been done in this field at home.

The clinical indexes of the two groups were compared, and the results showed that there was no significant difference in TC and TG levels between the two groups ($P > 0.05$). However, the quantities of SCr, BUN and 24h urine protein in the two groups were significantly lower than that of the control group ($P < 0.05$), which indicated that atorvastatin could effectively improve the renal function and normalize the renal function of the rats after renal transplant. Wang Shirong et al. [15] found that atorvastatin has effects on immune regulation and inhibition of cell proliferation and inflammatory expression, but also on renal function through regulation of inflammatory level. The expression level of osteopontin of rats after operation in the two groups showed that expression level of osteopontin of rats after operation in the study group was significantly lower than that in the control group ($P < 0.05$), which suggested that atorvastatin could inhibit the expression of osteopontin in rats, and the mechanism may be related to the inhibition of proliferation and migration of smooth muscle cells and the regulation of endothelial nitric oxide system.

IL-6 is a pluripotent inflammatory cytokine that plays a role in promoting cell proliferation and improving the body's immune response [16]. Studies have found that IL-6 is involved in the development and progression of many inflammatory diseases [17]. TNF-α is a proinflammatory cytokine and is mainly produced by mononuclear macrophages and T lymphocytes, it can not only play a role in killing tumor cells, but also can produce more inflammatory cytokines by stimulating T cells, so as to aggravate the inflammatory response of the body [18]. IL-17 is a proinflammatory cytokine, which has been found to play an important role in the occurrence and development of acute rejection in vivo [19]. In this study, the expression levels of various inflammatory cytokines of rats 7d after operation were compared between the two groups. The results showed that the levels of inflammatory cytokines of IL-2, IL-6, IL-17 and TNF-α in the study group were significantly lower than those in the control group ($P < 0.05$), indicating that atorvastatin could inhibit inflammatory reaction in the rats after renal transplant. IL-10 can influence the expression of proinflammatory cytokines by inhibiting the immune response mediated by macrophages and monocytes, thus to reduce the synthesis and release of proinflammatory cytokines, which is the main factor to maintain the balance of immune and inflammatory responses [20]. The expression of IL-10 was significantly higher in the study group than that in the control group ($P < 0.05$), which further indicated that atorvastatin could reduce the expression of inflammatory cytokines after renal transplant.

In conclusion, atorvastatin can effectively reduce the level of inflammatory cytokines and osteopontin expression in rats after renal transplant, which is beneficial to reduce interstitial inflammation and reduce cell rejection after renal transplant.

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