·论　著·

The effect of recipient kidney function by Ber coadministration CsA in rats of allogenic cardiac transplantation

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ABSTRACT: OBJECTIVE To investigate the effect of recipient kidney function by CsA coadministration Ber used to induce immune tolerance in rats of allogenic cardiac transplantation. **METHOD** The authors established the SD to Wister rats heterotopic cardiac transplantation model by Ono's method. Observe the cardiac allograft survival and levels of BUN and Cr in the recipients´plasma. The recipients were classified into 5 groups randomly after heterotopic cardiac transplantation were performed. Group A (Wistar to Wistar)): Received placebo intraperitoneal injected for 21days; Group B(SD to Wistar): Saline intraperitoneal injected for 21 days; Group C(SD to Wistar): CsA 2mg·kg^{-1}·day^{-1} intraperitoneal injected for 21 days; Group D(SD to Wistar): Ber 16 mg·kg^{-1}·day^{-1} gastrointubation for 21 days; Group E(SD to Wistar): Ber 16mg·kg^{-1}·day^{-1} gastrointubation coadministration CsA 2mg·kg^{-1}·day^{-1} ip for 21 days. **RESULTS** The levels of BUN and Cr in recipint plasma is lower evidently compare with the group with CsA ip simply. **CONCLUSION** Ber can reduce the renal toxicity in recipients by CsA which was intraperitoneal injected (ip) over a long period time.

KEY WORDS: Berberine; Cyclosporine; Immune tolerance; Heterotopic cardiac transplantation; Kidney function

小檗碱联用环孢素对受体肾功能的影响

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摘要: 目的 研究小檗碱联用环孢素诱导同种异基因移植免疫耐受后对受体肾功能的影响。**方法** 采用 Ono's 方法建立同种异基因大鼠心脏异位移植免疫耐受模型,将实验动物分为 5 组。对照组(Wistar-Wistar): 每天注射生理盐水共 21d; 安慰剂组(SD-Wistar): 每天注射生理盐水共 21d; CsA 组(SD-Wistar): 每天按 2mg·kg^{-1}剂量注射环孢素 A(Cyclosporine, CsA)共 21d; Ber 组(SD-Wistar)每天按 16mg·kg^{-1}剂量灌服小檗碱(Berberine, Ber)共 21d; CsA + Ber 组(SD-Wistar): 每天按 2mg·kg^{-1}剂量注射 CsA, 16mg·kg^{-1}剂量灌服 Ber 共 21d, 观察受体血清尿素氮(BUN)、肌酐(Cr)指标水平的变化。**结果** 联用 Ber 后,降低了受体长时间使用 CsA 后的尿素氮(BUN)和肌酐(Cr)水平。**结论** 联用 Ber 有助于减轻长期使用 CsA 后对受体肾功能的影响。

关键词: 小檗碱; 环孢素; 心脏异位移植; 免疫耐受; 肾功

Berberine (Ber), which popular name is HuangLian Su. Ber is a kind of quinoline alkaloid distilled from the dry branch of wampee tree or rhizomata of HuangLian, a kind of Chinese herb. It's medication can be used on multi-sorts treatments, antibacteria and resist tumor eg. Lately, some reasearchers not only find Ber have effect on suppression of experimental autoimmune tubulointerstitial nephritis and delay hypersensitivy (DTH), but also suppression effect of Ber on the activation and proliferation of T lymphocytes in vitro. Cyclosporine (CsA) is a kind of ring polypeptide with 11 amino acids, It have been used to treat organ and tissue transplantation, but it will have serious renal toxicity when it has been administered over a long period on the recipi-

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ent. To investigate the effect of recipient kidney function by CsA coadministration Ber used to induce immune tolerance, we performed the model of allogenic cardiac transplantation operation in rats by Ono's method.

1 MATERIALS AND METHODS

1.1 Animals

Adult femal inbreeding rats, SD rats as donor and Wistar rats as recipent, were purchased from First Military Medical University. they were housed in the clean facilities with temperature controlled. all the rats eat foods and drink water themselves freely.

1.2 Reagent And Experimental Equipment

CsA was purchased from Sandimmun company ; berberine was purchased Sigma company; BUN and Cr reagent kits were purchased from Beckman company. Beckman auto biochemistry detect facility; Leica microscope(Gemany); microsurgery appliance; electrocardiograph(ECG); Chloral hydrate; Ringer solution; Heparin.

1.3 Experimental Group

The recipients were classified into 5 groups randomly after heterotopic cardiac transplantation were performed.

Group A (Wistar to Wistar)): Received placebo intraperitoneal injected for 21 days; Group B (SD to Wistar) : Saline intraperitoneal injected for 21 day ; Group C (SD to Wistar): CsA 2mg · kg^{-1} · day^{-1} intraperitoneal injected for 21 days ; Group D(SD to Wistar): Ber 16 mg · kg^{-1} · day^{-1} gastrointubation for 21 days; Group E(SD to Wistar): Ber 6mg · kg^{-1} · day^{-1} gastrointubation coadministration CsA mg · kg^{-1} · day^{-1} ip for 21 days.

1.4 Herterotopic Cardiac Transplantation In Rats

In this procedure, donor rats and recipient rats were anesthetized with Chloral hydrate. The abdominal aorta and inferior vena cava (IVC) were dissected free from the surrounding tisses in the retroperitoneum. A clamp was positioned at the proximal side of bifurcation interrupting the flow in the aorta and IVC, another clamp was positioned just below the renal vessals in a similar manner. The ascending aorta was cut below the brachiocephalic cortery. The main pulmonary artery was cut proximal to its bifurcation. The pulmonary veins were ligated as a group. The donor heart was gently detached from the remaining connective tissue with blunt dissection and was placed in cold ringer solution at 4℃. The donor asending aorta was sutured end-to-side to the recipient abdominal aorta and the donor pulmonary artery was anastomosed to the recipient inferior vena cava. Having finished the suturing, the distal clamp was removed. Normally, the donor heart was filled with blood immediately, become bright red in color and began to contract.

1.5 Observation Of Transplantation Cardiac

Observed the transplantation cardiac operation had been performed by touching the abdominal every day. Examined the electrocardiograms of the transplantation cardiac and own cardiac in these rats by using ECG.

1.6 Observation Of The Levels Of BUN And Cr In The Recipients´Plasma

Examined the levels of BUN and Cr in the recipients´plasma from the caudal vein of rats in 3,20 day after the transplantation operation.

1.7 Statistical Methods

Expressing all the data in the form of mean ± deviation ($x \pm s$), we compared the mean BUN and Cr value of all the groups by One-Way Analysis of Variance, performed the multiple comparisons by using Scheffe's test, and detected the mean BUN and Cr value differences between 3-day and 20-day in each group by independent sample t test.

2 RESULTS

The levels of BUN and Cr in CsA group and CsA coadministration Ber group have difference evidently compare placebo group in 3, 20 day after admistration of medicament ($P < 0.05$ or $P < 0.01$), but the levels of Ber group have not difference evidently compare Placebo group($P > 0.05$); The levels of BUN and Cr in CsA group and CsA coadministration Ber group in 20 day have difference evidently compare Placebo group in 3 day after admistration of medicament ($P < 0.05$); The levels of BUN and Cr in CsA coadministration Ber group have difference evidently compare CsA group in 20 day after admistration of medicament ($P < 0.05$) (Table1 and fig1、2).

表 1 小檗碱联用环孢素 A 对受体肾功能的影响($\bar{x} \pm s$, $n = 5$ 或 6)

Tab 1 The effect of recipient kidney function by joint administration of Ber and CsA($\bar{x} \pm s$, $n = 5$ 或 6)

GROUP	BUN(mmol/L)		Cr(μmol/L)	
	3DAY	20DAY	3DAY	20DAY
Control ($n = 6$)	4.67 ± 0.61	4.85 ± 0.62	48.00 ± 4.34	45.83 ± 3.18
Placebo($n = 5$)	5.92 ± 0.98	5.92 ± 0.92	61.00 ± 8.46	64.80 ± 5.26
CsA ($n = 5$)	6.72 ± 2.20[1)]	11.02 ± 0.96[1)]	120.21 ± 8.17[1)]	159.00 ± 21.06[1)]
Ber ($n = 5$)	5.24 ± 0.96	6.46 ± 0.99	63.00 ± 2.55	66.60 ± 5.98
CsA + Ber($n = 5$)	5.90 ± 0.76[2,3)]	8.50 ± 0.58[2,3)]	92.20 ± 9.01[2,3)]	108.60 ± 8.56[2,3)]

Note: [1)] $P < 0.05$, [2)] $P < 0.01$ vs Placebo group; [3)] $P < 0.05$ vs CsA group ; $P > 0.05$ Ber vs Placebo group

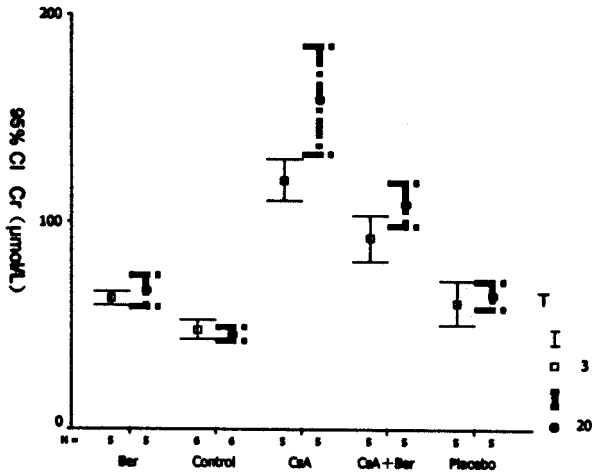

图 1 小檗碱联用环孢素后 3,20d 各组 Cr 值(μmol/L)

Fig 1 Comparison of the value of recipient Cr by joint administration of Ber and CsA between different groups in 3、20 day respectively.

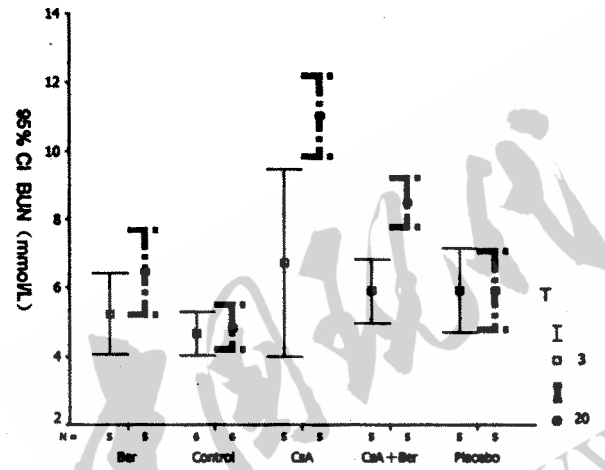

图 2 小檗碱联用环孢素后 3,20d 各组 BUN 值(mmol/L)

Fig 2 Comparison of the value of recipient BUN by joint administration of Ber and CsA between different groups in 3、20 day respectively.

3 Discussion

After organ and tissue transplantation, the immunosuppressant must be administered over a long period on the recipient, and the effective immunosuppressant should not only make implant in the immunologic tolerance but also have the best immunologic inhibition effect and the lowest renal toxicity. We can see from our experimental result that the group treated with CsA have the influence on the levels of Cr and BUN in the recipents´plasma, this is probably because CsA break the balance between the prostacyclin and thromboxane, increase the induction to renal small vessels contract stimulation and the Ca^{2+} transmembrane influx which lead the over contract reaction of small vessls´ smooth muscle, decrease the effective renal plasma flow and GFR(glomerular filtration rate), thus affect the the levels of Cr and BUN in the recipents´plasma, and harm the renal function. However, there is no effct on the levels of Cr and BUN in the recipents´plasma after only treat with Ber over a long period. The experimental result indicate that the levels of Cr and BUN in the recipents´plasma in the group treating both Ber and CsA over a long period is far lower than the group only treated with CsA, this is probably because that Ber can inhibit the Ca^{2+} influx in a does-dependent manner[7] and reduce the Ca^{2+} active transport. Ber also have a antagonistic effect on the pro-secrete ion medium and cAMP, cGMP, Ca^{2+}, and also can change the phosphorylation type of erythrocyte, increase the activity of protein kinase, decrease the Ca^{2+}-dependent K + efflux, thus enhance the transformative ability of erythrocyte, decrease blood viscosity, increase the blood flow and promote blood circulation, above all, improve the microcirculation hemodynamics, eventually, reduce the renal toxicity of CsA on the recipient. In conclusion, our experimental result shows that coadministration of Ber and CsA can reduce obviously the renal toxicity caused by single CsA treatment, which make a brilliant prospect for this application in the clinical research.

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