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• BRIEF REPORTS •

Protective effect of rhIL-1β on pancreatic islets of alloxan-induced diabetic rats

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Abstract

AIM: To observe the protective effect of rhIL-1β on pancreatic islets of alloxan-induced diabetic rats.

METHODS: Protection of rhIL-1β on pancreatic islets of alloxan-induced diabetic rats (n = 5) was demonstrated with methods of immunohistochemistry and stereology. The concentration of serum glucose was measured by GOD method and that of serum insulin by RIA.

RESULTS: The concentration of serum glucose increased but that of insulin decreased after administration of alloxan (150 mg/kg), and the volume density and numerical density of the islets were zero. In rhIL-1β pretreated rats, although the concentration of serum insulin decreased (from 11.9±3.0 mIU/L to 6.1±1.6 mIU/L, P<0.05), that of glucose was at normal level compared with the control group. As compared with alloxan group, the concentration of serum glucose in rhIL-1β pretreated rats decreased (from 19.4±8.9 mmol/L to 12.0±4.0 mmol/L, P<0.05) and the volume density increased(0/L to. 1/L, P<0.05).

CONCLUSION: rhIL-1β pretreatment may have protective effect on the islets of alloxan-induced diabetic rats.

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INTRODUCTION

The cytokine interleukin-1β (IL-1β) can not only promote immunological reaction but also regulate neuro-endocrine system[1]. Previous studies found that IL-1β could stimulate the central adrenalin system, promote production of PG, and downregulate glucose metabolism[2].

Diabetes mellitus implicates many organs and tissues. It has been found that IL-1β decreases serum glucose in experimental animals and may potentially be therapeutic for diabetes mellitus[3,4]. In this experiment, we observed changes in serum glucose and insulin in alloxan induced diabetic rats treated with IL-1β. In addition, we detected the variation of volume density and numerical density of insulin positive pancreatic islets by ABC immunohistochemistry and stereology.

MATERIALS AND METHODS

Animals

Twenty male Sprague-Dawley rats weighing 200 to 300 g were housed in a temperature-controlled room (24±1 ℃) with a 12-h light-dark cycle. The rats were provided with ordinary rat chow and water and divided into 4 groups (n = 5, every group): (1) Control group, each rat was injected with 2 mL saline every other day for 3 times, then injected with 2 mL saline on the 7th d; (2) rhIL-1β group, each rat was injected with 1×10^4 U rhIL-1β in 2 mL saline every other day for 3 times, then injected with 2 mL saline on the 7th d; (3) rhIL-1β pretreated group, each rat was injected with 1×10^4 U rhIL-1β in 2 mL saline every other day for 3 times, then was injected with 150 mg/kg alloxan in 2 mL saline on the 7th d; (4) Alloxan group, each rat was injected with 2 mL saline every other day for 3 times, then injected with 150 mg/kg alloxan in 2 mL saline on the 7th d.

Reagents

Guinea pig anti-rat insulin antibody and SPA-HRP were prepared by Professor Yun-Long Zhu (Department of Physiology) and Professor Cai-Fang Xue (Department of Parasitology) of our university respectively. DAB was purchased from Sigma.

Tissue preparation

Forty-eight hours after last injection of alloxan or saline, rats were anesthetized with ether and sacrificed by cervical dislocation. The blood was collected into heparinised tubes (50 kU/L) and centrifuged (3 000 g, 10 min, at room temperature). Plasma was aspirated and stored at -70 ℃ until assayed as described below. The pancreas was also removed and fixed in Bouin's solution overnight. Each piece was embedded in paraffin and 4-μm sections were prepared.

Immunohistochemistry

Four-micrometer sections from rat pancreas were employed for immunohistochemical analysis. Several dilutions of the antibody were tested to find the optimal staining concentration before the entire series was processed. The staining procedure was carried out as previously reported, but without protease treatment. Briefly, (1) the sections were deparaffinized in xylene, hydrated in ethanol, and blocked with 3 mL/L H_2O_2 in methanol for 30 min to remove endogenous peroxides, then treated with 30 mL/L normal goat serum for 40 min and rinsed in 0.01 mol/L PBS. (2) The sections were incubated at 4 ℃ for 24 h with primary antibody, guinea pig anti-rat insulin antibody (1:1 000 dilution, final concentration 5 mg/L); (3) then with secondary antibody, SPA-HRP (1:200 dilution), at room temperature for 1 h. (4) Peroxidative reaction was performed using DAB as chromogen. The sections were washed three times for 10 min after incubation.

All slides were stained at the same time and under identical conditions. Primary antibodies were replaced by irrelevant antibodies and normal guinea pig serum as specific antibody control. Primary antibody was replaced by PBS as negative control. Primary antibody was omitted as blank control.

Detection of serum glucose and insulin

The concentration of serum glucose was measured by routine GOD method[5] and the concentration of serum insulin was measured by RIA[6,7]. Every sample was measured three times and the results were displayed as mean±SD.

Morphometry

Five specimens from each group were used for morphometric analysis of slides processed for light microscopy. Two sections from each specimen were then selected and five different regions of each section were chosen for the measurement of volume density and number density by double blind method.

Statistical analysis

The results were calculated by the following formula; $Nv = 2/3\times\pi\times NA\times U (AT\times A)$; $Vv = A/AT$. Data were analyzed by χ^2 test. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Insulin expression in rat pancreas

There were more insulin immunoreactive cells in pancreas of control group and rhIL-1β group than in alloxan group. Immunoreactive cells were mainly located in the central region of the pancreas. Insulin immunopositive cells had dark-brown reaction products in the cytoplasm mostly and nuclei were not stained (Figure 1A, B). The number of insulin immunopositive cells in alloxan group decreased remarkably and there were only a few positive cells in each pancreatic islet (Figure 1C). The number of insulin immunopositive cells in rat pancreas of rhIL-1β pretreated group decreased slightly compared with that of control group and rhIL-1β group, whereas, the number of insulin immunopositive cells in rat pancreas of rhIL-1β pretreated group increased remarkably compared with that of alloxan group (Figure 1D).

Destructive effect of alloxan on rat pancreatic B cells

The concentration of serum glucose increased significantly but that of insulin decreased remarkably after administration of alloxan (150 mg/kg) for 48 h compared with those of control rats. At the same time, the volume density and numerical density of the islets were zero (Table 1).

Stimulatory effect of rhIL-1β on insulin secretion

Compared with control group rats, the concentration of serum insulin in rhIL-1β group rats increased significantly whereas that of glucose was at normal level. Immunohistochemistry and stereology data showed that there were no significant differences in the number density and volume density of the pancreatic islets between rhIL-1β group rats and control rats (Table 1).

Protective effect of rhIL-1β on pancreatic islets of alloxan-induced diabetic rats

In rhIL-1β pretreated group, when the rats were injected with

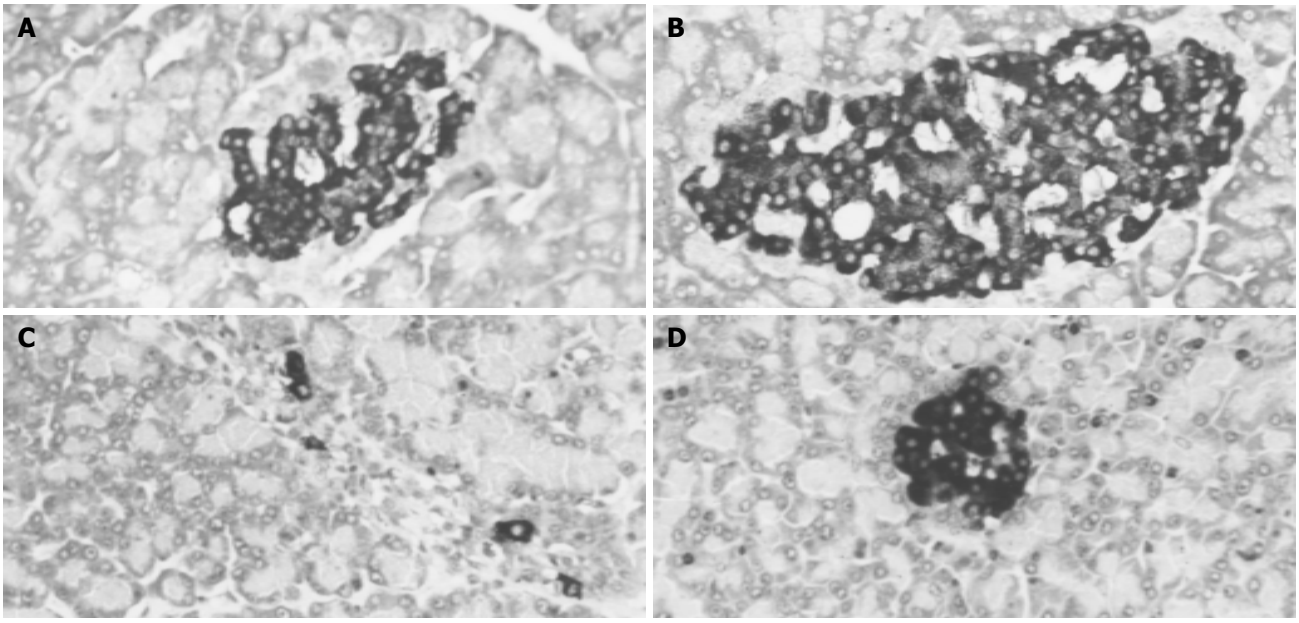

Figure 1 Insulin immunoreactive cells in pancreas. ABC method ×264 A: control group. B: rhIL-1β pretreated rat. C: alloxan-induced diabetic rat. D: rhIL-1β pretreated alloxan-induced diabetic rat.

Table 1 Protective effect of rhIL-1β on pancreatic islets of alloxan-induced diabetic rats

Group	Serum glucose (mmol/L, *n* = 5)	Serum insulin (mIU/L, *n* = 5)	Volume density (/L, *n* = 50)	Number density (/μm², *n* = 50)
Control	8.4±0.3	11.9±3.0	0.6±0.7	5.2±4.1
Alloxan	19.4±8.9[a]	4.7±1.0[a]	0[a]	0[a]
rhIL-1β	8.0±1.3	20.0±6.6[a]	0.6±0.5	5.6±5.3
rhIL-1β pretreated	12.0±4.0	6.1±1.6[a]	0.1±0.1[a]	5.0±5.7

[a]*P*<0.05 *vs* control.

alloxan for 48 h, although the concentration of serum insulin decreased significantly, that of glucose was at normal level compared with control group rats. Immunohistochemistry and stereology data showed that there were no significant differences in the number density of the pancreatic islets between rhIL-1β pretreated rats and control rats, whereas the volume density decreased markedly in rhIL-1β pretreated group (Table 1).

DISCUSSION

Interleukin 1β (IL-1β) is a multi-functional cytokine synthesized mainly by mononuclear/mo cells and is a key factor in the cytokine network[8,9]. IL-1β has many biological functions[9-21]. Previous study showed that IL-1β could decrease the serum glucose level and might be potentially a new drug for diabetes therapy[2-4]. In our experiment, when the rats were injected with alloxan (150 mg/kg) for 48 h, the concentration of serum glucose increased significantly, and that of insulin decreased remarkably. At the same time immunohistochemistry and stereology data showed that the value of number density and volume density of the pancreatic islets were zero. In rhIL-1β pretreated group, when the rats were injected with alloxan for 48 h, although the concentration of serum insulin decreased significantly, that of glucose was at normal level compared with control group rats. Immunohistochemistry and stereology data showed that there were no significant differences in the number density of the pancreatic islets between rhIL-1β pretreated rats and control rats, whereas the volume density decreased remarkably in rhIL-1β pretreated rats. Our results suggest that rhIL-1β has protective effect on pancreatic islets of alloxan-induced diabetic rats and provide the experimental evidence that rhIL-1β may be a new therapeutic drug for diabetes.

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