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• BRIEF REPORT •

Protective effects of cyclosporine A on T-cell dependent ConA-induced liver injury in Kunming mice

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INTRODUCTION

The T-cell dependent specific liver injury in mice induced by concanavalin A (ConA) is a newly established experimental liver injury model, which is considered more eligible for the study of pathophysiology of several human liver diseases, such as viral hepatitis and autoimmune hepatitis[1-9]. T cell activation and several cytokines release had been proven to play a critical role in ConA-induced liver injury[10-19]. Cyclosprine A (CsA), an effective in hibitor of activation of T lymphocyte, has been used widely in clinical treatment, especially in autoimmune diseases and organ transplantation[20-25]. In this study, we investigated the possible effect of CsA on ConA-induced liver injury in Kunming mice.

MATERIALS AND METHODS

Materials

Male Kunming mice were purchased from the animal experimental center of the Second Military Medical University, weight range 17g-21g, free access to water and food prior to the experiment. ConA and CsA were purchased from Dongfeng Ltd Shanghai and Sandoz Ltd respectively.

Methods

All the fifteen Kunming mice were divided into three groups randomly. ConA at a dose of 40mg·kg^{-1} was administered through the tail vein as a solution in pyrogen-free PBS at a volume of 300μL, which was the ConA group. CsA was injected subcutaneously twice at a dose of 130mg·kg^{-1} 15 and 1h before ConA challenge, which was used as the CsA group. PBS only in the corresponding volume served as controls.

Eight hours after ConA administration, the Kunming mice were sacrificed by cervical dislocation. Blood samples were obtained by puncture of heart with 25g·L^{-1} heparin. Liver specimen was fixed immediately in 100 mL·L^{-1} formalin/PBS for histological examination with HE stain. The degree of liver injury was assessed by determination of serum alanine aminotransferase (ALT) activity, serum TNF-α was determined by radioimmunoassay.

Statistics

The results were analyzed by Student's *t* test. The data were expressed as $\bar{x} \pm s$, and $P<0.05$ was considered to be significant.

RESULTS

ConA-induced liver injury in Kunming mice

Eight hours after ConA administration, two out of the five experimental mice were found dead in ConA only group, with elevated serum ALT 22 261 ± 2 523 nkat·L^{-1}. The concentration of serum TNF-α also increased significantly in ConA only group, increased more significantly than that of PBS only group 647±183ng·L^{-1} (Table 1).

Histological examination of liver specimen from ConA-treated mice showed diffuse cloudy swelling of the cytoplasm, spotty and necrotic foci were frequently present, severe agglutination of erythrocytes in the sinusoids of the liver were also observed. Lots of infiltrated lymphocytes in the portal area were the characteristic of this new liver injury model (Figure 1), indicating that lymphocyte may play an important role in the pathogenesis of ConA-induced liver injury, whereas no obvious tissue damage was found in the lung or kidney.

CsA protection

When pretreated with CsA (CsA group), serum ALT activity declined significantly (730 ± 266) nkat·L^{-1}, and the serum TNF-α was below the detective level (Table 1). No obvious hepatic necrosis or lymphocytes infiltration in the portal area was observed under light microscopy in the CsA group (Figure 2).

Table 1 ConA-induced liver injury in Kunming mice (n=5, $\bar{x}$±s)

	ALT- nkat·L^{-1}	TNF-α ng·L^{-1}	Dead
Con A only	22 261±2 523[b]	1230±240[b]	2
PBS only	647±183		0
CsA	730±266		0

[b]$P<0.05$, *vs* PBS only or CsA.

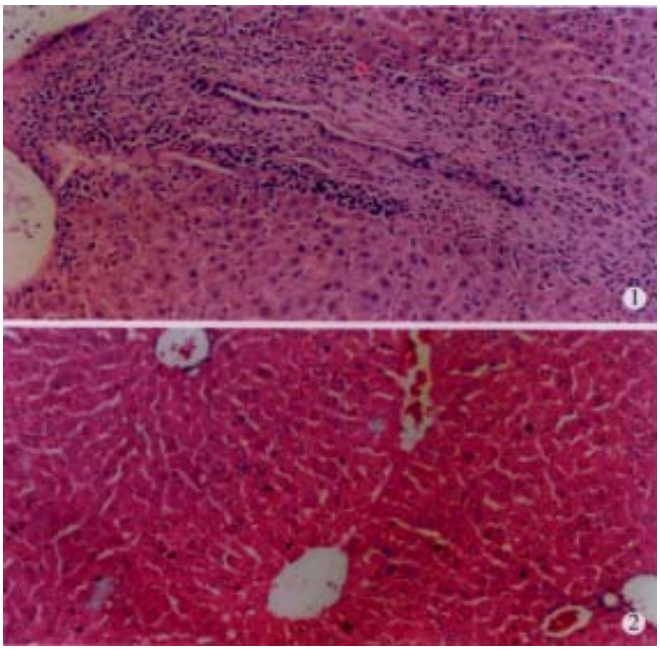

Figure 1 ConA (40 mg·kg^{-1}) induced liver injury. Hepatocyte necrosis and infiltration of lymphocytes in the portal area. HE×66
Figure 2 CsA pretreated, no obvious hepatocyte necrosis or infiltration of lymphocytes was observed. HE×66

DISCUSSION

ConA-induced specific liver injury in mice is a newly developed experimental animal model, which has been closely studied in the pathogenesis of the liver injury in recent years. T lymphocyte activation, cytokines release such as TNF-α, interferon-γ, and interleukines have been discovered to be involved in the pathogenesis of this liver injury model, especially the activation of T lymphocyte and the subsequent release of TNF-α are considered to play a much more important role in this experimental liver injury. The pathological process of ConA-induced liver injury was similar to what seen in several human liver diseases, such as viral hepatitis, and at least three types of autoimmune hepatitis. CsA is a specific inhibitor of T lymphocyte by inhibiting the transcription[21,23], and whether CsA has any protective effect on ConA-induced Kunming mice liver injury by inactivation of T lymphocytes has to be studied In our experiment, the ConA-induced specific liver injury was successfully duplicated in Kunming mice. The results (Table 1) showed that eight hours after ConA administration, the serum ALT activity was significantly increased compared with that in the control group (PBS only group). At the same time, two mice (2/5) died within eight hours. When pretreated with CsA, no death occurred, and the serum ALT level also declined significantly as compared with that of ConA group 730 ± 266 *vs* 22 261± 2 523 nkat·L^{-1}, P<0.01. The experimental results showed that CsA had potential protective effect on the ConA-induced liver injury in Kunming mice.

TNF-α has been proven to be the key cytokine in the destruction of hepatocyte in human liver diseases or liver injury animal model, such as acute and chronic viral hepatitis, especially in fulminant liver failure[26-41]. Anti-TNF antibody resulted in complete protection of ConA-induced liver injury in Balb/C mice[13]. We found that TNF-α increased significantly within eight hours when treated with ConA, but when pretreated with CsA before ConA administration, serum TNF-α became undetectable, hence the reduction of TNF-α might be due to the partial protective effects of CsA. Besides destruction of hepatocytes seen in the liver specimen of ConA group, lots of infiltrating lymphocytes in the portal area were also observed (Figure 1). When pretreated with CsA (CsA group), there was absence of lymphocytes infiltration in the portal area (Figure 2). The histological results gave a direct evidence that the protective effect of CsA in ConA-induced Kunming mice liver injury is through abrogating the activation of the T lymphocytes. The decline of serum TNF-α in CsA group may be a subsequent to T lymphocyte inactivation. $CD4^+$ lymphocyte was identified as the effector cells in the ConA-induced liver injury[3], however, in view of the fact that TNF-α synthesis is substantially higher in macrophages than in T lymphocytes, it seems likely that activated T lymphocytes might stimulate the macrophages to release TNF-α[15], but details of the concrete process worth further studies.

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