Dynamical influence of Cordyceps sinensis on the activity of hepatic insulinase of experimental liver cirrhosis

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BACKGROUND: ***Cordeceps sinensis* (CS) is a herb which can inhibit the liver fibrosis. Hyperinsulinemia is common in liver cirrhosis patients. The activity of insulin degrading enzyme could reflect the metabolism of insulin. This study was to detect the dynamical effects and mechanisms of CS on the activity of hepatic insulinase in CCl_4 induced liver cirrhosis in rats.**

METHODS: **Rats were randomly allocated into three groups: normal group, model group and CS group. The rats in the normal group were sacrificed at the beginning of experiment, and the other two groups were sacrificed randomly at the end of the third, sixth and ninth weeks. Blood and tissue specimens were taken. Biochemical assays were used to determine the changes of alanine transaminase (ALT), albumin levels in serum. And radioimmunological assays were used to determine the changes of hyaluronic acid (HA), insulin levels in serum and the activity of hepatic insulinase.**

RESULTS: **No significant differences were seen in the serum levels of ALT, albumin, HA between the CS group and the model group at the third and sixth weeks ($P>0.05$). The serum levels of ALT, HA in the CS group were lower than those in the model group at the ninth week ($P<0.05$), but the serum level of albumin in the CS group was higher than that in the model group at the ninth week ($P<0.05$). No significant differences were observed in the serum levels of insulin and the activity of hepatic insulinase between the CS and model groups at the third week and the normal group ($P>0.05$). The serum levels of insulin in the CS and model groups at the sixth and ninth weeks were higher than those in the normal group ($P<0.05$). But the activity of hepatic insulinase was lower than that in the normal group ($P<0.05$ or $P<0.01$). No significant differences were found in the serum levels of insulin and the activity of hepatic insulinase between the CS and model groups at the third, sixth and ninth weeks ($P>0.05$).**

CONCLUSIONS: **CS may decrease the damage to hepatocyte by CCl_4, and inhibit hepatic fibrogenesis. Six weeks after CCl_4 administration, the activity of hepatic insulinase began decreasing. CS could not inhibit the decrease of the activity of hepatic insulinase.**

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KEY WORDS: **Cordyceps sinensis; liver cirrhosis; insulinase**

Introduction

Cordyceps sinensis (CS) is a herb which is well tolerated and verified by its inhibition on liver fibrosis.[1-7] Hyperinsulinemia is common in liver cirrhosis patients. The activity of insulin degrading enzyme could reflect the metabolism of insulin. In this study, we established an animal model of chronic hepatitis to liver fibrosis and liver cirrhosis, which was interferred by CS. The effect of CS on hyperinsulinemia was investigated in the experimental hepatic fibrogenesis rats by detecting the changes of the activity of hepatic insulin degrading enzyme (IDE).

Methods

Animals

Wistar rats weighing 200-300 g were obtained from the Experimental Animal Center of Chongqing University of Medical Sciences, Chongqing, China. The rats were housed 3 or 4 per cage and subjected to a 12-day/12-night cycle with unrestrictive access to basic food. All animals were treated according to the National Guidelines for the Care of Animals in China.

Preparation for CS suspension

CS was purchased from Baoding Pharmaceutical Company, Baoding, China. CS and double-distilled water were mixed in proportion of 1:3 and subjected to full vibration.

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Establishment of animal model: carbon tetrachloride (CCl_4)-plus-ethanol induced hepatic fibrosis

Sixty-seven male Wistar rats were randomly assigned into normal control group, model control group, and CS group. At the beginning of the experiment, the rats in the model control group and CS group were subjected to hypodermic injection of CCl_4 suspension (40% in bean oil) at a dose of 0.3 ml/100 g of body weight twice a week. Besides, the rats in these two groups also received 5% ethanol solution as the only fluid to drink. The rats in the normal control group received hypodermic injection of bean oil at the same dose and frequency as did the other two groups while drinking water. Ten days after the CCl_4 administration (for 3 times), the CS group was given CS suspension orally at a dose of 1 ml/100 g body weight daily. In the meantime, three rats in the model control group were randomly sacrificed to evaluate histological change of the liver while the rats in the normal control group were given saline orally at a dose of 1 ml/100 g body weight daily. All the administrations lasted 9 weeks.

Collection of specimens

At the end of the 9th week, the rats in each group were sacrificed under amobarbital sodium anesthesia. Blood was collected through cardio-puncture. The fasting insulin level in serum and the serum HA level were determined by radioimmunological assay.

Liver homogenate preparation

Part of the right lobe of the liver was flushed with iced sodium bicarbonate buffer. According to the weigh/volume ratio (1 g:100 ml), liver tissue and iced sodium bicarbonate buffer were added into a homogenenizer, homogenated at 15 000 rpm for 10 seconds five times. Subsequently, the homogenated fluid was centrifuged 340×g at 4 ℃ for 10 minutes. The sedimentation was thrown and the supernatant was centrifuged 1058×g at 4 ℃ for 20 minutes, and recentrifuged 17 388×g at 4 ℃ for 10 minutes. The supernatant was then used to detect the activity of insulinase.

Detection of the activity of insulinase in liver homogenate supernatant

0.2 ml ^{125}-I insulin and 1.3 ml incubation buffer were mixed with 0.5 ml liver supernatant in a water bath at 37 ℃. 0.2 ml 5% trichloroacetic acid was added to the mixed 0.2 ml incubation buffer for ending the reaction at 0 minute and 25 minutes after the reaction. The fluid was centrifuged 905×g at normal temperature for 30 minutes. 0.3 ml supernatants at 0 minute and 25 minutes were respectively used to detect the reactive counts. The volume of protein in the supernatant was detected by the biuret reaction. The activity of insulinase could be calculated with the following formula: EIDEA=Pn/Sa.E.t.W (Pn: reactive count; Sa: specific activity; E: γ count ratio; t: incubation time; W: volume of protein).

Statistical analysis

The data were presented as means ± SD. The significance of any differences were evaluated using *t* test.

Results

Change of serum levels of ALT, albumin and HA in rats

No significant differences were observed in the serum levels of ALT, albumin, and HA between the CS and model groups at the third and sixth weeks ($P>0.05$). The serum levels of ALT, HA in the CS group were lower than those in the model group at the ninth week ($P<0.05$), but the serum level of albumin in the CS group was higher than that in the model group at the ninth week ($P<0.05$, Table 1).

Change of levels of fasting serum insulin and the activity of hepatic insulinase in rats

No significant difference was found in the serum levels of insulin and the activity of hepatic insulinase between the CS and model groups at the third week and the normal group ($P>0.05$). The serum levels of insulin in the CS and model groups at the sixth and ninth weeks were higher than those in the normal group ($P<0.05$). But the activity of hepatic insulinase was

Table 1. Change of serum levels of ALT, albumin and HA in rats (mean ± SD)

Group	*n*	ALT (U/L)	Albumin (g/L)	HA (μg/mL)
Normal	7	64±12	31±2	155±56
3rd model	5	79±18	26±1	261±107
6th model	5	395±6	28±3	180±100
9th model	6	224±180	24±1	294±104
3rd CS	5	81±41[a]	26±1[a]	226±14[a]
6th CS	5	216±4[a]	27±3[a]	275±200[a]
9th CS	8	98±34[b]	27±1[b]	202±80[b]

Vs the same week's model group. a: $P>0.05$; b: $P<0.05$.

Table 2. Change of the levels of fasting serum insulin and the activity of hepatic insulinase in rats (mean ± SD)

Group	*n*	Insulin (MIU/L)	EIDEA (U/mg)
Normal	7	24±2	0.57±0.09
3rd model	5	52±19[a]	0.40±0.12[a]
6th model	5	53±3[b]	0.26±0.19[b]
9th model	6	50±31[b]	0.31±0.12[c]
3rd CS	5	51±46[d]	0.51±0.08[ad]
6th CS	5	34±14[d]	0.31±0.20[bd]
9th CS	8	41±31[d]	0.26±0.09[cd]

Vs normal control group. a: $P>0.05$; b: $P<0.05$; c: $P<0.01$. Vs the same week's model group. d: $P>0.05$.

lower than that in the normal group ($P < 0.05$, or $P < 0.01$, Table 2).

Discussion

CS could regulate immune function of humans and could prevent the development of immune-injured cirrhosis. Research has shown that it could alleviate inflammatory cell infiltration and hepatocyte degeneration and necrosis, and inhibit deposition of collagen I and III.[1-7] Moreover, it could make the collagen that have already formed resolved and reabsorbed. It is promising in therapy of virus-induced hepatitis and cirrhosis.[8-12] In this study, the serum levels of ALT and HA in the CS group were markedly lower than those in the model control group, and the albumin level of the former group was higher than that of the latter, indicating that CS could alleviate the destruction of hepatocyte, inhibit fibrogenesis, and promote hepatocyte regeneration.

The activity of insulinase could reflect the metabolic rate of insulin. There is tremendeous amount of insulinase in hepatocyte, 50%-80% of which is eliminated by the liver in normal condition.[13] Because the decrease of hepatocytes when cirrohsis occurs in the liver, the absorption and deactivation of insulinase by the liver will decline. In this study, we detected the activity of insulinase in liver homogenate of experimental hepatofibrosis in rats in an attempt to determine the degeneration activity of the liver toward insulin. The results showed that 3 weeks after the experiment there was no significant difference among the CS, model control, and normal control groups since the model had not completely established. But after 6 and 9 weeks, the activity of insulinase in the liver tissue of both CS and model control groups was lower than that of the normal control group, with a serum insulin level of the former higher than that of the latter. As a result, the activity of insulinase declined in cirrhosis and the deactivation of insulin reduced, thus hyperinsulinism occurred. This mechanism is different from that of hyperinsulinism in type II diabetes. In type II diabetes, insulin-resistance is probably related to overly fast degeneration of insulin.[14-17] Overly fast degeneration of insulin may lead to deficiency of insulin that is internalized to cell, thus stimulating pancreatic islet B cell to excrete more insulin for compensation. In this study, no significant difference was seen in the activity of insulinase between the CS and model control groups, indicating that CS could not change the decline of the activity of insulinase, hence could not ameliorate hyperinsulinism.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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