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Acute effects of huperzine A and tacrine on rat liver

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KEY WORDS cholinesterase inhibitors; huperzine A; tacrine; liver; acute toxicity test

ABSTRACT

AIM: To observe the acute effects of huperzine A and tacrine on rat liver. **METHODS:** Changes of liver coefficient, serum biochemistry, and histopathology were detected after single dose. *In vitro* cytotoxicity was assessed by determining extracellular and intracellular amount of lactate dehydrogenase in cultured hepatocytes. **RESULTS:** Both huperzine A and tacrine raised liver coefficient and increased serum aspartate aminotransferase and alanine aminotransferase. Tacrine induced liver histopathologic changes. The acute effects of huperzine A on liver could be redressed by atropine, while effects of tacrine on liver could not. Concentration-dependent *in vitro* cytotoxicity occurred with tacrine, but not with huperzine A. **CONCLUSION:** The acute effects of huperzine A on rat liver are not related to hepatotoxicity. The acute effects of tacrine on rat liver are related to hepatotoxicity.

INTRODUCTION

Physiological homeostasis of organism may be disturbed by acute exposure to xenobiotics, especially chemical agents with high activity. The occurrence of acute exposure to xenobiotics usually results from self-medication, suicide or homicide attempts, drug addiction, or exposure to toxins. Now, the incidence of acute exposure to xenobiotics has a significant trend to increase and get great hazard for the people.

Huperzine A (HupA) and tacrine (TAH) are acetylcholinesterase (AChE) inhibitors approved for the treatment of Alzheimer's disease (AD)^[1]. The 50 % inhibitory concentration (IC₅₀) of HupA for AChE is 82 nmol/L, and IC₅₀ of TAH for AChE is 93 nmol/L. The acute 50 % lethal dose (LD₅₀) in mice (*po*) is 17.31

mmol/kg for HupA and 199.83 mmol/kg for TAH^[2]. A series of studies have been conducted to evaluate the toxicity of HupA and TAH. However, there is no report documenting the acute effects of HupA and TAH on liver, the most important organ for detoxification. In the present study, attempts have been made to observe acute effects of HupA and TAH on rat liver.

MATERIALS AND METHODS

Chemicals and reagents HupA was provided by Department of Phytochemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences. TAH was purchased from Sigma Chemical Co, USA. The reagents used for serum biochemistry detection were purchased from Roche and Sigma Co. All other reagents were of analytical grade and purchased from commercial sources.

Animals and treatment Forty-eight Sprague-Dawley rats (about 2-month old) were supplied by Shanghai Experimental Animal Center, Chinese Acad-

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emy of Sciences (Grade II, Certificate No 005). The rats were randomly divided into eight groups (A to H) and each group contained 3 male and 3 female. Group A was treated with distilled water, group B and C with HupA 0.1 and 2 mg/kg, group D with HupA 2 mg/kg and atropine 0.05 mg/kg, group E and F with TAH 2 and 40 mg/kg, group G with TAH 40 mg/kg and atropine 0.05 mg/kg, group H with atropine 0.05 mg/kg, respectively. HupA and TAH were given orally once. Atropine was given subcutaneously five times (once every hour) after administration of HupA and TAH. Animal facilities were maintained under 12-h light/dark cycle (light on at 7:30 AM), (22±2) °C and 50 %-70 % relative humidity. Food and water are allowed *ad libitum*.

Serum biochemistry Blood samples were taken at 24 h after dosing. Serum was separated for biochemical assay; parameters included total bilirubin (BIL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), and albumin (ALB). The assay was performed with a biochemical autoanalyzer (Roche Cobas, Switzerland).

Histopathology Each liver was weighed and liver coefficient (liver to body ratio) was calculated. Liver tissues were fixed in 10 % buffered formaldehyde, embedded in paraffin, sectioned at approximately 6 microns, and stained with hematoxylin and eosin (HE) for histologic examination.

Hepatocyte culture Techniques for isolating single hepatocytes are based on the description^[3]. Briefly, the technique used a two-step *in situ* perfusion of the liver with collagenase to digest the connective tissue and differential centrifugation to isolate parenchymal hepatocytes. Monolayer cultures of hepato-

cytes were established in Eagle's minimal essential medium with nonessential amino acids (MEM) supplemented with insulin (0.1 μmol/L), dexamethasone (0.1 μmol/L), gentamicin (50 μg/L) and 15 % FBS on collagen-coated 24-well plates at a density of 2×10⁵ cells/well. When the cells were plated, viability detected by trypan blue exclusion was greater than 90 % for all experiments. Cells were allowed 2 h for attachment before treatment.

Cytotoxicity Cells were exposed to HupA and TAH (0 to 1 mmol/L) for 4 h in serum-free media and then the toxicity was determined by measuring extra- and intracellular levels of lactate dehydrogenase (LDH). Extracellular LDH was assayed in the media. Intracellular LDH was assayed following addition of media containing 0.5 % Triton-X100 to lyse the cells. The ratio of intracellular LDH to total LDH was calculated. LDH was determined using a Roche biochemical autoanalyzer. Experiments were performed in triplicate with each compound.

Statistics Data were expressed as mean±SD and analyzed using analysis of variance (ANOVA) followed by Newman-Keuls test. *P*<0.05 was considered statistically significant.

RESULTS

Liver coefficient Changes of liver coefficient are shown in Tab 1. For female rats, significant increase of liver coefficient was noted in high dose groups of HupA and TAH. When combined with atropine, the liver coefficient in HupA group was redressed, but it was still higher in TAH-treated rats. For male rats, HupA-induced increase of liver coefficient was not found, while in TAH group the liver coefficient was

Tab 1. Changes of liver coefficient, ALT and AST of rats treated with huperzine A (HupA) or tacrine (TAH) were determined at 24 h after dosing. HupA1: 0.1 mg/kg; HupA2: 2 mg/kg; TAH1: 2 mg/kg; TAH2: 40 mg/kg; Atropine: 0.05 mg/kg. n=6. Mean±SD. ^b*P*<0.05, ^c*P*<0.01 vs control.

Groups	Coefficient (Female)	Coefficient (Male)	ALT/U·L ⁻¹	AST/U·L ⁻¹
Control	3.88±0.08	4.51±0.09	34±4	55±7
HupA1	3.86±0.05	4.59±0.06	35±4	61±6
HupA2	4.07±0.06 ^b	4.55±0.07	93±3 ^c	125±54 ^b
HupA2+Atropine	3.89±0.10	4.46±0.12	39±6	61±9
TAH1	3.90±0.11	4.59±0.09	33±6	62±8
TAH2	4.25±0.08 ^b	4.80±0.16 ^b	118±63 ^b	108±67 ^b
TAH2+Atropine	4.17±0.07 ^b	4.71±0.05 ^b	90±50 ^b	122±35 ^c
Atropine	3.90±0.06	4.54±0.09	34±5	62±12

significantly increased no matter it was combined with atropine or not.

Serum biochemistry Twenty hours after dosing, significant increase of serum AST and ALT occurred both in HupA- and TAH-treated rats. Changes of AST and ALT were shown in Tab 1. About 3-fold increase of ALT and 2.5-fold increase of AST were noted in rats treated with 2 mg/kg HupA, but they were redressed to normal range when combined with atropine. As for TAH (40 mg/kg), about 4-fold increase of ALT and 2-fold increase of AST were observed, and they could not be redressed by atropine. No significant change of serum BIL, ALP, TP and ALB was noted after 24 h treated with HupA or TAH.

Histopathology HupA-related histological changes of liver were not found. In TAH-treated rats, the liver tissues showed the features of hepatocyte swelling and Disse's space disappearance in midzonal and pericentral regions of the liver lobule. Steatosis and necrosis were not observed. Histology of liver from rats treated with HupA and TAH was shown in Fig 1.

Cytotoxicity Cytotoxic effects obtained after 4-h exposure to HupA and TAH were illustrated in Fig 2. Concentration-dependent decrease of intracellular LDH to total LDH occurred with TAH. At concentration of 200 $\mu\text{mol/L}$ TAH, the percent of intracellular LDH to total LDH was less than 50%. At concentration of 1 mmol/L TAH, the percent of intracellular LDH to total LDH was 9.6%. Concentration-dependent decrease of intracellular to total LDH was not observed in HupA-treated hepatocytes. At concentrations from 0 to 1 mmol/L of HupA, the percent of intracellular LDH to total LDH was no less than 70%.

DISCUSSION

In the present study, we examined the changes of liver coefficient and serum biochemistry after single dose of HupA and TAH in rats. Both HupA and TAH raised liver coefficient and increased serum ALT and AST indicating that liver was affected during the acute exposure to HupA and TAH. HupA and TAH are AChE inhibitors, which may increase ACh at the celiac ganglion and thus increase sympathetic activity via the hepatic nerve^[4]. It is well known that the sympathetic nervous system plays an important role of the micro-circulation in the liver^[5]. ACh is released in the celiac ganglion and causes an action potential that propagates through the hepatic nerve and induces the decreasing sinusoidal vascular space and tissue perfusion^[6]. So it

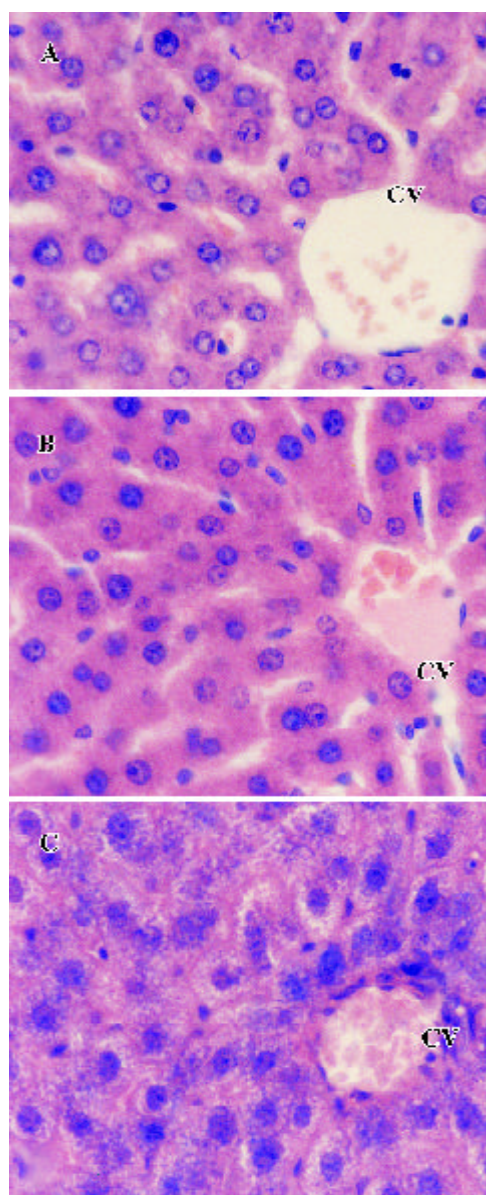


Fig 1. Histology of liver from rats after 24-h treatment with HupA and TAH. HE staining, $\times 132$. CV: central vein. (A) Control. (B) HupA 2 mg/kg. (C) TAH 40 mg/kg.

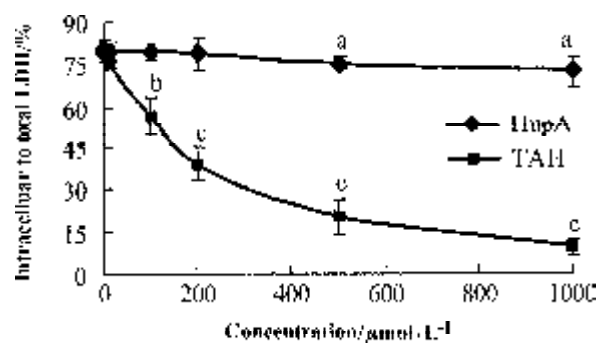


Fig 2. Percent of intracellular to total LDH in hepatocytes treated with HupA and TAH for 4 h. $n=3$. Means \pm SD. ^a $P>0.05$, ^b $P<0.05$, ^c $P<0.01$ vs control.

is possible for overdose of HupA and TAH to get adverse effect on liver because of AChE inhibition and ACh increase in celiac ganglion.

Atropine, an anticholinergic reagent, inhibits the actions of ACh and cholinomimetic drugs with the mechanism of selectively blocking muscarine cholinoreceptor. Nicotine cholinoreceptor can also be blocked when dose of atropine was high enough. In clinical, high dose of atropine was used to relieve vasospasm, relax peripheral vascular, and ameliorate microcirculation. In the present study, HupA-induced rise of liver coefficient and increase of AST and ALT in serum were redressed when combined with atropine. While for TAH, the significant increase of liver coefficient and serum AST and ALT could not be redressed by atropine. It is thus suspected that the acute effects of HupA on liver are related to AChE inhibition. As for TAH, the acute effects on liver are related not only to AChE inhibition, but also to cytotoxicity.

Histopathologic and cytotoxic studies confirmed the suspect. Histologic changes showed the occurrence of TAH-induced hepatotoxicity, while HupA-related histologic changes of liver were not found. Cultured hepatocytes were used in this study, which eliminated extrahepatic effects that could be as potential contributors to hepatic effect in whole animals^[7]. Concentration-dependent decrease of intracellular LDH to total

LDH occurred with TAH, but not with HupA, which indicated that HupA was not hepatotoxic *in vitro*. In conclusion, the acute effects of HupA on rat liver are not related to hepatotoxicity. The acute effects of TAH on rat liver are related to hepatotoxicity. It is recommended to protect liver during acute exposure to TAH.

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