

Effect of matrine on Kupffer cell activation in cold ischemia reperfusion injury of rat liver

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Abstract

AIM: To study the effect of matrine on activation of Kupffer cell during cold ischemia and reperfusion injury in rat orthotopic liver transplantation (OLT).

METHODS: 168 syngeneic SD rats were randomly divided into four groups: untreated group, small-dose treated group, large-dose treated group and sham operation group. After 5 hours of preservation in Ringer's (LR) solution, orthotopic implantation of the donor liver was performed. At 1 h, 2 h, 4 h and 24 h after reperfusion of the portal vein, 6 rats were killed in each group to collect the serum and the liver for assay and pathology.

RESULTS: Matrine markedly inhibited the activation of Kupffer cells and their release of tumor necrosis factor (TNF). TNF cytotoxicity level at 2 h decreased significantly by matrine treatment (7.94 ± 0.42 , 2.39 ± 0.19 and 2.01 ± 0.13 U/ml, respectively; $P < 0.01$), so did the other three time points. The level of hyaluronic acid (HA) and alanine transaminase (ALT) decreased significantly in both treated groups, and matrine treatment markedly ameliorated focal necrosis of hepatocytes, inflammatory cells aggregating, rounding and detachment of sinusoidal endothelial cells (SEC). And no significant difference was observed between the treated groups.

CONCLUSION: Matrine can inhibit the activation of Kupffer cell and prevent the donor liver from cold preservation and reperfusion injury in rat orthotopic liver transplantation.

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INTRODUCTION

Preservation injury continues to be a major clinical problem in orthotopic liver transplantation (OLT) with a 10% incidence of primary nonfunction^[1,2]. Additionally, there is clinical

evidence that severe preservation injury is associated with an increase in liver graft rejection^[3]. Therefore, it is clinically and pathophysiologically important to elucidate the mechanism and prevention of cellular injury during hepatic ischemia and the subsequent reperfusion. Kupffer cells become activated during cold preservation and subsequent reperfusion, as has been demonstrated by enhanced endocytosis of carbon particles^[4], an increased release of TNF- α ^[5-8] and ultrastructural changes^[9]. When activated, they generate a plethora of inflammatory cytokines and oxygen-derived free radicals, which play a particularly important part in the pathogenesis of hepatic ischemia and reperfusion injury^[10].

Matrine (matridin-15-one) is a typical lupine alkaloid along with lupinine, sparteine and cytisine, and has an absolute structure of 5S, 6S, 7R, 11R^[11] (Figure 1). This alkaloid, isolated from kinds of *Sophora* plants in *Leguminosae*, shows pharmacological effects as anti-inflammation^[12], immunity-inhibition^[13], B and C hepatitis virus inhibition^[14], anti-tumor^[15] and anti-arrhythmia activity^[16], and has been used in treatment of chronic viral hepatitis. Pharmacological studies revealed no obvious side-effect of matrine^[17]. The present study was designed to evaluate the effects of matrine on Kupffer cell activation caused by preservation and reperfusion with an orthotopic liver transplantation model.

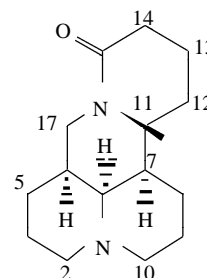


Figure 1 Chemical structure of (+)-Matrine

MATERIALS AND METHODS

Reagents

Matrine, a parenteral solution (50 mg/5 ml), was purchased from Ming Xing Pharmaceutical Factory, Guangzhou, China. HA RIA kit was from Shanghai Ocean Research Biomedical Technology Center, China. Recombinant human TNF (1000 units/ml) was from Genzyme Co. in Boston, America. Rat TNF- α ELISA kit was purchased from Bender MedSystems Co. in Vienna, Austria. Endotoxin quantitation kit was from Shanghai Medical Test Center, China.

Animals

168 male inbred SD rats weighing 200 to 220 g were purchased from the Animal Center of Jin Ling Hospital (Nanjing, China). All rats were provided with standard laboratory chow and water and housed in accordance with institutional animal care

policies. Prior to being used in the study, the rats were fasted for 12 hours and were allowed free access to water.

Experimental design

Rat orthotopic liver transplantation was performed using the technique of Kamada and Calne^[18] under ether anesthesia, and the hepatic artery was not reconstructed. Ringer's (LR) solution was used for perfusion. The liver graft was preserved for 5 h in Ringer's solution at 4 °C, then transplanted orthotopically. The explantation of the recipient liver required <10 minutes and the rewarming time of the graft (i.e. clamping of the portal vein and the subhepatic vena cava during implantation) did not exceed 18 minutes.

The animals were randomly assigned into four experimental groups as follows: (1) a control group in which the recipients were injected ip Normal Saline (N.S, 1ml) before laparotomy. (2) a small-dose treated group (ST) in which the recipients were injected ip matrine (40 mg·Kg⁻¹) before surgery. (3) a large-dose treated group (LT), in which the transplantation was performed following the injection of matrine (80 mg·Kg⁻¹) as above. (4) a sham operation group (Normal), in which the liver was mobilized as the others without hepatectomy to exclude the influence of surgery.

At 1 h, 2 h, 4 h and 24 h after reperfusion of the portal vein, 6 rats were killed in each group to collect the blood sample via the infrahepatic vena cava and the median lobe of liver for assay. The serum was separated and stored at -70 °C until analysis. Washed with cold Saline solution, the liver samples were stored immediately in liquid nitrogen until analysis.

Hepatic enzyme assays

Alanine aminotransferase (ALT) activity of the serum samples collected at 4 h and 24 h after reperfusion was determined by automatic biochemistry analyzer (HITACHI 7 600).

Assessment of HA plasma level

HA plasma levels of those samples collected at 1 h, 2 h, and 4 h after reperfusion were determined in duplicate using a HA RIA kit according to the manufacturer's protocols.

Plasma endotoxin level

Endotoxin plasma levels of the samples collected at 1h and 2h were determined in duplicate using a limulus amoebocyte lysate assay kit according to the manufacturer's instruction.

Plasma TNF cytotoxicity determination

Plasma TNF cytotoxicity of all samples were determined with TNF cytotoxicity L929 assays as described^[19,20]. Each sample was assayed in duplicate wells.

Hepatic TNF- α assay

Coomassie Light Blue assay was used for protein quantitation of all hepatic samples. Hepatic levels of TNF- α were assayed with a rat TNF- α ELISA kit from the Bender MedSystems corporation. This was an enzyme-linked immunosorbent assay for the quantitation of natural or recombinant rat TNF- α levels. The assay was carried out according to the manufacturer's instructions.

Light microscopy

Six hepatic specimens were collected at 4 hr after reperfusion of portal vein in each group. The liver specimens for light microscopy were fixed with 10 % formalin and then embedded in paraffin. The sections were stained with hematoxylin and eosin for histologic examination.

Transmission electron microscopy

For transmission electron microscopy, liver fragments of approximately 1 mm³ were fixed in 2 % glutaraldehyde containing 0.1 M phosphate buffer for 3 hours. After washing in phosphate buffer, specimens were postfixed with osmium tetroxide, dehydrated in graded alcohols, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under an electron microscope (JEM-1200EX).

Statistical analysis

The results are expressed as mean \pm SD. Data were analyzed using Statistical Analysis System (SAS). One-way analysis of variance (ANOVA) was used for multiple comparisons with Student-Newman-Keuls (snk) test. A *P* value of < 0.05 was considered statistically significant.

RESULTS

Effect of matrine on ALT serum levels

ALT serum levels in both treated groups decreased significantly compared with control values at different time points after reperfusion, and two folds-dose matrine provided the large-dose treated group with a significant decrease in ALT. (Figure 2).

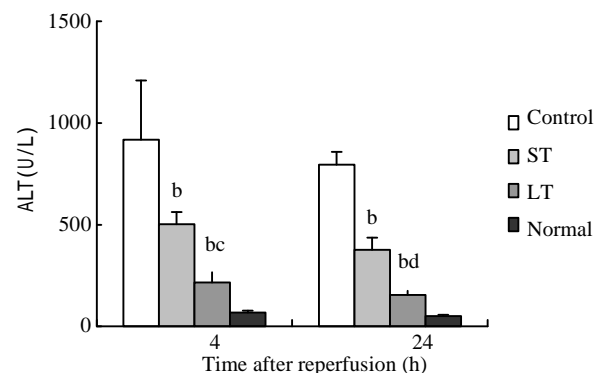


Figure 2 Effect of matrine on ALT plasma level after 5-hour cold preservation and reperfusion in rat orthotopic liver transplantation. (*n*=6 at each time); b: very significantly different from control (*P*<0.01); c: significantly different from the small-dose treated group (*P*<0.05); d: very significantly different from the small-dose treated group (*P*<0.01).

Effect of matrine on HA plasma levels

Compared with the sham operation group, a significant elevation of serum HA was observed in the other three groups at different time points, with the peak level at 2 h after surgery. HA levels at different time points were ameliorated markedly by matrine treatment, and two folds-dose matrine provided the large-dose treated group with a significant elevation in HA. (Figure 3).

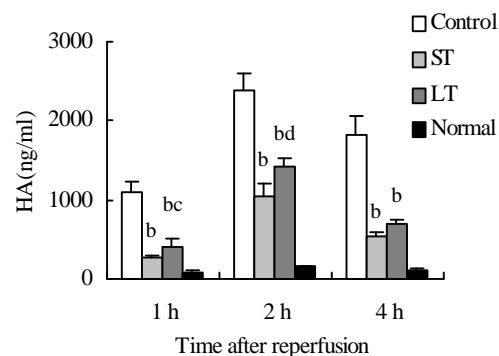


Figure 3 Effect of matrine on plasma HA level after 5-hour cold preservation and reperfusion in rat orthotopic liver

transplantation. ($n=6$ at each time); b: very significantly different from control ($P<0.01$); d: very significantly different from the small-dose treated group ($P<0.01$).

Effect of matrine on plasma endotoxin levels

In both treated groups, plasma endotoxin levels increased significantly compared with control values at 1 h and 2 h after reperfusion. No significant difference was noted between the two treated groups (Figure 4).

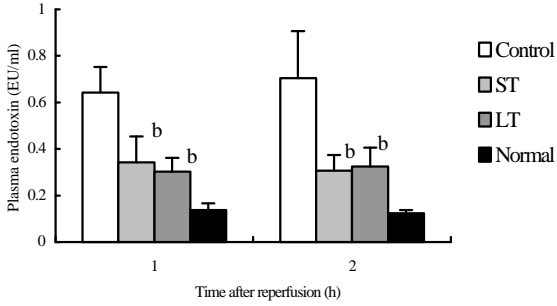


Figure 4 Effect of matrine on endotoxin plasma level after 5-hour cold preservation and reperfusion in rat orthotopic liver transplantation. ($n=6$ at each time) b: very significantly different from control ($P<0.01$)

Effect of matrine on plasma TNF cytotoxicity

Almost no plasma TNF cytotoxicity was detected in the sham operation group. In the other three groups, the maximum TNF values were obtained 2 h after surgery as shown in Figure 5. TNF cytotoxicity level at 2 h decreased significantly by matrine treatment (7.94 ± 0.42 , 2.39 ± 0.19 and 2.01 ± 0.13 U/ml, respectively; $P<0.01$), so did the other three time points. No significant difference was noted between the two treated groups.

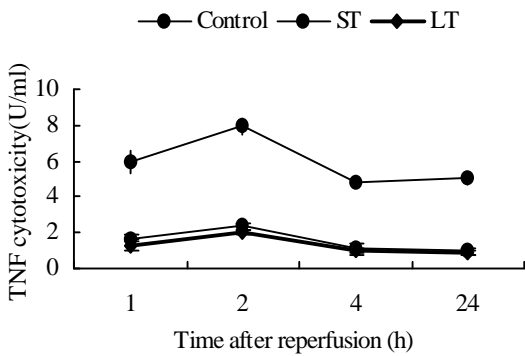


Figure 5 Effect of matrine on TNF cytotoxicity after 5-hour cold preservation and reperfusion in rat orthotopic liver transplantation. ($n=6$ at each time).

Effect of matrine on hepatic TNF- α levels

The hepatic TNF- α level of sham operation group was low (56.8 ± 13.2 pg/mgprot). In the other three groups, the maximum TNF- α levels were obtained at 2 h after surgery as shown in Figure 6, and TNF- α levels at different time points post reperfusion were significantly decreased by matrine treatment, but no significant difference was noted between the two treated groups.

Light microscopy

In sham operation group, the histological findings indicated that the degree of liver cell injury, Kupffer cell hyperplasia,

and inflammatory cell infiltration in portal areas and sinusoids were mild. As shown in Figure 7, histological examination revealed some focal necrosis of hepatocytes, extensive congestion, and inflammatory cells aggregating in hepatic sinusoid lumen in the control group, and the obvious Kupffer cell hyperplasia and rounding and detachment of SEC were observed, too. These were ameliorated markedly in both treated groups, and no significant difference was observed between the two treated groups.

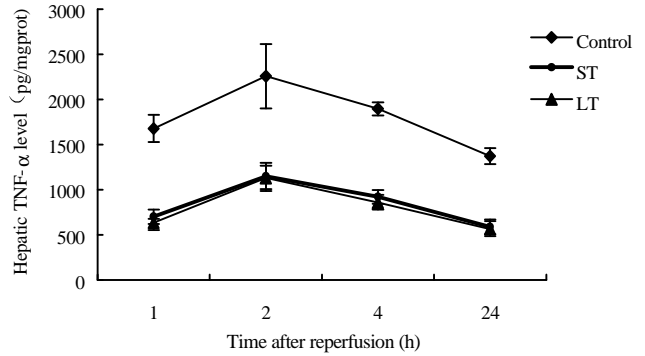


Figure 6 Effect of matrine on hepatic TNF- α level after 5-hour cold preservation and reperfusion in rat orthotopic liver transplantation. ($n=6$ at each time)

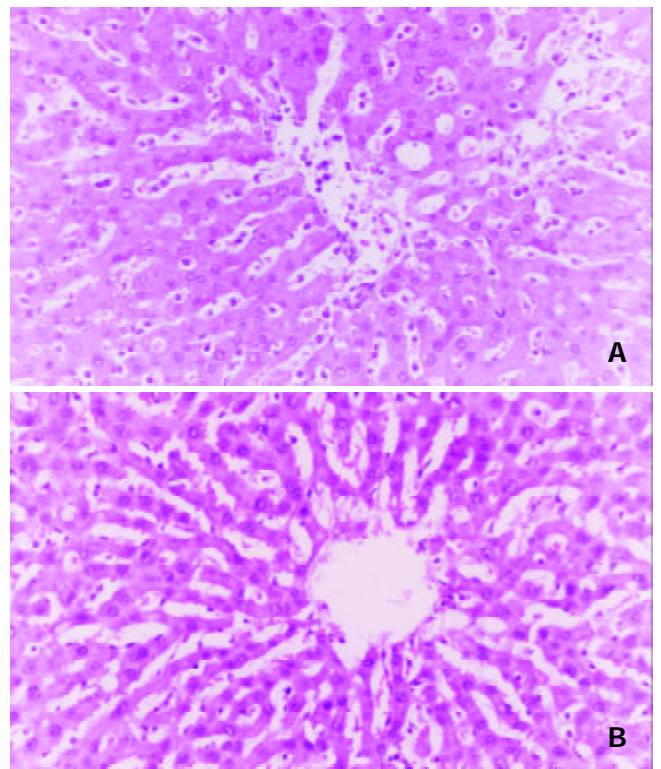


Figure 7 Histological appearance of the rat liver at 4 hours after reperfusion A: control group; B: a small-dose treated group. (HE staining paraffin-embedded 5 μ m thick-sections. $\times 200$)

Transmission electron microscopy

Samples taken from the control group showed obvious activation of Kupffer cells, including increase of cytoplasmic processes, irregular nucleus and many phagolysosomes, hyaline vacuoles, and granules in cytoplasm. (Figure 8) The treated groups showed a nearly normal ultrastructure of Kupffer cells in the samples examined compared with the control. This was reflected in

rounding of Kupffer cell, regular nucleus, and a reduction in the amount of cytoplasmic processes. No significant differences were noted between the two treated groups.

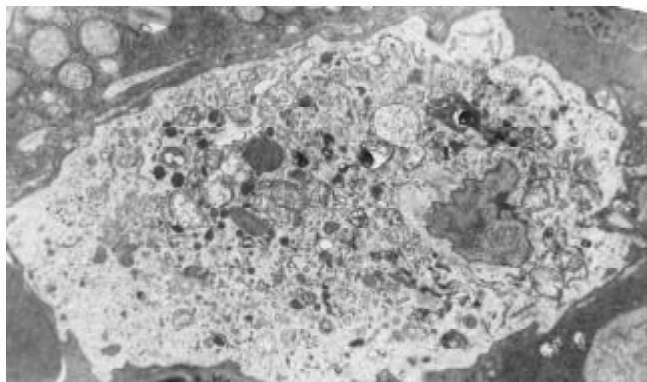


Figure 8 Electron microscopic pictures of rat liver in control group 4 hours after reperfusion. (Original magnifications:×6 000)

DISCUSSION

With donor shortage becoming worse, preservation injury continues to be a major clinical problem with a 10 % incidence of primary nonfunction^[1,2]. Kupffer cells play an important part in mediating ischemia and reperfusion injury after hepatic transplantation. It is likely that they are primed during cold preservation and then activated at the time of reperfusion. The addition of an inhibitor of Kupffer cell activation to liver preservation solutions may further increase the duration of cold storage times and also improve the outcome of grafts^[21-23].

In the present investigation, rat livers were kept for 5 hours in cold Ringer's solution (4 °C), exceeding the safe limit of 4 hours^[24]. Under these preservation and transplantation conditions (i.e., a portal vein clamping time of less than 20 minutes), a postoperative survival rate of about 40 % was obtained. Thus, 5 hours in cold Ringer's solution, although a severely compromising condition, should allow an adequate assessment of the mechanisms of cold ischemia and reperfusion injury that lead to primary nonfunction. Usually, the rats without matrine treatment recovered well from anesthesia; however, their clinical status began to deteriorate within 4 to 6 hours, and nonsurvivors died within 24 hours, mostly between 10 to 20 hours.

After reperfusion, serum endotoxins immediately bind to lipopolysaccharides binding protein (LPSBP) and are usually trapped by Kupffer cells^[25]. Kupffer cells exhibited progressive rounding, ruffling of the cell surface, polarization, appearance of wormlike densities, vacuolization, and degranulation. After activation, Kupffer cells produce TNF- α and induce neutrophil chemotaxis and activation^[5,26]. TNF- α has potent proinflammatory actions, which can induce IL-8 synthesis and up-regulate the expression of adhesion molecules giving rise to increased leukocyte-sinusoidal endothelial cell interactions^[23,27], which result in further cytokine production. In our study, serum endotoxin levels at different time points post transplantation were ameliorated markedly by matrine treatment, and the results have confirmed matrine markedly inhibited the activation of Kupffer cells and their release of TNF, inflammatory cell infiltration and injury of sinusoidal endothelial cell.

The serum ALT and HA levels were determined as functional indices of hepatocyte and sinusoidal endothelial cell damage, respectively. ALT and HA levels at different time

points post-transplantation improved markedly by matrine treatment, and their pathological changes of liver graft ameliorated, too. Two folds-dose matrine treatment can't give rats the better therapeutic effect, and no obvious side effect was noted in our study.

In conclusion, the results of this study demonstrated the inhibition effect of matrine on Kupffer cell activation and its protective effect against the cold ischemia reperfusion injury of the graft in liver transplantation.

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