Matrine Protects Sinusoidal Endothelial Cells From Cold Ischemia and Reperfusion Injury in Rat Orthotopic Liver Transplantation

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Abstract. The effect of matrine on cold ischemia and reperfusion injury of sinusoidal endothelial cells (SEC) was investigated in rats using an orthotopic liver transplantation (OLT) model. Syngeneic Sprague-Dawley (SD) rats were randomly assigned to 4 groups of 32 rats: untreated group (controls), low-dose treated group, high-dose treated group, and sham operation group (normals). After 5 hr of preservation in Ringer's solution, orthotopic implantation of the donor liver was performed. At 1, 2, 4, and 24 hr after reperfusion, 6 rats from each group were killed to collect blood and to excise the median hepatic lobe; the other 8 rats were observed to assess the 1-wk survival rate post-transplantation. All transplant recipients in the untreated group (controls) died within 48 hr, mostly between 10 to 20 hr. Matrine treatment increased the 1-wk survival rate to 75% in both treated groups. Plasma levels of hyaluronic acid (HA) at 1, 2, and 4 hr post-implantation were decreased significantly by matrine treatment. The immunohistochemical expression of intercellular adhesion molecule-1 (ICAM-1) in rat liver decreased significantly in both treated groups, and the pathological changes of SEC were ameliorated. Matrine markedly inhibited the activation of Kupffer cells and their release of tumor necrosis factor (TNF). Hepatic malondialdehyde (MDA) levels and superoxide dismutase (SOD) activities were improved by matrine administration. In conclusion, matrine can protect SEC from cold ischemia and reperfusion injury after rat orthotopic liver transplantation. *(received 19 September 2002; accepted 3 January 2003)*

Keywords: liver transplantation, matrine, reperfusion injury, hepatic sinusoidal endothelial cells

Introduction

Preservation injury continues to be a major clinical problem in orthotopic liver transplantation (OLT), with 10% incidence of primary nonfunction [1-3]. The underlying mechanism of cold ischemia and reperfusion injury that leads to primary nonfunction is unknown. Cold ischemia and reperfusion injury typically cause rounding and detachment of the sinusoidal endothelial cells (SEC) [4]. The degree of SEC injury has been shown to correlate with graft viability after transplantation in animal [5-8] and clinical [9] studies. SEC impairment [10] and/or liver microcirculatory disturbances [11,12] that are mediated by oxygen radicals [13], activation of Kupffer cells [14,15], or sinusoidal accumulation of leukocytes [16], have been proposed as primary causes of storage-related graft failure.

Matrine (matridin-15-one) is a typical lupine alkaloid related to lupinine, sparteine and cytisine, (Fig 1) [17]. This alkaloid, isolated from certain

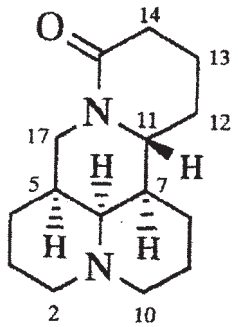

Fig. 1. The chemical structure of (+)-matrine.

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Sophora plants of the *Leguminosae*, has pharmacological effects (eg, anti-inflammation [18], immunoinhibition [19], anti-liver fibrosis [20,21], and antiarrhythmia [22]); it has been used for the treatment of chronic liver disease. Matrine markedly inhibited the activation of rat Kupffer cells and decreased the serum levels of TNF and IL-6; these findings suggest that matrine may have a protective effect on liver injury [18].

The present study evaluated the effects of matrine on SEC injury caused by preservation and reperfusion using an orthotopic rat liver transplantation model. Hyaluronic acid (HA) elimination and intercellular adhesion molecule-1 (ICAM-1) expression were used as indices of sinusoidal endothelial cell function. Activation of Kupffer cells and their release of tumor necrosis factor (TNF), the hepatic level of malondialdehyde (MDA), and hepatic superoxide dismutase (SOD) activity were studied to assess the protective effects of matrine.

Materials and Methods

Reagents. Matrine was purchased as a parenteral solution (50 mg/5 ml) from Ming Xing Pharmaceutical Factory, Guangzhou, China. The radioimmunoassay kit for hyaluronic acid (HA) was purchased from Shanghai Ocean Research Biomedical Technology Center, Shanghai, China. The mouse-anti-rat CD54 (ICAM-1) monoclonal antibody, MCA773, was purchased from Serotec Co. (UK). Recombinant human TNF (1000 units/ml) was purchased from Genzyme Co. (Boston, MA).

Animals. Male inbred SD rats (n = 224; body wt 200 to 220 g) were obtained from the Animal Center of Jin Ling Hospital (Nanjing, China). All rats were provided with laboratory chow and water ad libitum and were housed and treated in accordance with institutional animal care policies. Prior to their use in this study, the rats were fasted for 12 hr, but were allowed unrestricted access to water.

Experimental Design. Rat liver transplantation was performed by the technique of Kamada and Calne [23] under ether anesthesia. Ringer's (LR) solution was used for hepatic perfusion. The graft liver was preserved for 5 hr in Ringer's solution at 4°C and then transplanted orthotopically. Explantation of the recipient liver required <10 min; the rewarming time of the graft (clamping the portal vein and subhepatic vena cava during implantation) was 20 min.

The rats were randomly assigned into 4 experimental groups as follows: (A) an untreated group (controls) in which 32 recipients received ip injection of normal saline solution (NS, 1 ml) 30 min before laparotomy; (B) a low-dose treated group (LT) in which 32 recipients received ip injection of matrine (40 mg/kg, body wt) 30 min before surgery; (C) a high-dose treated group (HT), in which 32 recipients received ip injection of matrine (80 mg/kg, body wt) 30 min before surgery; and (D) a sham operated-group, in which the liver was simply mobilized without hepatectomy or transplantation to exclude the influence of surgery.

At 1, 2, 4, and 24 hr after reperfusion of the portal vein, 6 rats from each group were killed to collect blood samples from the infrahepatic vena cava and to excise the median hepatic lobe. Serum and plasma samples were separated and stored at -70°C until analysis. The liver samples were washed with cold saline solution and stored immediately in liquid nitrogen until analysis. The other 8 rats in each group were kept to assess their survival at 1-wk post-transplantation.

Serum ALT assay. Hepatic ischemia and reperfusion injury leads to an increase of serum alanine aminotransferase (ALT) activity. The ALT levels of serum samples collected 4 and 24 hr after reperfusion were assayed by an automatic biochemistry analyzer (Hitachi model 7600).

Plasma HA assay. Diminution of SEC function and viability leads to an increase of plasma hyaluronic acid (HA) concentration. HA levels of plasma samples collected 1, 2, and 24 hr after reperfusion were assayed in duplicate using the HA RIA kit according to the manufacturer's instructions.

Plasma TNF cytotoxicity. The TNF-induced cytotoxicity of all plasma samples was determined in duplicate by TNF cytotoxicity L929 assays, as described by Chang [24,25].

Plasma nitrate plus nitrite assays. The sum of plasma nitrate plus nitrite levels was used as an index of the nitric oxide level, decrease of which is a marker of microcirculatory disturbances. Samples were collected for these assays at 1 and 2 hr after reperfusion of the portal vein in each group. Nitrate was measured as nitrite after enzymatic conversion by nitrate reductase using the Griess reaction, as described by Schmidt et al [26]. Values obtained by this procedure represent the sum of plasma nitrite and nitrate. The detection limit of the assay was 5 μmol/L; the assays were all performed in duplicate.

Hepatic lipid peroxide levels and SOD activities. Hepatic lipid peroxide levels and SOD activities, which were used as indices of antioxidant effects, were assayed in liver samples collected at 1, 2, and 4 hr after reperfusion. Lipid peroxidation was assayed as the MDA level by the thiobarbituric acid method of Ohkawa [27] using tetraethoxypropane as the standard. Hepatic SOD activities were assayed by the method of Winterbourn et al [28].

Histochemical Analysis of ICAM-1. ICAM-1 was assayed in hepatic samples collected at 1, 2, and 4hr after reperfusion. Embedded in OCT-compound, they were stored in liquid nitrogen until analysis. Cryostat frozen sections (6 μm) were fixed in cold acetone for 10 min and immunohistochemical staining was performed immediately according to manufacturers' protocol. In brief, the primary antibody (anti-rat-ICAM-1 monoclonal antibody) was incubated in RPMI 1640 medium including 10% fetal bovine serum (1 hr at room temperature). The primary antibody was diluted 1/50 v/v. Blocking for nonspecific protein binding was accomplished by adding normal goat serum. Diaminobenzidine hydrogen peroxide was the chromogen; the sections were counterstained with hematoxylin. Mouse IgG1 negative control was used to verify the specificity of the reaction to ICAM-1.

Table 1. Effect of matrine on 1-wk survival rates of rats after orthotopic liver transplanation (n = 8/group).

Group	Days to death	1-wk survival
Controls[a]	<1,<1, <1, 2, 1, <1, <1, 1	0%
LT[b]	>7, 6, >7, >7, >7, 5, >7, >7	75%
HT[c]	5, >7, >7, >7, 5, >7, >7, >7	75%
Normals[d]	>7, >7, >7, >7, >7, >7, >7, >7	100%

[a] Liver transplantation, without matrine pretreatment.
[b] Transplantation, with matrine pretreatment (40 mg/kg, ip).
[c] Transplantation, with matrine pretreatment (80 mg/kg, ip).
[d] Sham operation without matrine pretreatment.

Light microscopy. Liver specimens were obtained from 6 rats in each group at 4 hr after reperfusion of the portal vein. The specimens were fixed with formalin, embedded in paraffin, and stained with hematoxylin and eosin for histologic examination.

Transmission electron microscopy. Liver fragments (approximately 1 $mm^{3)}$ collected at 4 hr after reperfusion were fixed in 2.5% glutaraldehyde containing 0.1 M phosphate buffer for 3 hr. After washing in phosphate buffer, the 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 analyses. Results are expressed as mean ± SD. Data were analyzed by the Statistical Analysis System program (SAS, Chicago, IL). One-way ANOVA was used for multiple comparisons, in conjunction with the Student-Newman-Keuls test. A p value <0.05 was considered significant.

Results

Effect of matrine on survival post-transplantation. All rats in the control group died within 48 hr after orthotopic liver transplantation (mostly between 10 to 20 hr). Matrine increased the 1-wk survival rate to 75% in both treated groups (Table 1).

Effect of matrine on serum ALT levels. Serum ALT levels in both of the matrine-treated groups were decreased significantly in comparison to the control rats at 4 and 24 hr after orthotopic liver transplantation and reperfusion (Fig. 2).

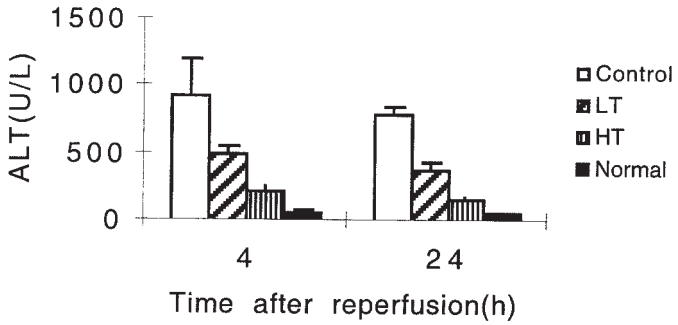

Fig. 2. Effect of matrine on serum ALT level after 5-hr cold preservation and reperfusion in rat orthotopic liver transplantation (n = 6 at each time). Both treated groups (LT, HT) are different from controls ($p < 0.01$) at 2 and 4 hr post-reperfusion; the high-dose treated group (HT) is different from the low-dose treated group (LT) ($p < 0.05$) at 2 and 4 hr.

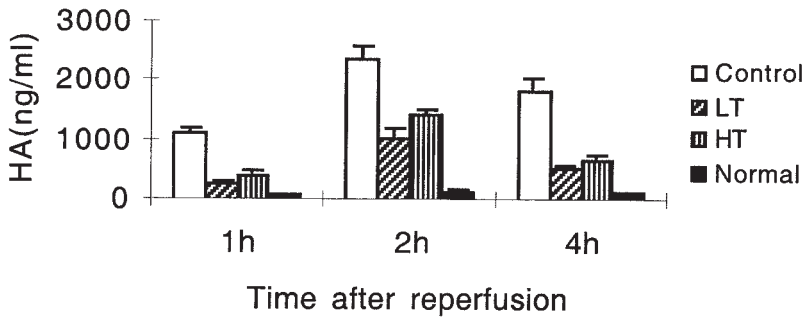

Fig. 3. Effect of matrine on plasma hyaluronic acid (HA) levels after 5-hr cold preservation and reperfusion in rat orthotopic liver transplantation (n = 6 at each time). Both treated groups differ from controls ($p < 0.01$) at 1, 2, and 4 hr post-reperfusion; the high-dose treated group differs from the low-dose treated group ($p < 0.05$) at 1 and 2 hr.

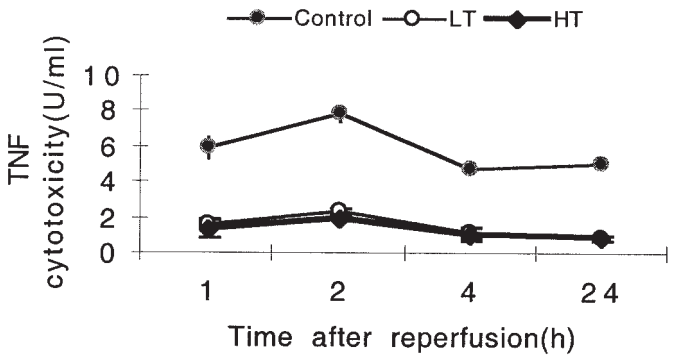

Fig. 4. Effect of matrine on TNF cytotoxicity after 5-hr cold preservation and reperfusion in rat orthotopic liver transplantation (n = 6 at each time). Both treated groups differ from controls ($p < 0.01$) at 1, 2, 4 and 24 hr post-reperfusion

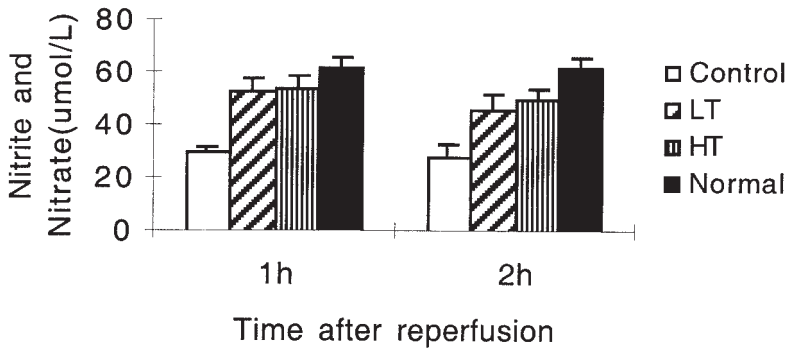

Fig. 5. Effect of matrine on plasma nitrate and nitrite levels after 5-hr cold preservation and reperfusion in rat orthotopic liver transplantation. (n = 6 at each time). Both treated groups (LT, HT) are different from controls ($p < 0.01$) at 1 and 2 hr post-reperfusion.

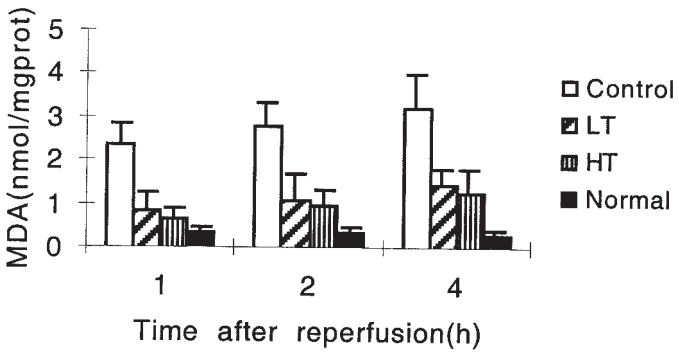

Fig. 6. Effect of matrine on hepatic MDA levels after 5-hr cold preservation and reperfusion in rat orthotopic liver transplantation. (n = 6 at each time). Both treated groups (LT, HT) are different from controls ($p < 0.01$) at 1, 2, and 4 hr post-reperfusion.

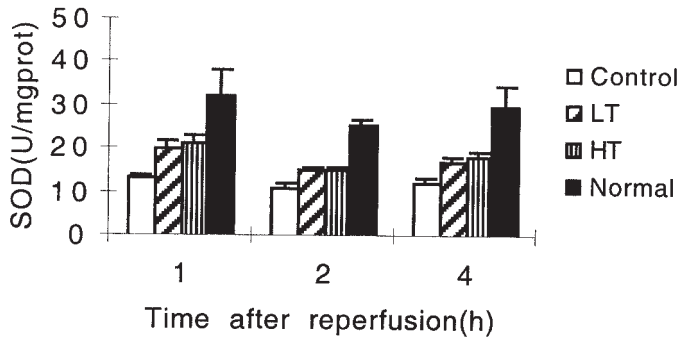

Fig. 7. Effect of matrine on hepatic SOD levels after 5-hr cold preservation and reperfusion in rat orthotopic liver transplantation. (n = 6 at each time). Both treated groups (LT, HT) are different from controls ($p < 0.01$) at 1, 2, and 4 hr post-reperfusion.

Effect of matrine on plasma HA levels. Compared to the sham-operated group, significant elevations of serum HA were observed at 1, 2, and 4 hr post-reperfusion, with the peak HA level at 2 hr, in the 3 groups that received orthotopic liver transplantation HA levels at the 3 time points were ameliorated markedly at both doses of matrine treatment (Fig. 3).

Effect of matrine on plasma TNF cytotoxicity. Almost no plasma TNF cytotoxicity was detected in the sham-operated group. In the other 3 groups, the maximum TNF values were obtained at 2 hr after surgery, as shown in Fig 4. TNF cytotoxicity level at 2 hr was decreased significantly by matrine treatment (7.94 ± 0.42 U/ml in controls; 2.39 ± 0.19 U/ml in LT group; 2.01 ± 0.13 U/ml in HT group; $p < 0.01$). Similar results were observed at other time points (Fig. 4).

Effect of matrine on plasma nitrate plus nitrite levels. In both of the matrine-treated groups and in the sham-operated (normal) group, the sum of the plasma nitrate plus nitrite levels was increased significantly at 1 and 2 hr after reperfusion, compared to the controls that received orthotopic liver transplants. No significant difference was noted between the two matrine-treated groups (Fig. 5).

Effect of matrine on hepatic lipid peroxides. Hepatic MDA levels in both of the matrine-treated groups were significantly lower at 1, 2, and 4 hr after reperfusion, compared to the controls that received orthotopic liver transplants. No significant difference was noted between the two matrine-treated groups (Fig. 6).

Effect of matrine on hepatic SOD activity. Hepatic SOD levels in both of the matrine-treated groups were significantly increased at 1, 2, and 4 hr after reperfusion, compared to the controls that received orthotopic liver transplants. No significant difference was noted between the two matrine-treated groups (Fig. 7).

Effect of matrine on hepatic ICAM-1 expression There was almost no expression of ICAM-1 in the livers from the sham-operated group. Expression of ICAM-1 was prominent in sinusoidal endothelial cells of livers from the control group that received orthotopic liver transplants. ICAM-1 expression was also evident in some hepatocytes and in the sinusoidal space, with strongest expression at 4 hr post-perfusion (Fig 8A). In both of the matrine-treated groups, the hepatic expression of ICAM-1 was markedly decreased, compared to the controls that received orthotopic liver transplants. No significant difference was noted between the two matrine-treated groups. (Fig. 8B)

Light microscopy. In the sham-operated group, microscopic examination showed that the degree of liver cell injury and the infiltration of inflammatory cells in portal areas and sinusoids were mild. As shown in Fig 9A, histological examination revealed extensive congestion and some inflammatory cells aggregating in hepatic sinusoid lumen in the control group. Rounding and detachment of SEC were observed. These were ameliorated markedly in both of the matrine-treated groups; no significant difference was observed between the 2 treated groups (Fig. 9B).

Transmission Electron Microscopy. Hepatocytes and SECs of liver from the sham-operated rats showed normal appearance after harvest. Samples taken from the untreated control group showed typical injury to the endothelial cells, including rounding, swelling, detachment from the hepatocyte plate, loss of cytoplasmic processes, swollen mitochondria, and focal loss of fenestration. The matrine-treated groups showed reduced severity of the ultrastructural changes of SEC, compared to the controls (Fig. 10). This was reflected in a reduced amount of cellular debris in the sinusoidal space. Moreover, the mitochondria were not swollen so severely. No significant differences were noted between the two matrine-treated groups.

Discussion

Prolongation of the permissible preservation time means that harvested organs can be transported for longer times and over wider areas. The development

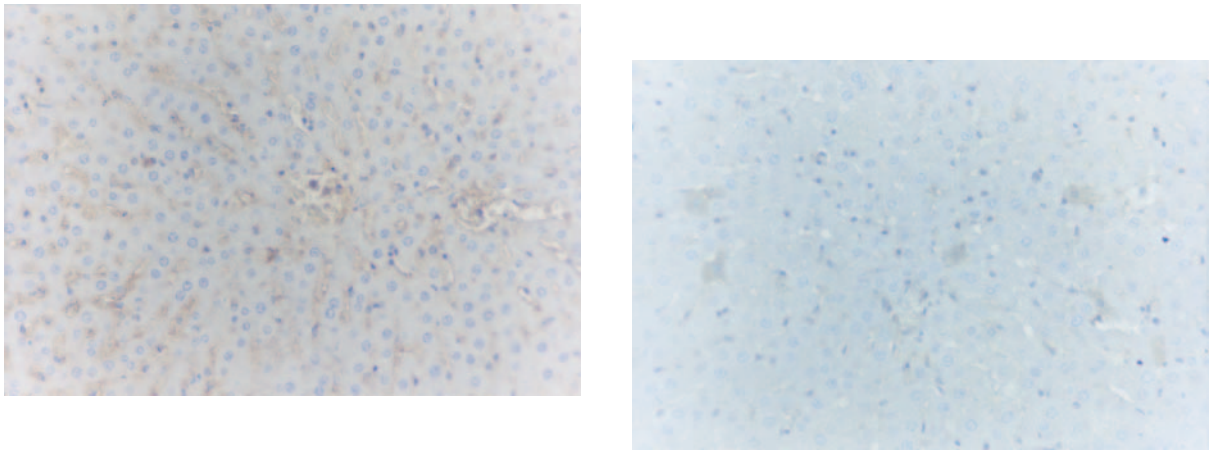

Fig 8. Immunohistochemical expression of ICAM-1 in rat liver at 4 hr after reperfusion. A (left panel): control group; B (right panel): low-dose matrine-treated group. ICAM-1 is prominent in sinusoidal endothelial cells in almost all lobules in A and is seen in some hepatocytes and the sinusoidal space. ICAM-1 expression is markedly decreased at the corresponding sites in B. (x 200)

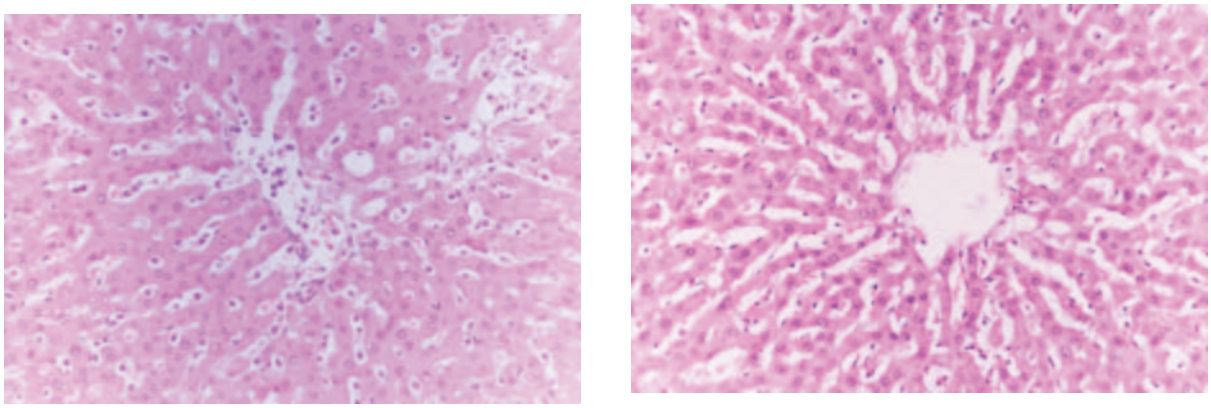

Fig 9. Light microscopic appearance of rat liver at 4 hr after reperfusion. A (left panel): control group; B (right panel): low-dose treated group. Focal necrosis of hepatocytes, inflammatory cell aggregates in hepatic sinusoid lumen, and Kupffer cell hyperplasia with rounding and detachment of SEC are seen in A. These are ameliorated markedly in B. (H&E, x 200)

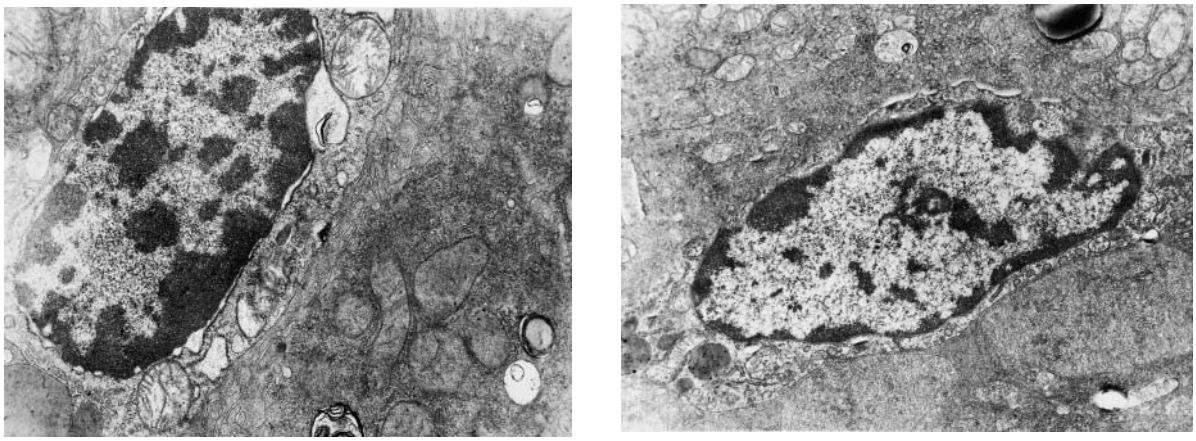

Fig 10. Electron microscopic photographs of rat liver at 4 hr after reperfusion. A (left panel): control group; B (right panel): low-dose treated group. Typical injuries to the endothelial cells are seen in A. These are ameliorated markedly in B. (x 6,000)

of University of Wisconsin (UW) solution has extended the successful preservation time to 24 hr [29]. Nevertheless, preservation injury continues to be a major clinical problem, with a 10% incidence of primary nonfunction [1-3]. The sinusoidal endothelial cells of the liver are susceptible to injury by extended cold preservation [30]. It has been proposed that the preservation-reperfusion injury is mainly due to changes in the SEC surface [4,5]. SEC damage causes marked microcirculatory disturbances, leukocyte and platelet adhesion, diminished blood flow, and continuation of the ischemic process, leading to massive hepatic necrosis [31,32]. It appears that SEC injury after preservation is linked to graft failure.

In the present investigation, rat livers were kept for 5 hr in cold Ringer's solution (4°C), exceeding the safe limit of 4 hr [33]. Under these preservation and transplantation conditions (ie, portal vein clamping time 20 min), a postoperativ e survival rate of about 40% was obtained. Thus, 5 hr in cold Ringer's solution, although a severely compromising condition, should permit an adequate assessment of the mechanisms of cold ischemia and reperfusion injury that lead to primary nonfunction. Rats without matrine treatment usually recovered well from anesthesia, but their clinical status began to deteriorate within 4 to 6 hr, and nonsurvivors died within 24 hr, mostly between 10 to 20 hr. Matrine treatment significantly increased the survival rate post-transplantation.

Loss of SEC function and viability leads to an increase of plasma HA concentration. Plasma HA levels at different time points post-transplantation were ameliorated markedly by matrine treatment, and the high-dose matrine treatment resulted in significant elevation of plasma HA, compared to the low-dose treated group. It seems that increasing the dose of matrine did not improve the therapeutic effect, which agrees with the results of Lin et al [17]. The severity of the ultrastructural changes of SEC was reduced markedly by matrine treatment.

The precise protective mechanism of matrine is unknown. A "no-reflow phenomenon," which is a microcirculatory injury attributed to formation of intracapillary thrombi from infiltration of inflammatory cells, has been recently stressed as one factor in the pathogenesis of reperfusion injury. After hepatic ischemia and reperfusion, Kupffer cells release inflammatory cytokines (TNF-α,and others) [34,35]. Inflammatory cytokines activate the sinusoidal endothelial cells and hepatocytes to express ICAM-1 on their membrane surfaces and also to release leukocyte-activating factors such as interleukin-1 or platelet-activating factor in the blood [36], which induce the expression of lymphocyte-function-associated antigen-1 (LFA-1) on the surface of neutrophils, resulting in adhesion of ICAM-1 and LFA-1. Thrombi of neutrophils may then be formed in hepatic sinusoids, causing peripheral microcirculatory failure at the inflammatory sites. Our results have confirmed that matrine markedly inhibited the activation of Kupffer cells and their release of TNF. As an indication of the destruction of cellular membrane structures, lipid peroxidation products, estimated as hepatic MDA levels, were decreased by matrine treatment. Our results suggest that matrine treatment decreases the expression of ICAM-1 and the adhesion of inflammatory cells to SEC, and increases the production of nitric oxide, resulting in suppression of the microcirculatory injury caused by hepatic reperfusion.

Matrine has been shown to inhibit the activation of Kupffer cells and the release of TNF-α, IL-1, and IL-6 in vitro and in vivo [17,37,38]. The results of this study indicate that matrine treatment inhibited the expression of ICAM-1 by SEC and the sinusoidal accumulation of inflammatory cells. These may point to a possible mechanism for matrine's protective effect on SEC during cold ischemia and reperfusion injury.

In conclusion, this study demonstrated a protective effect of matrine against cold ischemia and reperfusion injury of SEC following hepatic transplantation.

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References

1. Strasberg SM, Howard TK, Molmenti EP, Hertl M. Selection of the donor liver: risk factors for poor function after orthotopic liver transplantation. Hepatology 1994; 20:829-838.
2. Jaeschke H. Preservation injury: mechanisms, prevention and consequences. J Hepatology 1996;25:774-780.
3. Ploeg RJ, D'Alessandro AM, Knechtle SJ, Stegall MD, Pirsch JD, Hoffmann RM, Sasaki T, et al. Risk factors for primary dysfunction after liver transplantation: a multivariate analysis. Transplantation 1993;55:807-813.
4. Gao W, Bentley RC, Madden JF, Clavien PA. Apoptosis of sinusoidal endothelial cells is a critical mechanism of prevention injury in rat liver transplantation. Hepatology 1998;27:1652-1660.
5. Marzi I, Zhong Z, Lemasters JJ, Thurman RG.. Evidence that graft survival is not related to parenchymal cell viability in rat liver transplantation. The importance of nonparenchymal cells. Transplantation 1989;48:463-468.
6. Momii S, Koga A. Time-related morphological changes in cold-stored rat livers: a comparison of Euro-Collins solution with UW solution. Transplantation 1990;50: 745-750.
7. M.R Schon, O Kollmar, N Akkoc, et al. Cold ischemia affects sinusoidal endothelial cells while warm ischemia affects hepatocytes in liver transplantation. Transplant Proc 1998;30:2318-2320.
8. Takei Y, Marzi I, Gao W, Gores GJ, Lemasters JJ, Thurman RG. Leukocyte adhesion and cell death following orthotopic liver transplantation in the rat. Transplantation 1991;51:959-964.
9. Chazouilleres O, Calmus Y, Vaubourdolle M, Ballet F. Preservation-induced liver injury. J Hepatol 1993;18:123-134.
10. Gao W, Bentley RC, Madden JF, Clavien P. Apoptosis of sinusoidal endothelial cells is a critical mechanism of preservation injury in rat liver transplantation. Hepatology 1998;27:1652-1660.
11. Imamura H, Brault A, Huet PM. Effects of extended cold preservation and transplantation on the rat liver microcirculation. Hepatology 1997;25:664-671.
12. Koeppel TA, Lehmann TG, Thies JC, et al. Impact of N-acetylcysteine on the hepatic microcirculation after orthotopic liver transplantation. Transplantation 1996;61: 1397-1402.
13. Connor HD, Gao W, Nukina S, Lemasters JJ, Mason RP, Thurman RG. Evidence that free radicals are involved in graft failure following orthotopic liver transplantation in the rat: an electron paramagnetic resonance spin trapping study. Transplantation 1992;54:199-204.
14. Marzi I, Knee J, Buhren V, Menger M, Trentz O. Reduction by superoxide dismutase of leukocyte-endothelial adherence after liver transplantation. Surgery 1992;111: 90-97.
15. Clifford A. Brass, Thomas G. Roberts. Hepatic free radical production after cold storage: Kupffer cell-dependent and -independent mechanisms in rats. Gastroenterology 1995; 108:167-1175.
16. Clavien PA, Harvey PRC, Sanabria JR, Cywes R, Levy GA, Strasberg SM. Lymphocyte adherence in the reperfused rat liver: mechanisms and effects. Hepatology 1993;17:131-142.
17. Okuda, S., Yoshimoto, M., Tsuda, K., Utzugi, N. Über die absolute Konfiguration des Matrins. Chem Pharm Bull 1966;14:314-318.
18. Lin W, Zhang JP, Hu ZL, Qian DH. Inhibitory effect of matrine on lipopolysacchride-induced tumor necrosis factor and interleukin-6 production from rat Kupffer cells. Acta Pharm Sin 1997;32:93-96.
19. Liang P, Bo AH, Xue GP, et al. Study on the mechanism of matrine on immune liver injury in rats. World Chinese J Digest 1999;7:104-108.
20. Zhang JP, Zhang M, Cheng J, et al. Matrine inhibits production and actions of fibrogenic cytokines released by mouse peritoneal macrophages. Acta Pharmacol Sin 2001;22:765-768.
21. Zhang JP, Zhang M, Zhou JP, et al. Antifibrotic effects of matrine on in vitro and in vivo models of liver fibrosis in rats. Acta Pharmacol Sin 2001;22:183-186.
22. Ai J, Gao HH, He SZ, et al. Effects of matrine, artemisinin, and tetrandrine on cytosolic [Ca2+] in guinea pig ventricular myocytes. Acta Pharmacol Sin 2001;22: 512-515.
23. Kamada N, Calne RY. Orthotopic liver transplantation in the rat:Technique using cuff for portal vein anastomosis and biliary drainge. Transplantation 1979,28: 47-51.
24. Chang NS. TGF-α induction of novel extracellular proteins that trigger resistance to TNF cytotoxicity in murine L929 fibroblasts. J Biol Chem 1995;270:7765-7772
25. Chang NS. Hyaluronidase induces murine L929 fibrosarcoma cells resistant to tumor necrosis factor and Fas cytotoxicity in the presence of actinomycin D. Cell Biochem. Biophys 1996;27:109-132
26. Shmidt HHHW, Warner TD, Nakane M, Forstermann U, Murad F. Regulation and subcellular location of nitrogen oxide synthases in RAW264.7 macrophages. Mol Pharmacol 1992;41:615-624.
27. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-358
28. Winterbourn CC, Hawkins RE, Brian M, Carrell RW. The estimation of red cell superoxide dismutase activity. J Lab Clin Med 1975;85:337-342.
29. Todo S, Nery J, Yanaga K, et al. Extended preservation of human liver grafts with UW solution. JAMA. 1989;261: 711-714.
30. Wang L, Florman S, Roayaie S, et al. Differential in vivo recovery of sinusoidal endothelial cells, hepatocytes, and Kupffer cells after cold preservation and liver transplantation in rats. Transplantation 1998;66:573-578.
31. Arai S, Mochida S, Ohno A, et al. Decreased expression of receptors for vascular endothelial growth factor and

sinusoidal endothelial cell damage in cold-preserved rat livers. Transplant Proc 1999;31:2668-2672.
32. Cywes R, Brendan J, Mullen M, et al. Prediction of the outcome of transplantation in man by platelet adherence in donor liver allografts. Transplantation 1993,56:316-323.
33. Sumimoto R, Jamieson NV, Wake K, et al. 24-hour rat liver preservation using UW solution and some simplified variants. Transplantation 1989;48:144-146.
34. Shibuya H, Ohkouchi N, Tsukamoto S, et al. Tumor necrosis factor-α induced, superoxide-mediated neutrophil accumulation in cold ischemic/reperfusion rat liver. Hepatology 1997;26:113-120.
35. Carrick JB, Martins O, Snider CC, et al. The effect of LPS on cytokine synthesis and lung neutrophil influx after hepatic ischemia/reperfusion injury in the rat. J Surg Res 1997;68:16-23.
36. Shito M, Wakabayashi G, Ueda M, et al. Interleukin 1 receptor blockade reduces tumour necrosis factor production, tissue injury, and mortality after hepatic ischemia-reperfusion in the rat. Transplantation 1997;63:143-148.
37. Hu ZL, Zhang JP, Wan MB, et al. Effect of matrine on mouse hepatitis and tumor necrosis factor production induced by propionibacteriun acnes/lipopolysaccharides. Acta Pharm Sin 1996;31:662-665.
38. Hu ZL, Zhang JP, Yu XB, et al. Effect of matrine on lipopolysaccharides/D-galactosamine-induced hepatitis and tumor necrosis factor release from macrophages in vitro. Acta Pharmacol Sin 1996;17:351-353.