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[原著] Protective effects of lidocaine in hepatic ischemia : reperfusion injury in-vivo and ex-vivo

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Protective effects of lidocaine in hepatic ischemia/ reperfusion injury in-vivo and ex-vivo

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ABSTRACT

We investigated the effect of lidocaine on hepatic ischemia/reperfusion (I/R) injury in the rat both in vivo (Experiment A, groups 1 to 3) and ex vivo (Experiment B, groups 4 to 7). In Experiment A, hepatic ischemia were performed for 60 min at room temperature. In groups 1 and 2, physiological saline (group 1, n=5) or 10 mg/kg of lidocaine (group 2, n=5) was injected through the peripheral vein, before the hepatic ischemia. In group 3 (n=5), 10 mg/kg of lidocaine was injected twice at reperfusion and before hepatic ischemia. The hepatic tissue blood flow in group 1 was lower than in group 2 or 3 at 5 min to 90 min after reperfusion. Serum transaminase level was significantly lower in group 3 compared to that in group 1 at 2 hours after reperfusion. In Experiment B, livers were removed from the rat and preserved in saline at room temperature for 60 min, followed by 120 min of reperfusion with oxygenated perfusate at 37℃. The livers were perfused with Krebs-Henseleit solution (group 4, n=5). The perfusate was supplemented with lidocaine in group 5 (n=5), 3.5×10^6 of neutrophil in group 6 (n=5), and both lidocaine and neutrophils in group 7 (n=5). The levels of GOT, GPT and LDH were all significantly lower in group 7 than in group 6. These data thus suggest that lidocaine plays a protective role in hepatic I/R injury by stabilizing both the hepatocytes and the neutrophil membrane. *Ryukyu Med. J., 19(1)11~16, 1999*

Key words: lidocaine, liver, ischemia/reperfusion injury, in-vivo, ex-vivo

INTRODUCTION

Lidocaine is a well known local anesthetic with pronounced antiarrhythmic and anticonvulsant properties. Lidocaine is also known to inhibit the release of superoxide anion from the neutrophils in vitro[1]. These effects of lidocaine have been reported to potentially reduce the size of infarction in patients with acute myocardial infarction[2]. Lidocaine, is also well known to be a membrane-stabilizer and has also been reported to be effective in reducing the size of myocardial infarction in an experimental coronary arterial ligation model[2]. However, there has yet to be a study regarding the effect of lidocaine on ischemia/reperfusion (I/R) injury in the rat liver. Based on these facts, we investigated the protective effects of lidocaine on hepatic I/R injury, using both in-vivo and ex-vivo models of hepatic I/R injury in rats.

MATERIALS AND METHODS

Male Wistar rats, weighing from 250 to 300 g, were purchased from "Ryukyu Biotec" and used for the Experiment.

Protocol of Experiment A (in-vivo model)

The rats were anesthetized with ether. Then they were injected with 100 units of heparin intravenously and laparotomized through combined midline and midtransverse incisions. The inferior vena cava and portal vein were isolated and then the hepatic artery and portal vein were clamped for 60 min at room temperature, following the intra-venous administration of 1 ml of physiological saline in group 1 (n=5) or 10 mg/kg of lidocaine in group 2 and 3 (n=5, in each group) at ten min before hepatic ischemia. In group 3, an additional 10 mg/kg of lidocaine was given at reperfusion. The hepatic tissue blood flow (TBF) was measured continuosly using laser doppler flowmetry. The serum glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) were measured at 120 min after reperfusion. The animals were sacrificed at 120 min after reperfusion. Finally a microscopic examination of the hepatic structure was performed.

Protocol of Experiment B (ex-vivo model)

The rats were anesthetized with ether. Then they were injected with 100 units of heparin intravenously and laparotomized through combined midline and midtransverse incisions. The inferior vena cava and portal vein were isolated and then the hepatic artery was ligated and cut off. An intramedic PE-10 polyethylene catheter was inserted into the common bile duct and a 20G eraster catheter was inserted into the abdominal aorta. The liver was flushed with saline at room temperature through the abdominal aorta. Then the liver was removed and preserved in saline for 60 min at room temperature. After preservation, the liver was placed on a platform of the liver circulation apparatus which was equipped with a recirculating perfusion-aeration chamber and a temperature control system. A 14G eraster catheter was inserted into the portal vein and the liver was reperfused from the portal vein with 200 ml of Krebs-Henseleit (K-H) solution which was saturated with 95 % O_2 and 5 % CO_2 for 2 hours at 37℃. The perfusate composition was as follows: NaCl 118.4 mM; $NaHCO_3$ 25 mM; KCl 4.8 mM; KH_2PO_4 1.2 mM; $MgSO_4$ 1.2 mM and D-glucose 0.01 mM. During perfusion, the flow rate of the perfusate through portal vein was maintained at 2.0 ml/g·liver/min. The perfusion pressure was then monitored continuously in an open gas capillary tube connected to the inflow. The livers were perfused with Krebs-Henseleit (K-H) solution in group 4 (n=5). Two mg/kg of lidocaine was added to the K-H solution immediately after reperfusion followed by continuous lidocaine infusion at the rate of 2 mg/kg/min during 120min of reperfusion period in group 5 (n=5). Neutrophils (3.5×10^6/ml) were added to K-H solution immediately after reperfusion in group 6 (n=5). In group 7 (n=5), 2 mg/kg of lidocaine and neutrophils (3.5×10^6/ml) was added to the K-H solution immediately after reperfusion followed by continous lidocaine infusion at a rate of 2 mg/kg/min during the 120 min reperfusion period. The intrahepatic nitric oxide (NO) levels measured using an NO monitor (Model NO-501, Inter Medical, Tokyo, Japan), the intrahepatic partial pressure of oxygen (PO_2) measured by a PO_2 monitor (PO_2-100, Inter Medical, Tokyo, Japan), the portal pressure and the bile output were all monitored continously. The GOT, GPT, lactic dehydrogenase (LDH) levels in perfusate from the liver were analyzed before and 5, 15, 30, 60, 90, 120 min after reperfusion. Myeloperoxidase (MPO) activitiy was also analyzed in the tissue homogenate obtained from the liver at 120 min after reperfusion.

Measurement of intra-hepatic nitric oxide (NO) Level: The intra-hepatic NO concentration was determined with an NO-selective electrode and an NO monitor. This electrode, made from PG/IR alloy and coated with a three-layered membrane (KCl, NO-selective silicone resin, and a normal silicone membrane), is specific and senstive to NO. NO was measured by the current based on the electrochemical reaction $NO+2H_2O\rightarrow NO_3^-+4H^++3e^-$. The electrode was implanted into the hepatic parenchyma, thus avoiding any fluctuation due to surgical manipulation[3]. The intra-hepatic NO level was measured from 0 to 120 min after reperfusion in Experiment B.

Tissue preparation and Myeloperoxidase Assay: Myeloperoxidase (MPO) was extracted from the homogenized tissue by suspending the material in 0.5 % hexadecyltrimethylammonium bromide (HTAB) (Sigma Chemical Co., St. Louis, MO) in 50 mM potassium phosphate buffer, pH 6.0, followed by sonication in an ice bath for 10 seconds. The specimens underwent 3 cycles of freezing and thawing, followed by re-sonication. The suspensions were then centrifuged at 40,000×g for 15 min, and the resulting supernatant or pellet was then used for the spectrophotometrical assay. In brief, 0.1 ml of the material was mixed with 2.9 ml of 50 mM phosphate buffer (pH 6.0), containing 0.167 mg/ml of o-dianisidine dihydrochloride (Sigma Chemical Co.) and 0.0005 % hydrogen peroxide. Changes in absorbance at 460 nm were measured with a spectrophotometer with a recording attachment. One unit of MPO activity was defined as that degrading one micromole of the peroxide per minute at 25℃.

Preparation of neutrophils: The heparinized blood was collected from the abdo minal aorta in male Wister rats. The leukocytes, consisting of both mononuclear and polymorphonuclear leukocytes, were separated into two distinct bands in discontinious gradients of a mono-poly resolving medium (Dainihon Phamaceutical Co., LTD., Osaka, Japan). These neutrophils were then washed twice with saline. The cell suspension was centrifuged at 250×g for 12 min at room temperature. The final cell conconcentration was then deter mined by an electronic counting device. Using this procedure, the leukocyte preparation was found to contain (90 % neutrophils as deter mined by Wright-Giemsa staining while the cell viability was around 98 %.

Statistical analysis: The data were expressed as the mean±SE (standard error) and were analyzed using an analysis of variance (ANOVA), when appropriate in Experiment. Since groups 2 and 3 served as negative controls, the values of group 1 were always compared with the values of groups 2 and 3. Then since groups 5, 6 and 7 served as negative controls, the values of group 4 were always compared with the values of groups 5, 6 and 7. Differences among the groups were considered to be statistically significant at a P value of less than 0.05.

RESULTS

Experiment A (in-vivo model)

In group 1, hepatic TBF ranged from 7.29±2.69 to 10.4±3.9 ml/min/100g liver weight. In groups 2 and 3, however, TBF ranged from 10.0±2.9 to 19.3±5.3 and

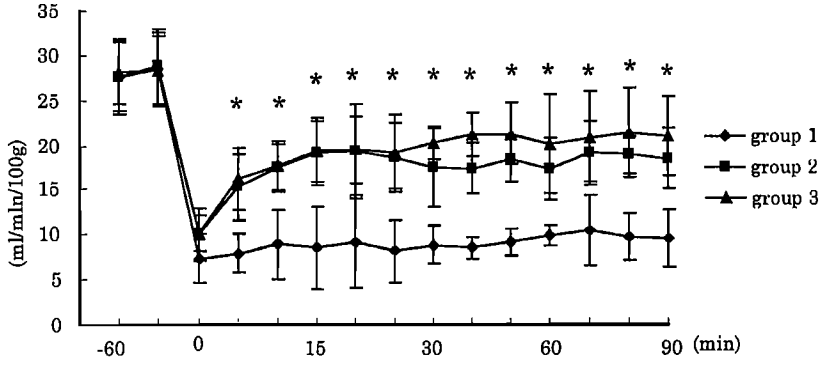

Fig. 1 Time course of changes in the hepatic tissue blood flow in groups 2 and 3 showed a statistical differences in comparison to group 1 at 5 min to 90 min after reperfusion (in-vivo model). The data represent the means ± S.E. (*p < 0.05)

from 10.1±2.0 to 21.7±5.1 ml/min/100g liver weight respectively, which was significantly statistical different from group 1 from 5 min to 90 min after reperfusion (Fig. 1). The serum transa minase level (GOT, GPT) was significantly lower in group 3 at 2 hours after reperfusion (341±28, 265±48 IU/L), compared to group 1 (3380±1495, 2153±1277 IU/L). In the electron microscopic exa mination, the disappearance of the intramitochondrial ground substance, nuclear deformation and a tendency of chromatin aggregation were observed in group 1. In group 3, however, such observations were significant.

Experiment B (ex-vivo model)

Intra-hepatic NO: The NO levels were given as a percentage of the value measured before reperfusion. The intra-hepatic NO level decreased after reperfusion in groups 4, 5 and 6. At every point evaluated from 5 to 120 min after reperfusion, the NO levels in group 7 always demonstrated the highest values among those levels of all groups but no significant difference was observed between the groups (group 4: 80.3±109.3 %, group 5: 47.1±30.8 %, group 6: 55.6±21.2 %, group 7: 111.8±117.2 % at 120 min after reperfusion).

Portal pressure: The portal pressure immediately decreased after reperfusion and there after continued to decrease until 60 min after reperfusion in all groups. At 120 min after reperfusion, the portal pressure was 15.2 ±2.6 cmH_2O in group 4, 17±1.9 cmH_2O in group 5, 26± 7.7 cmH_2O in group 6, and 27.2±5.2 cmH_2O in group 7. However, no significant differences were observed between the four groups at any point after reperfusion.

Intra-Hepatic PO_2: The intra-hepatic PO_2 in group 4 showed no change during reperfusion. In group 5, the PO_2 levels increased at 5 min after reperfusion and thereafter decreased gradually. In groups 6 and 7, the PO_2 levels increased at 15 min after reperfusion and no change was seen from 30 to 120 min after reperfusion. At 120

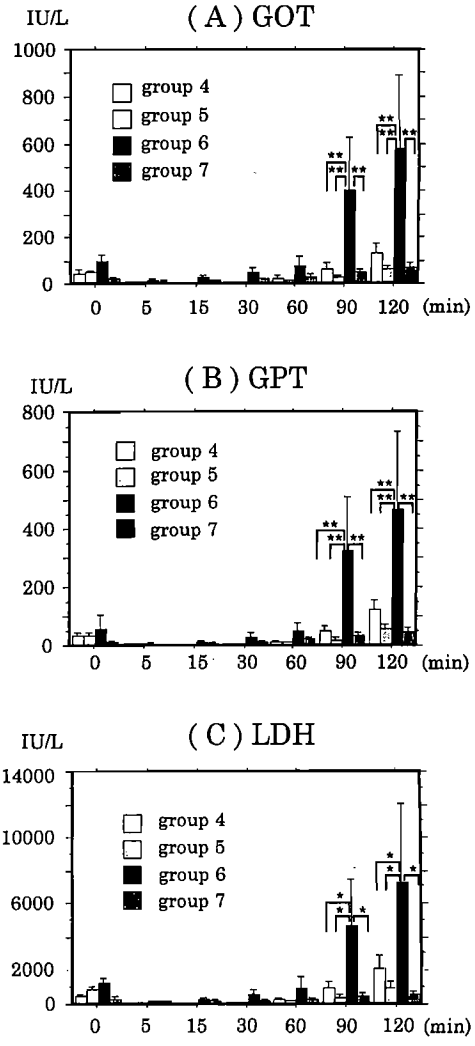

Fig. 2 Time course of changes in GOT (A), GPT (B) and LDH (C) level after reperfusion (ex-vivo model). The data represent the means ± S.E. (*p < 0.05)

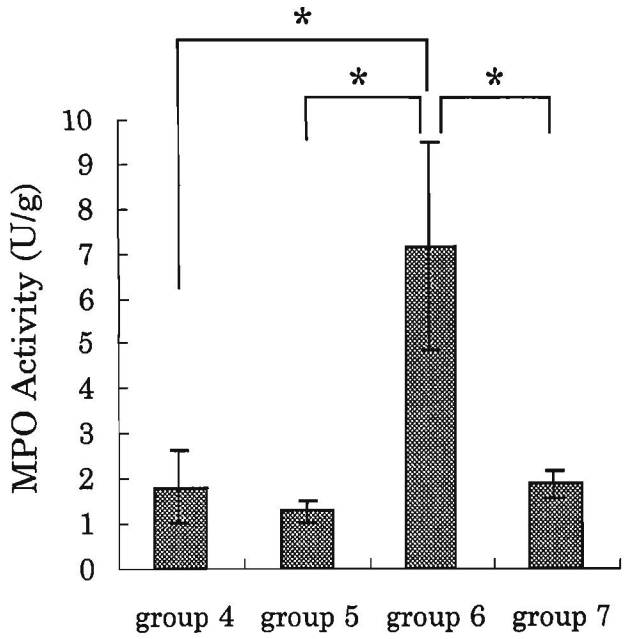

Fig. 3 Changes in the myeloperoxidase activity after 120 min reperfusion (ex-vivo model). The data represent the means ± S.E. (*p < 0.05)

min after reperfusion, the intra-hepatic PO_2 levels were 232±61.9 mmHg in group 4, 233±14.5 mmHg in group 5, 139±81.7 mmHg in group 6 and 141.8±64.7 mmHg in group 7. However no significant differences were observed among the four groups.

Bile output: Bile output increased gradually and peaked at 120 min after reperfusion. The levels of bile output were higher in group 4 (0.0024±0.002 ml/min), group 5 (0.0054±0.0054 ml/min) and group 7 (0.0021 ±0.0014 ml/ min) than in group 6 (0.0005±0.0005 ml/ min), at 120 min after reperfusion. However, no significant differences were observed between these groups.

Levels of liver enzymes: The release of hepatic enzymes (GOT, GPT and LDH) in the perfusate was meassured as an index of hepatic injury and is shown in Fig. 2. These levels increased immediately after reperfusion (GOT: 43.2±44.8 IU/L in group 4, 45.3±18.7 IU/L in group 5, 96.7±52.8 IU/L in group 6, 17.8±15.3 IU/L in group 7, GPT: 31.3±34.3 IU/L in group 4, 36±19.6 IU /L in group 5, 58.3±84.7 IU/L in group 6, 9.5±10.7 IU /L in group 7, LDH: 473±265.4 IU/L in group 4, 850± 527.2 IU/L in group 5, 1232.7±479.6 IU/L in group 6, 318.3±307.2 IU/L in group 7) but decreased in groups 4, 5, and 7 at 5 min. But in group 6 these levels increased from 30 min until 120 min after reperfusion. The GPT and LDH levels were significantly lower in groups 5 and 7 than in group 4 at 120 min. The GOT, GPT and LDH levels were also significantly higher in group 6 (740 ± 659 IU / L, 620 ± 528 IU / L, 10243 ± 7604 IU / L,

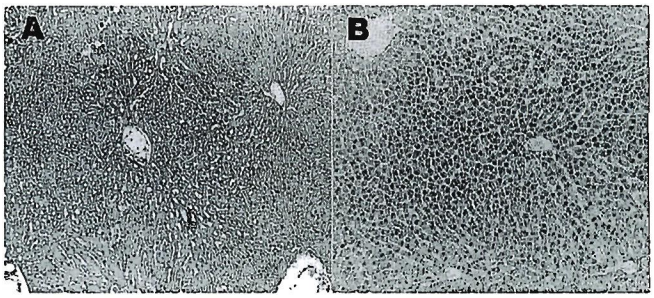

Fig. 4 The light microscopic findings of the liver after 120 min reperfusion (ex-vivo model). A: group 6 ; B: group 7

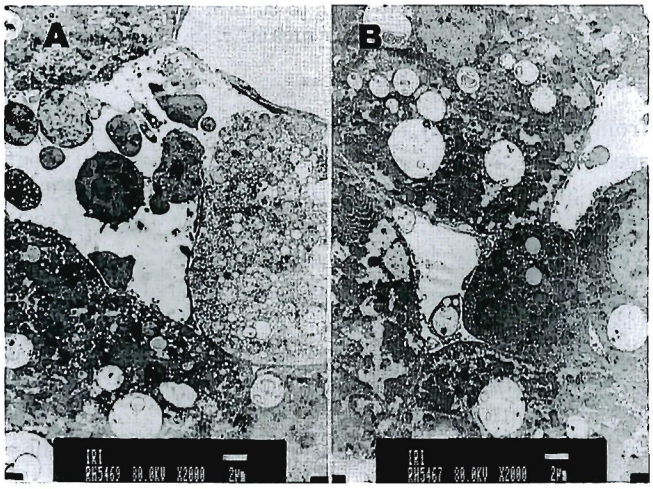

Fig. 5 The electron microscopic findings of the liver after 120 min reperfusion (ex-vivo model). A: group 6; B: group 7

respectively) than in group 4 (134±103 IU/L, 124±74 I U/L, 2163±1737 IU/L, respectively), group 5 (61±47 IU /L, 54±52 IU/L, 990±802 IU/L, respectively) and group 7 (68±47 IU/L, 46±29 IU/L, 576±455 IU/L, respectively) at 120 min after reperfusion (Fig. 2).

Myeloperoxidase Activity: After 120 min reperfusion, the MPO activities of the liver tissue were significantly higher in group 6 (7.15±3.27 U/g) than in the other groups (1.81±1.12 U/g in group 4, 1.26±0.33 U/g in group 5, 1.89±0.52 U/g in group 7) (Fig. 3).

Number of Neutrophils: The number of neutrophils added to the perfusate were $3.5\pm0.06\times10^6$ and 3.53±0.1 ×106 in groups 6 and 7, respectively, which were reduced to $0.75\pm0.5\times10^6$ (78.6 % reduction) and $1.53\pm0.65\times10^6$ (56.4 % reduction) at 120 min after reperfusion.

Histological Findings of the liver: The light microscopic findings showed no significant differences in groups 4, 5 and 7 at 120 min after reperfusion. The findings in group 6 showed hepatocyte vacuolization, however, these findings improved in group 7 based on the microscopic findings (Fig. 4). Trypan blue uptake by

the nuclei of parenchymal and nonparenchymal cells were little identified in all groups at 120 min after reperfusion. The electron microscopic findings revealed mitochondrial swelling and the destruction of its crista at 120 min after reperfusion in all groups. But fine vesiculation of the rough endoplasmic reticulum were observed in group 7, which were not found in group 6 (Fig. 5).

DISCUSSION

Lidocaine, which is extensively used as an antiarrhythmic agent, has been reported to reduce the size of myocardial infarction in the coronary arterial ligation model, in which lidocaine exerted a tissue protective effect mainly by inhibiting neutrophil adherence to the endothelium[2]. Lidocaine is also found to be effective as a free radical scavenger which inhibits the lipid peroxidation processes during reperfusion after brain ischemia[4]. These effects of lidocaine have been reported in the brain, lung and heart, both in vitro and in vivo studies[4-8]. However, these effects of lidocaine have not yet been clarified in the liver. Based on these facts, we investigated the effects of lidocaine in hepatic ischemia reperfusion injury, espesially regarding its relation to the neutrophil activity.

In our first study in vivo, TBF of lidocaine ad ministered animals were significantly higher compared to that of the saline injected control animals. The histological findings and hepatic enzyme levels also improved after lidocaine ad ministration compared to those of the control group. These data indicated that lidocaine was useful in reducing hepatic I/R injury by improving the peripheral circulation in the hepatic I/R injury. However, in this in vivo model, additional factors other than neutrophils, such as the no-reflow phenomenon, may also be involved in hepatic injury[9], and the mechanism of lidocaine in reducing the hepatic I/R injury could thus not be clearly elucidated.

In our second study, we used ex-vivo perfusion of the liver, to investigate the role of lidocaine in reducing the neutrophil mediated hepatic I/R injury. The ex vivo model has been used in many I/R injury studies of the heart, lung and liver, and was also thought to be appropriate to study the isolated factors in hepatic I/R injury[10-12].

In this ex-vivo model, we first investigated the effects of lidocaine on the peripheral circulation of the liver, which was implicated to improve in our in-vivo study. Since NO is known to play a vital role in vascular relaxation[13], and the tissue PO_2 levels and the bile output also expresses the peripheral circulation of the liver, these parameters were monitored. However, in this ex-vivo perfusion model, these parameters did not improve after the addition of lidocaine, even in the animals in which hepatic I/R injury improved with lidocaine. These data suggested that lidocaine did not have any direct effect on the peripheral circulation of the liver in neutrophil mediated hepatic I/R injury.

On the other hand, lidocaine effectively supressed the release of liver enzymes, which was caused by neutrophil administration in the ex-vivo perfusion model. Since lidocaine is known to decrease accumulation of ions such as potassium, sodium, and calcium, into the intra- and extracellular spaces during ischemia[14] and a well known membrane-stabilizer[2], the administration of lidocaine might stabilize the membranes of hepatocytes, thus resulting in a reduced enzyme release.

We also monitored the MPO activity as an index of neutrophil accumulation in the liver. After 120 min of reperfusion, the MPO activity was significantly higher only in the neutrophil administered group, but was suppressed by the administration of additional lidocaine. Superoxide anion is reported to mediate neutrophil accumulation in the reperfused liver after warm ischemia[15]. Neutrophil elastase is also reported to increase the rate of leukocyte adherence and extravasation normally induced by ischemia reperfusion[16]. In the present study, the number of neutrophils in the perfusate tended to increase in the lidocaine ad ministered group. Thus these facts combined with the decreased MPO activity and hepatic I/R injury in the lidocaine treated animals might suggest that lidocaine mediated these effects by controlling the release of super oxide or elastase from the neutrophils.

In the light microscopic findings, no significant differences in hepatic damage were observed. However in the electron microscopic findings, the hepatic damage observed in neutrophil administered group improved after the addition of lidocaine. Regarding the histological findings, no differences in neutrophil infiltration were observed between the groups, thus suggesting that lidocaine inhibited the neutrophil activity without inhibiting the process of its infiltration. Since lidocaine was reported to inhibit the neutrophil adherence to the endothelium[6] and also impaired the release of the superoxide anion of human neutrophils in vitro[1], we thus speculate that lidocaine stabilized the neutrophil membrane and inhibited the release of neutrophil enzyme in our experiment.

In conclusion, we investigated the potential role of lidocaine in the model of hepatic ischemia/reperfusion injury both in vivo and in vitro. The findings of this study therefore suggest that lidocaine is able to reduce warm ischemia/reperfusion injury in the liver by stabilizing both the hepatocytes and the neutrophil membrane.

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