

Liver injury after intermittent or continuous hepatic pedicle clamping and its protection by reduced glutathione

Li Zhou, Jing-An Rui, Rou-Li Zhou, Xue-Min Peng, Shao-Bin Wang, Shu-Guang Chen, Qiang Qu and Yu-Pei Zhao

Beijing, China

BACKGROUND: The debate is still going on about selection of several clamping patterns during hepatectomy. The aim of this study was to assess the safety and preference of normothermic intermittent or continuous hepatic pedicle clamping and confirm the protective effect of reduced glutathione (GSH).

METHODS: Thirty-two adult male healthy Sprague-Dawley (SD) rats were divided into groups of intermittent clamping and GSH absent (IA), continuous clamping and GSH absent (CA), intermittent clamping and GSH present (IP) and continuous clamping and GSH present (CP). The clamping manners were successively 40 minutes in continuous clamping groups and two cycles of 20 minutes with an interval of 5 minutes in intermittent clamping groups, and reperfusion periods were 60 minutes. Experimental parameters included levels of malonaldehyde (MDA) and Cu/Zn superoxide dismutase (SOD), pathological and ultrastructural changes in liver tissues, activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in sera.

RESULTS: In the same group, the activities of ALT and AST were significantly higher in post-clamping rats than in pre-clamping rats ($P < 0.05$), but no significant differences were noted in levels of MDA and Cu/Zn SOD ($P > 0.05$). The differences of all values between post-reperfusion rats and pre-clamping rats were significant ($P < 0.05$). Pathological and ultrastructural changes could be observed, but no irreversible injury was present. The comparison of the groups showed that the values at relevant time points between the intermittent and continuous groups were not significantly different ($P > 0.05$). The values were significantly different between the GSH absent and present groups after

reperfusion ($P < 0.05$). The morphological damages were also obviously alleviated in the GSH present group.

CONCLUSIONS: Normothermic intermittent or continuous hepatic pedicle clamping could cause reversible liver ischemia/reperfusion injury when the clamping time lasts 40 minutes. The injury extent seems to be similar. Continuous clamping should be regarded as a proper method in liver surgery. GSH has been confirmed as an effective agent in preventing post-clamping liver injury.

(*Hepatobiliary Pancreat Dis Int* 2004; 3: 209-213)

KEY WORDS: hepatic pedicle clamping; hepatic ischemia/reperfusion injury; reduced glutathione; protection

Introduction

Intraoperative bleeding is always one of the key problems in liver surgery. It has been demonstrated by some authors that massive hemorrhage during partial hepatectomy contributes to morbidity and mortality.^[1,2] Besides, subsequent blood transfusion facilitates recurrence of hepatic malignancies, including both primary and secondary tumors, and decreases survival of the patients.^[3-5] Thus, control of intraoperative hemorrhage appeared to be very important in hepatic resection. Since Pringle^[6] first reported temporary normothermic occlusion of hepatic inflow by clamping the porta hepatis in the early twentieth century, the technique named Pringle maneuver has been used worldwide. But clamping-caused hepatic ischemia/reperfusion (I/R) injury has been increasingly recognized with controversy on intermittent or continuous clamping in hepatic surgery. Each clamping was experimentally supported by some animal models.^[7-11] Intermittent clamping was reported to alleviate liver injury in a long-period of ischemia in contrast to continuous clamping.^[7-9] In a short-period of occlusion, however, liver injury was similar between intermittent and continuous clampings,^[10] even lightened liver injury was present in some animals with continuous clamping.^[11] We still lack comparative data on liver in-

Author Affiliations: Department of General Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing 100032, China (Zhou L, Rui JA, Wang SB, Chen SG, Qu Q and Zhao YP); Department of Cell Biology, Peking University Health Science Center, Beijing 100083, China (Zhou RL and Peng XM)

Corresponding Author: Li Zhou, MD, Department of General Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing 100032, China (Tel: 86-10-88068150; Fax: 86-10-6605 2572; Email: lizhou02@hotmail.com)

© 2004, Hepatobiliary Pancreat Dis Int. All rights reserved.

jury in 40 minutes of hepatic pedicle clamping in rats up to the present.

Reduced glutathione (GSH) is an endogenous potent antioxidant that plays an important role in protection against I/R injury of many organs, such as the liver.^[12] Bilzer et al^[13] reported that continuous reperfusion of cold preserved rat liver with GSH solution can prevent hepatocellular damage and deterioration of hepatic circulation. It was also verified that GSH ester (GSHE) could protect rat liver from reperfusion injury by providing intra- and extracellular GSH.^[14] But whether pulsating import of GSH takes similar protective effect under normothermic hepatic pedicle clamping remains unknown.

In view of the above-mentioned facts, the present study aimed to clarify hepatic ischemia and postischemia injury in 40 minutes of intermittent or continuous clamping of the porta hepatis and explore the protective effect of GSH against the injury under pulsating import.

Methods

Animal groups

Thirty-two male adult healthy Sprague-Dawley (SD) rats, weighing 300-350 g, were used in experiments. All rats fed with standard biscuit were divided into four groups according to intermittent or continuous clamping and GSH presence or absence, i. e., IA group (intermittent clamping and GSH absent), CA group (continuous clamping and GSH absent), IP group (intermittent clamping and GSH present), and CP group (continuous clamping and GSH present). Each group consisted of 8 rats.

Surgical procedures

The rats were initially anesthetized by intraperitoneal injection of 3% pentobarbital sodium (40 mg/kg), followed by middle incision. The hepatic pedicles including the portal vein, hepatic artery and bile duct were clamped with a microvessel clip. The clamping lasted for two cycles of 20 minutes with an interval of 5 minutes in the IA and IP groups, and continued for 40 minutes in the CA and CP groups. The reperfusion period was 60 minutes in all groups. Reduced glutathione (50 mg/kg Laboratoro Farmaceutico C. T., USA) was injected into the portal vein in 5 minutes before clamping and at the time point of immediate reperfusion in the IP and CP groups. And the same amount of sterile normal saline was injected at the same time point in the IA and CA groups. The samples of liver tissue and blood in the inferior vena cava were obtained at three time points of pre-clamping, post-clamping and postreperfusion. Each sample of liver tissue was divided instantly into three portions and stored respectively in liquid nitrogen, 10% formaldehyde solution, and 3% glutaraldehyde solution for detection of malonaldehyde (MDA) and Cu/Zn su-

peroxide dismutase (SOD) contents, routine pathological examination, and electron microscopy. Blood samples were directly separated into serum in order to detect the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Preparation of liver tissue supernatant

The sample of liver tissue frozen in liquid nitrogen was weighed and added sterile normal saline to a scale of 100 mg sample/ml saline. The mixture was ground into homogeneous. After centrifugation at 3000 rpm/min, the supernatant was taken and stored in a refrigerator set at -20 °C.

Quantification of MDA and Cu/Zn SOD content in liver tissue

The quantification of liver tissue MDA was carried out according to Vardareli et al's method.^[15] The content of Cu/Zn SOD in liver tissue was detected with a Cu/Zn SOD radioimmune assay kit (saturation method, Northern Institute of Biological Technology, Beijing, China).

Biochemical assay of the serum activities of ALT and AST

The serum activities of ALT and AST were all detected with a Hitachi 7150 automatic biochemical analyzer (Hitachi Co., Ltd., Tokyo, Japan).

Pathological examination with light microscope

One portion of liver tissue samples was fixed with 10% formaldehyde, embedded with paraffin, stained with hematoxylin and eosin, and subsequently observed under a light microscope.

Transmission electron microscopy of samples

The sample for transmission electron microscopy (TEM) was washed and fixed with 3% glutaraldehyde at once. The subsequent procedures included washing with sucrose solution, gradient dehydration with acetone, and infiltration and embedding with epoxy resin. Finally the sample was observed under a transmission electron microscope (JEM 1230, Japan).

Statistical analysis

The values at the relevant time points between the groups were compared with the independent-samples *t* test, and the differences in the values between various time points in the same group were revealed by the paired-samples *t* test. The analysis was all finished with a SPSS 10.0 software for windows. The *P* values less than 0.05 or 0.01 were considered significant or extremely significant.

Results

Table. Values of all quantitative parameters

Time/Point	Pre-clamping	Post-clamping	Post-reperfusion
<i>n</i>	8	8	8
MDA (nmol/mg tissue)			
CA group	0.50±0.07	0.55±0.11	0.99±0.33 *
IA group	0.47±0.06	0.51±0.11	1.03±0.22 *
CP group	0.47±0.08	0.52±0.12	0.66±0.14 **
IP group	0.47±0.08	0.48±0.10	0.59±0.15 **
Cu/Zn SOD (ng/mg tissue)			
CA group	0.91±0.15	0.89±0.13	0.56±0.15 *
IA group	0.92±0.25	0.77±0.11	0.50±0.11 *
CP group	0.91±0.18	0.85±0.16	0.74±0.16 **
IP group	0.89±0.17	0.80±0.16	0.77±0.19 **
ALT(U/L)			
CA group	51±12	1642±141 *	1882±159 *
IA group	49±13	1691±131 *	1844±138 *
CP group	49±7	1574±121 *	1614±152 **
IP group	51±11	1515±146 **	1576±187 **
AST(U/L)			
CA group	102±14	1571±118 *	1714±136 *
IA group	103±17	1546±151 *	1650±139 *
CP group	103±15	1448±135 *	1475±129 **
IP group	104±17	1427±128 *	1491±153 **

*: $P < 0.01$ vs before clamping; #: $P < 0.05$ vs IA or CA group.

MDA content in liver tissue

In the same group, the MDA values were extremely higher after reperfusion than before clamping ($P < 0.01$). No significant differences were observed in the values before and after clamping ($P > 0.05$). Differences were seen at the time point of postreperfusion between the IP and IA groups ($P < 0.01$), and extremely significant or significant differences between the CP and CA groups ($P < 0.05$). But the values at other time points were not significantly different ($P > 0.05$, Table).

Cu/Zn SOD content in liver tissue

The contents of Cu/Zn SOD after reperfusion reduced more significantly than those before clamping in the relevant group ($P < 0.01$). But the values after clamping were not varied significantly in spite of their decreasing ($P > 0.05$). The values after reperfusion in the GSH present (IP and CP) groups were significantly higher than those in the GSH absent (IA and CA) groups ($P < 0.05$). Such differences were not observed at other time points ($P > 0.05$, Table).

Serum ALT activity

The activity of serum ALT elevated markedly after clamping and continued to increase after reperfusion in each group ($P < 0.01$). At the time point after reperfusion, extremely significant differences were noted between the IP and IA groups and between the CP and CA groups ($P < 0.01$). And the value after clamping in the IP group was significantly lower than that in the IA

group ($P < 0.05$); but at other time points, the difference was not significant ($P > 0.05$, Table).

Serum AST activity

Extremely significant differences existed in the activity of serum AST between time points after clamping, after reperfusion, and before clamping in the same group ($P < 0.01$). Comparison of the groups showed that the value at the time point after reperfusion in the IP group was significantly lower than that in the IA group ($P < 0.05$), and that the differences between the values in the CP and CA groups at the same time point were extremely significant ($P < 0.01$). Such difference was not found at other time points between the groups ($P > 0.05$, Table).

Morphological changes under light microscopy

The appearance, size and structure of hepatocytes seemed to be normal before clamping. Their distribution was cord-like. Dilation, stasis and edema of hepatic sinusoids were not present. At the time point after clamping, patchy ballooning degeneration and disturbance in arrangement of liver cells were obviously observed in the IA and CA groups with distinct stasis of hepatic sinusoids. The hepatocytes in the IP and CP groups showed marked vacuolar degeneration and arrangement disturbance, accompanied by a certain degree of stasis of hepatic sinusoids. But no obvious evidence of patchy necrosis of liver cells was present. After reperfusion, the relatively severe vacuolar degeneration and arrangement disturbance of hepatocytes remained in the IA and CA groups, but hepatic sinusoid stasis improved remarkably. The figure, size, structure and distribution of liver cells in the IP and CP groups returned to near normal, but a little bit of blood was detained in hepatic sinusoids.

Ultrastructural changes under electron microscopy

Before clamping the porta hepatis, karyomorphism and organelle structure of hepatocytes were all normal, nuclear membrane was smooth, and chromatin was homogeneous. After clamping, irregular karyomorphism (nuclear shrinkage), increased heterochromatin and secondary lysosomes, retrogressed and decreased rough surfaced endoplasmic reticulum, and vacuolized organelles (mitochondria) and cytoplasm were observed in all four groups. But no evidence of severe irreversible changes (nuclear pyknosis, etc.) was found. At the time point after reperfusion, the nuclear figure and organelle structure of hepatocytes were basically normalized in the IA and CA groups, but the amount of cell organs remained low. Mitochondria and rough surfaced endoplasmic reticulum adjacent to the nucleus were aggregated obviously in the IA group. The ultrastructure of liver cells almost recovered to normal in the IP and CP groups, without evidence of injury.

Discussion

Because massive intraoperative hemorrhage causes high risk of morbidity and mortality in hepatic resection^[1,2] and tumor progression is associated with subsequent blood transfusion.^[3-5] Advances in skill of limiting intraoperative bleeding have run through the whole history of liver surgery in the 20th century. Despite Heaney^[16] and Fortner^[17] introduced normothermic and hypothermic total hepatic vascular isolation respectively, the interruption method used most widely is still the Pringle maneuver,^[6] i. e. normothermic temporary occlusion of hepatic inflow by clamping of the porta hepatis. Within nearly one century's history of the technique, a controversy has persisted on intermittent and continuous clampings. Comparison of experimental data showed that the two maneuvers lead to similar liver injury^[10] (even lightened injury in continuous clamping^[11]) in a short-period, but intermittent clamping reduces hepatocellular damage in a long period of ischemia.^[7-9] Besides, it is worthy of notice that definition of long- or short-term clamping varies in different species. However, the study of hepatic injury caused by the two occlusion maneuvers under 40 minutes of clamping remains inadequate, because most hepatectomies can be finished within this interruption term according to our experience.^[18] Using hepatic ischemia/reperfusion model in rats, we compared the extent of liver damage by markers of MDA, Cu/Zn SOD, ALT, AST, routine histology and ultrastructural morphology in the present study. It was demonstrated that oxygen derived free radical (ODFR) played a crucial role in liver ischemia/reperfusion injury as a main mechanism of hepatic injury after clamping.^[19] Previously, the oxygen free radical was thought to be the initiator of a cascade reaction that led to damage of the cell membrane by lipid peroxidation, mainly occurred in unsaturated fatty acids.^[20,21] MDA as the stable final product of lipid peroxidation reaction, its tissue level could be used as a good marker of extent of lipid peroxidation-induced cellular injury.^[22] Thus, it is also an indirect index for production of ODFR. The process of ODFR development during ischemia and reperfusion is always followed by a drain of its scavengers and reduction of Cu/Zn SOD indicates damage of the free radical scavenging system due to ODFR.^[23] Thus, ODFR production and subsequent hepatic damage could be evaluated by the decreased extent of Cu/Zn SOD. The activities of ALT and AST are regularly tested for liver injury. To get direct proof, we studied morphologically with light or electron microscopy. In our study, markers of liver injury changed more significantly after reperfusion than before clamping in the same group, and histologically tissue or cellular damage but irreversible lesion was not present. These results indicated that clamping the porta hepatis for 40 minutes could induce reversi-

ble hepatic injury in rat. Others^[24,25] reported that liver damage due to occlusion of hepatic inflow 40 minutes was reversible in rats and humans, respectively. This finding was consistent with ours. Moreover, no significant differences were found in values at all three time points between the intermittent (IA and IP) clamping groups and continuous (CA and CP) groups, and morphological findings also suggested analogous degree of injury. Hence the present study showed that the two styles of clamping, intermittent and continuous, lead to similar extent of postischemic liver injury after 40-minute clamping of the porta hepatis.

GSH has been clarified as a low molecule weight tripeptide compound with abundant thiol consisting of cysteine, glycine and glutamic acid.^[12] Its protective effect against I/R is chiefly invoked by neutralizing free radicals directly or indirectly^[26] and resisting lipid peroxidation.^[27] The current study has confirmed that other antioxidants fail to suppress lipid peroxidation when GSH level is reduced,^[28] suggesting its central effect in antioxidation. Because the liver is the most capable organ for synthesizing GSH^[12] and transporting large amounts of it into blood,^[29] the antioxidant is of even more importance in hepatic I/R. The importance of GSH is also verified by the phenomenon described by Stein et al^[30] that I/R injury merely occurs in GSH-deficient rat liver. The *in vitro* experiment^[13] has demonstrated the role of GSH in preventing I/R. Liu et al^[31] also reported that continuous infusion of GSH from the portal vein attenuated hepatic reperfusion injury by 55%, and that exogenous GSH had protective effect. In the present study, the values of MDA, ALT and AST were significantly lower but the values of Cu/Zn SOD were higher after reperfusion in the GSH present groups than in the GSH absent groups. Histological damage was also alleviated. These results indicated that pulsating import of GSH solution from the portal vein could also protect the liver from I/R injury. Because its main mechanism is scavenging of ODFR, the first target certainly is reperfusion injury. With a low molecule weight, GSH easily enters cell and could play its antioxidation role more effectively. Because of its effectiveness and convenience, pulsating import in the portal vein should be a very good route for GSH administration during hepatic surgery.

We conclude that 40-minute hepatic pedicle clamping causes reversible liver injury in rats, but no marked difference is seen in injury extent between intermittent and continuous clampings. In addition, humans have well developed portosystemic collaterals,^[32,33] and human tolerance to I/R is thought to be better compatible to that of rats. Continuous clamping for 40 minutes should be regarded as a suitable occlusion in clinical practice because of its superiorities in manipulation and less bleeding. GSH is a potent protectant against I/R injury

caused by clamping and pulsating import of GSH from the portal vein is of clinical significance.

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.

References

- Nagao T, Goto S, Kawano N, Mizuta T, Omori Y, Kawano N, et al. Hepatic resection for hepatocellular carcinoma. Clinical features and long-term prognosis. *Ann Surg* 1987;205:33-40.
- Ekberg H, Tranberg KG, Andersson R, Jeppsson B, Bengmark S. Major liver resection; perioperative course and management. *Surgery* 1986;100:1-8.
- Stephenson KR, Steinberg SM, Hughes KS, Vetto JT, Sugarbaker PH, Chang AE. Perioperative blood transfusions are associated with decreased time to recurrence and decreased survival after resection of colorectal liver metastases. *Ann Surg* 1988;208:679-687.
- Asahara T, Katayama K, Itamoto T, Yano M, Hino H, Okamoto Y, et al. Perioperative blood transfusion as a prognostic indicator in patients with hepatocellular carcinoma. *World J Surg* 1999;23:676-680.
- Makino Y, Yamanoi A, Kimoto T, El-Assal ON, Kohno H, Nagasue N. The influence of perioperative blood transfusion on intrahepatic recurrence after curative resection of hepatocellular carcinoma. *Am J Gastroenterol* 2000;95:1294-1300.
- Pringle JH. Notes on the arrest of hepatic hemorrhage due to trauma. *Ann Surg* 1908;48:541-549.
- Isozaki H, Adam R, Gigou M, Szekely AM, Shen M, Bismuth H. Experimental study of the protective effect of intermittent hepatic pedicle clamping in the rat. *Br J Surg* 1992;79:310-313.
- Kimura N, Muraoka R, Horiuchi T, Tabo T, Uchinami M, Yokomachi J, et al. Intermittent hepatic pedicle clamping reduces liver and lung injury. *J Surg Res* 1998;78:11-17.
- van Wagenveld BA, van Gulik TM, Gelderblom HC, Scheepers JJ, Bosma A, Endert E, et al. Prolonged continuous or intermittent vascular inflow occlusion during hemihepatectomy in pigs. *Ann Surg* 1999;229:376-384.
- Hardy KJ, Tancheroen S, Shulkes A. Comparison of continuous versus intermittent ischemia-reperfusion during liver resection in an experimental model. *Br J Surg* 1995;82:833-836.
- van Wagenveld BA, van Gulik TM, Gabeler EE, van der Kleij AJ, Obertop H, Gouma DJ. Intrahepatic tissue pO₂ during continuous or intermittent vascular inflow occlusion in a pig liver resection model. *Eur Surg Res* 1998;30:13-25.
- Shan XQ, Aw TY, Jones DP. Glutathione-dependent protection against oxidative injury. *Pharmacol Ther* 1990;47:61-71.
- Bilzer M, Paumgartner G, Gerbes AL. Glutathione protects the rat liver against reperfusion injury after hypothermic preservation. *Gastroenterology* 1999;117:200-210.
- Grattagliano I, Vendemiale G, Lauterburg BH. Reperfusion injury of the liver; role of mitochondria and protection by glutathione ester. *J Surg Res* 1999;86:2-8.
- Vardareli E, Saricam T, Koken T, Degirmenci I, Aral E, Erenoglu E. The effect of alpha-tocopherol and pentoxifylline on ischemia-reperfusion induced liver injury in rats. *Hepatogastroenterology* 1998;45:1505-1508.
- Heaney JP, Stanton WK, Halbert DS, Seidel J, Vice T. An improved technique for vascular isolation of the liver: experimental study and case reports. *Ann Surg* 1966;163:237-241.
- Fortner JG, Shiu MH, Kinne DW, Kim DK, Castro EB, Watson RC, et al. Major hepatic resection using vascular isolation and hypothermic perfusion. *Ann Surg* 1974;180:644-652.
- Rui JA, Wang SB, Chen SG, Zhou L, Wei X, Han K, et al. A surgery oriented serial treatments of 191 patients with large primary liver cancers. *Chin J Oncol* 2001;23:417-419.
- Kobayashi H, Nonami T, Kurokawa T, Sugiyama S, Ozawa T, Takagi H. Mechanism and prevention of ischemia-reperfusion-induced liver injury in rats. *J Surg Res* 1991;51:240-244.
- McCord JM. Oxygen-derived free radicals in postischemic tissue injury. *N Engl J Med* 1985;312:159-163.
- Chen HM, Chen MF, Shyr MH. Prostacyclin analogue (OP-2507) attenuates hepatic microcirculatory derangement, energy depletion, and lipid peroxidation in a rat model of reperfusion injury. *J Surg Res* 1998;80:333-338.
- Comporti M. Lipid peroxidation and cellular damage in toxic liver injury. *Lab Invest* 1985;53:599-623.
- Liu XH, Kato H, Araki T, Itoyama Y, Kato K, Kogure K. An immunohistochemical study of copper/zinc superoxide dismutase and manganese superoxide dismutase following focal cerebral ischemia in the rat. *Brain Res*, 1994;644:257-266.
- Xiong CL, Hu H, Wei W, Chen XP, Wu ZD. Liver sinusoidal endothelial cell damage during normothermic ischemia and reperfusion of rat. *Chin J Surg* 2000;38:297-299.
- Tu KQ, Liang CF, Lun ZJ, Zhang YL, Hou JL. The clinical studies of pathologic and ultrastructural alterations of liver after hepatic vascular occlusion under normothermia in human. *Chin J Bases Clin General Surg* 1998;5:226-227.
- Kaplowitz N, Aw TY, Oolhtens M. The regulation of hepatic glutathione. *Annu Rev Pharmacol Toxicol* 1985;25:715-744.
- Tirmenstein MA, Reed DJ. Characterization of glutathione-dependent inhibition of lipid peroxidation of isolated rat liver nuclei. *Archs Biochem Biophys* 1988;261:1-11.
- Ozaki M, Nakamura M, Teraoka S, Ota K. Ebselen, a novel anti-oxidant compound, protects the rat liver from ischemia-reperfusion injury. *Transpl Int* 1997;10:96-102.
- Deneke SM, Fanburg BL. Regulation of cellular glutathione. *Am J Physiol* 1989;257:L163-L173.
- Stein HJ, Oosthuizen MMJ, Hinder RA, Lamprechts H. Oxygen free radical and glutathione in hepatic ischemia-reperfusion injury. *J Surg Res* 1991;50:398-402.
- Liu P, Fisher MA, Farhood A, Smith CW, Jaeschke H. Beneficial effects of extracellular glutathione against endotoxin-induced liver injury during ischemia and reperfusion. *Circ Shock* 1994;43:64-70.
- Nagasue N, Yukaya H, Suehiro S, Ogawa Y. Tolerance of the cirrhotic liver to normothermic ischemia. A clinical study of 15 patients. *Am J Surg* 1984;147:772-775.
- Delva E, Camus Y, Nordlinger B, Hannoun L, Parc R, Deriaz H, et al. Vascular occlusions for liver resections. Operative management and tolerance to hepatic ischemia; 142 cases. *Ann Surg* 1989;209:211-218.

Received December 1, 2003

Accepted after revision February 3, 2004