Histol Histopathol (2004) 19: 281-289
DOI: 10.14670/HH-19.281

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

Ischemic preconditioning: tolerance to hepatic ischemia-reperfusion injury

A. Serafín[1], L. Fernández-Zabalegui[1], N. Prats[2], Z.Y. Wu[3], J. Roselló-Catafau[1] and C. Peralta[1]

[1]Department of Experimental Pathology, Instituto de Investigaciones Biomédicas de Barcelona-Consejo Superior de Investigaciones Científicas, Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain, [2]Veterinary School, Universitat Autònoma de Barcelona, Barcelona, Spain and [3]General Surgery Division, Renji Hospital, Shanghai, China

Summary. Hepatic ischemia-reperfusion (I/R) injury still remains an unresolved problem in both liver resectional surgery and liver transplantation and may be responsible for liver failure, lung injury and death. The current review summarizes the findings reported to date on the effectiveness of ischemic preconditioning against liver and lung damage associated with hepatic I/R injury and the underlying protective mechanisms. The effect of ischemic preconditioning on the mechanisms potentially involved in hepatic I/R injury, including alterations in energy metabolism, neutrophil accumulation, microcirculatory disturbances, formation of proinflammatory mediators, such as endothelin and tumor necrosis factor-alpha, and reactive oxygen species generation have been evaluated. In this review, we address the role of preconditioning in the increased vulnerability of fatty livers to hepatic I/R injury. The effectiveness of ischemic preconditioning versus pharmacological strategies that could simulate the benefits of liver preconditioning has been also discussed.

Key words: Ischemia, Reperfusion, Liver, Preconditioning, Steatosis

Hepatic ischemia-reperfusion injury

The ischemia-reperfusion (I/R) injury is an important cause of liver damage occurring during surgical procedures that include hepatic resections and liver transplantation (Clavien et al., 1992; Huguet et al., 1992; Strasberg et al., 1994). An established consequence of hepatic I/R is the induction of important pulmonary pathological alterations, such as the adult respiratory distress syndrome associated with human liver transplantation and multiple organ failure associated with primary graft failure (Matuschak et al., 1987; Colletti et al., 1990a; Clavien et al., 1992). I/R injury is a complex pathophysiology with a number of contributing factors. The ischemic insult associated with the reduction in cellular adenosine triphosphate (ATP) levels and the consequent lactate accumulation can lead to sublethal cell injury (Clavien et al., 1992; Rosser et al., 1995; Kukan and Haddad, 2001), which is aggravated by the formation of reactive oxygen species (ROS) from various intracellular sources, including xanthine/xanthine oxidase (XOD) (Muller et al., 1996; Pesonen et al., 1998; Fan et al.,1999). In addition, formation of proinflammatory mediators, including endothelin (ET) and tumor necrosis factor-alpha (TNF), and the neutrophil accumulation can further enhance the injury (Schmid-Schonbein, 1987; Colletti et al., 1990b; Jaeschke et al., 1990; Goto et al., 1994; Peralta et al., 1996, 2000, 2001a; Sawaya et al., 1999). Microcirculatory alterations lead to underperfused areas in the liver and may cause ischemic injury (Horton and Fairhurst, 1987; Vajdova et al., 2000; Serracino-Inglott et al., 2001). It has been suggested that a potential explanation for the association of liver I/R injury with pulmonary insufficiency involves liver-derived TNF and xanthine/XOD release, following hepatic I/R (Muller et al., 1996; Pesonen et al., 1998; Fan et al., 1999). The mechanisms involved in the pathophysiology of hepatic I/R have been extensively explained in previous reviews (Clavien et al., 1992; Lichtman and Lemasters, 1999; Bilzer and Gerbes, 2000; Lentsch et al., 2000; Serracino-Inglott et al., 2001; Jaeschke, 2003).

Ischemic preconditioning on hepatic i/r injury

Over the recent years, a surgical strategy known as ischemic preconditioning, firstly described in the heart by Murry et al. (1986), has been developed to reduce hepatic I/R injury. Ischemic preconditioning is an endogenous protective mechanism by which short periods of ischemia, separated by short reperfusions, confer a state of protection against subsequent sustained

Offprint requests to: Dr. Joan Roselló-Catafau, Department of Experimental Pathology, Instituto de Investigaciones Biomédicas de Barcelona, IDIBAPS-CSIC, C/ Rosellón 161, 6ª y 7ª planta, 08036-Barcelona, Spain. Fax: 34-933638301. e-mail: jrcbam@iibb.csic.es

I/R injury. Ischemic preconditioning reduces necrosis and apoptotic cell death and enhances survival in normothermic hepatic ischemia and liver transplantation (Peralta et al., 1997; Yin et al., 1998; Yadav et al., 1999; Ricciardi et al, 2001; Fernandez et al., 2002). The benefits of ischemic preconditioning on lung damage associated with hepatic I/R have also been demonstrated (Peralta et al., 1999b, 2001a, 2002b; Fernandez et al., 2002). The histological findings are shown in Fig. 1. It is important to remark that the protective effects of ischemic preconditioning have recently been evidenced in normothermic conditions associated with human hepatic resections (Clavien et al., 2000).

Mechanisms of ischemic preconditioning

Although there are several hypotheses, the precise protective mechanisms of ischemic preconditioning against hepatic I/R injury remain to be elucidated. The molecular basis for ischemic preconditioning consists of a sequence of events: in response to the triggers of ischemic preconditioning, a signal must be rapidly generated which is then transduced into an intracellular message leading the amplification of the effector mechanism of protection (Cutrin et al., 2002).

Adenosine and nitric oxide

There is evidence indicating that in response to preconditioning, substances released by the liver, including adenosine and nitric oxide (NO) are able to active protective mechanisms in normothermic conditions as well in cold ischemia associated with liver transplantation (Peralta et al., 1998, 1999a; Yin et al. 1998; Howell et al., 2000). In fact, the optimal ischemic time window to induce preconditioning in the liver is

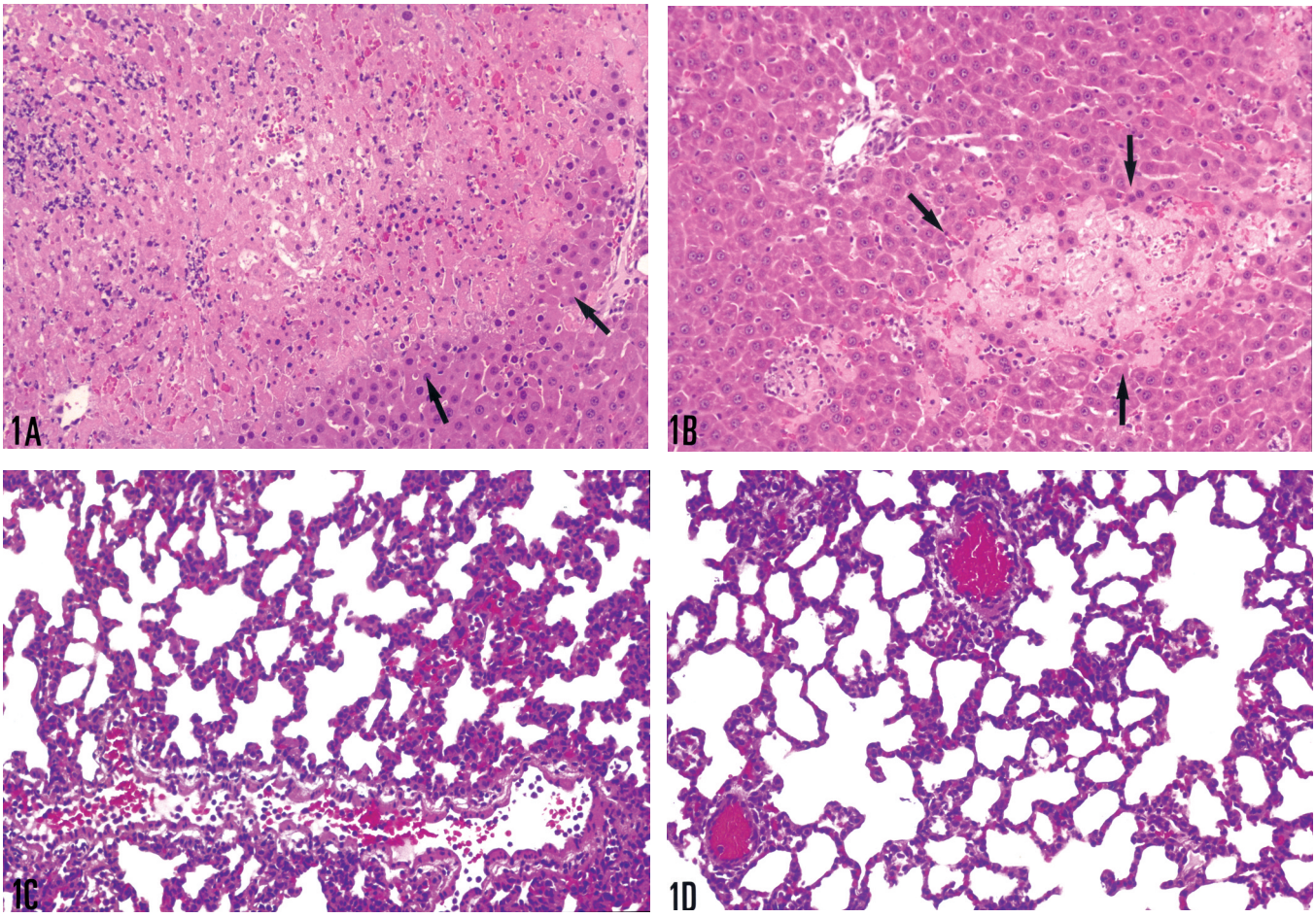

Fig. 1. Histological lesions in liver **(A-B)** and lung **(C-D)** after liver transplantation from liver grafts undergoing 8 h of cold ischemia. The histological study of the liver in transplantation show multifocal and extensive areas of coagulative necrosis with neutrophil infiltration and hemorrhage at 24 h of reperfusion (arrow) **(A)**. In contrast, fewer and smaller areas of hepatocyte necrosis are observed when preconditioning was performed (arrow) **(B)**. In the lung, the transplantation group shows margination and adhesion of neutrophils to the endothelium, a diffuse augmentation of the cellularity in the alveolar walls, and a slight thickening of the alveolar walls **(C)**. In contrast, no apparent vascular margination of neutrophils and only a very and non-diffuse thickening of alveolar walls is observed in the preconditioned group **(D)**. H&E, original magnifications, x 214

determined by at least two factors: an adenosine concentration high enough to induce NO generation through the activation of adenosine A2 receptors together with a low xanthine concentration to avoid the deleterious effects of this metabolite (Peralta et al., 1998). NO generation induced by preconditioning is likely to be derived from constitutive NO synthase (NOS). It has been reported that NOS activation following ischemic preconditioning occurs within a few minutes and preconditioning does not promote any difference in the inducible NOS activity after hepatic I/R (Peralta et al., 1996; Rudiger and Clavien, 2000; Koti et al., 2002).

Signalling pathways in ischemic preconditioning

Data reported in cultured hepatocytes points to the activation of adenosine receptors coupled to G proteins as a potential pathway that initiates the preconditioning response (Carini et al., 2001a,b). The receptor-coupled G protein, through different signals induces the activation of protein kinase C (PKC), which in turn affords phosphorylation of effector molecules, including p38 mitogen-activated protein kinase (p38 MAPK) (Fig. 2) (Carini et al., 2001a,b). Other effector molecules, such as ATP-sensitive K^+ channel (KATP), 5'nucleotidase and cAMP-protein kinase A activation (PKA), have been reported in the heart (Fig. 2)(Wyatt et al., 1989; Simkhovich et al., 1998; Tong et al., 2000; Ricciardi et al., 2001a). The activation of these intracellular signalling pathways, such as p38 MAPK, not only triggers increased tolerance of the hepatocytes and endothelial cells against ischemic insults but also causes quiescent cells to enter the cell cycle and to initiate a regenerative response (Teoh et al., 2002). Recently, it has been reported that NO can induce preconditioning of hepatocytes by promoting the sequential activation of guanylate cyclase, cyclic GMP-dependent kinase (cGK) and p38 MAPK (Carini et al., 2003). In addition, it has been reported that PKC could induce nuclear factor transcriptions in the heart, including nuclear factor transcription factor B (NF-κB), which govern the expression of protective genes responsible for the protection conferred by preconditioning (Maulik et al., 1998; Li et al., 2000). Several proteins have been proposed as possible effectors, including antioxidant enzymes like Mn-SOD and heat-shock proteins (HSP) (Carroll and Yellon, 1999; Bolli, 2000). Both the PKC and NF-κB activation as well as the increase in HSP expression induced by ischemic preconditioning have been demonstrated in the liver (Kume et al., 1996; Carini et al, 2001a,b; Teoh et al., 2002). In contrast to the heart, the role of PKC in liver preconditioning could be independent of NF-κB (Ricciardi et al, 2002). The possibility that NF-κB could induce HSP expression in liver preconditioning remains to be elucidated. In addition, it has been suggested that liver preconditioning through NF-κB could induce the activation of the transcription factor, signal and activator of transcription 3 (STAT3) (Teoh et al., 2003). The possibility that

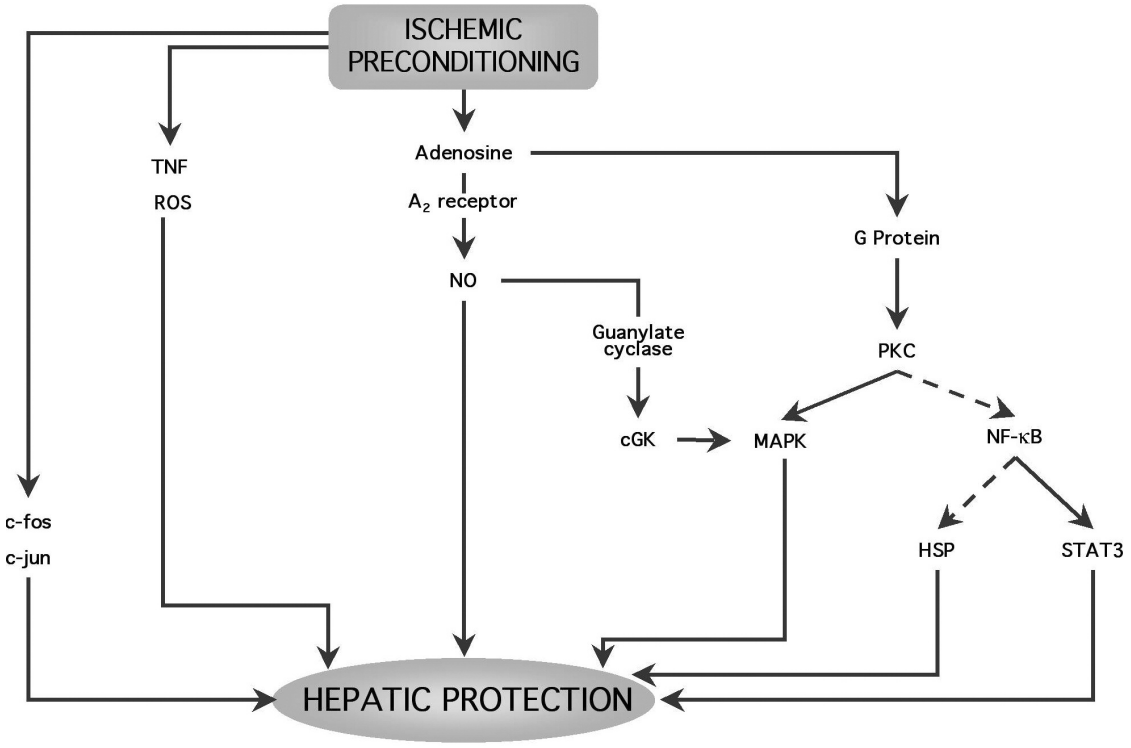

Fig. 2. Diagram showing the signalling pathways in ischemic preconditioning. Please see text.

preconditioning could decrease the transcription levels of immediate early genes, including c-fos and c-jun, protecting against hepatic I/R injury has also been suggested (Ishii et al., 2001; Saito et al., 2001).

In addition to the potential signalling pathways that trigger the above-mentioned ischemic preconditioning, the existence of parallel signalling pathways in preconditioning could be considered. In this line, recent works indicate that the preconditioning period may induce ROS burst (Sindram et al., 2002) or low TNF release (Teoh et al., 2003), thus protecting against subsequent hepatic I/R injury. Moreover, the inhibition of NF-κB activation induced by liver preconditioning has been demonstrated (Funaki et al., 2002) (see Fig. 2). The apparent controversial data on the effect of preconditioning on NF-κB activation in liver could be explained by the different in vivo experimental models of hepatic I/R evaluated.

Energy metabolism

Besides producing adenosine, the brief period of preconditioning was able to induce the activation of adenosine monophosphate-activated protein kinase (AMPK) (Peralta et al., 2001b). Once activated, AMPK responds by phosphorylating multiple downstream substrates aimed to conserve the existing ATP levels thus reducing the intermediate glucolytic and lactate accumulation during warm ischemia (Fig. 3). Its beneficial effect on energy metabolism seems to be independent of NO because the inhibition of NO synthesis in preconditioned group or the administration of NO donor before ischemia has no effect on energy metabolism (see Peralta et al., 2001b).

Neutrophil accumulation and microcirculatory alterations

The possibility that ischemic preconditioning could modulate the neutrophil accumulation and the microcirculatory alterations following hepatic I/R has been reported (Howell et al., 2000; Koti et al., 2002; Shinoda et al., 2002; Glanemann et al., 2003). In the field of liver transplantation, preconditioned grafts demonstrated improved hepatic tissue blood flow and decreased hepatic vascular resistance after cold storage (Ricciardi et al., 2000, 2001a,b). Similarly, beneficial effects of preconditioning on microcirculatory alterations following hepatic I/R have been reported in normothermic hepatic ischemia (Iwasaki et al., 2002; Shinoda et al., 2002; Glanemann et al., 2003). It has been suggested that preconditioning may act through the release of NO, which has vasodilator effects, and inhibits the vasoconstrictive effects of both stellate cells and inflammatory mediators such as ET (Peralta et al., 1996, 2000; Koti et al., 2002).

Although there are numerous studies on normothermic hepatic ischemia and liver transplantation which indicate the benefits of preconditioning on

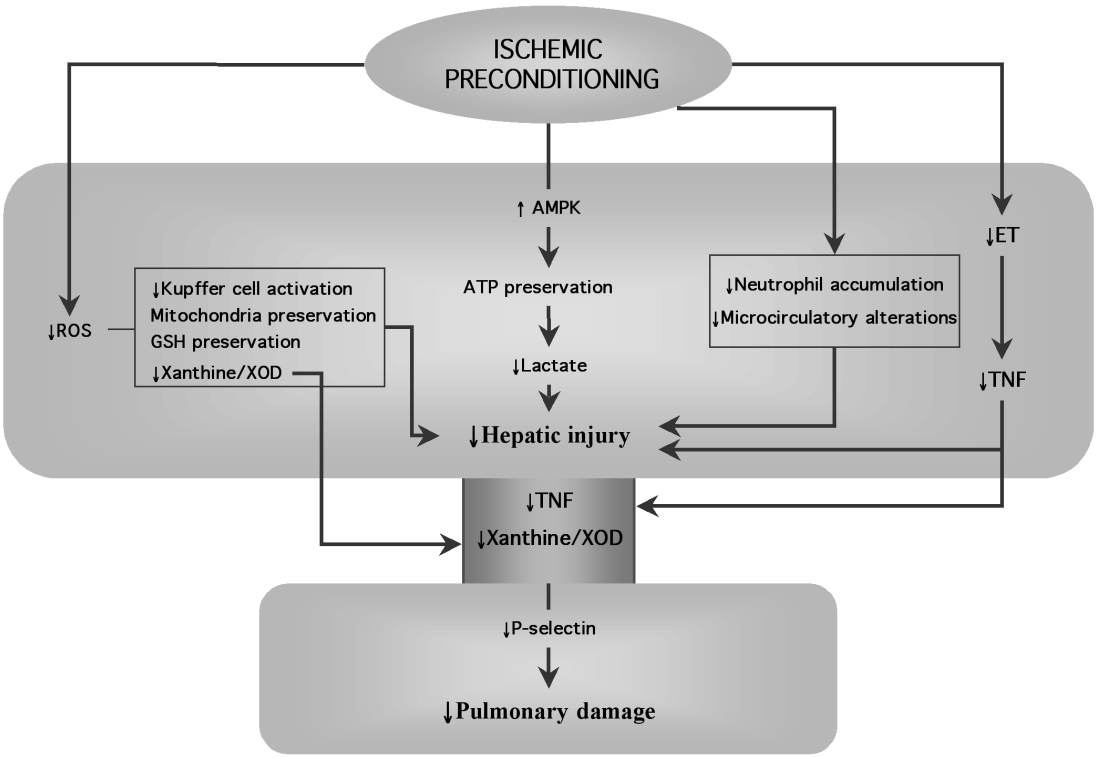

Fig. 3. Effect of preconditioning on the mechanisms responsible for hepatic I/R injury. In this diagram the effect of preconditioning on the energy metabolism, neutrophil accumulation, microcirculatory disturbances, formation of proinflammatory mediators such as endothelin and tumor necrosis factor-alpha and reactive oxygen species generation is represented. Please see text.

neutrophil accumulation following hepatic I/R (Howell et al., 2000; Koti et al., 2002; Shinoda et al., 2002; Glanemann et al., 2003), the mechanisms by which preconditioning modulate the hepatic leukocyte/endothelial cell interactions remain to be elucidated. Experimental data in normothermic ischemia showed reduced leucocyte adherence in sinusoids and postsinusoidal venules after the induction of preconditioning (Sawaya et al., 1999; Zapletal et al., 1999; Howell et al., 2000). However, there is some evidence indicating that the expression of adhesion molecules in the liver is not altered after preconditioning (Peralta et al., 2001a; Funaki et al., 2002). It has been suggested that the extensive vascular injury during reperfusion eliminates, in part, the sinusoidal endothelial cell barrier and that the neutrophil has direct accesss to the hepatocyte (Jaeschke, 2003). Thus, it could be hypothesized that preconditioning reduces neutrophil accumulation by preserving the endothelial cell integrity. Future investigations on the mechanisms by which preconditioning reduces neutrophil accumulation following hepatic I/R may help to design new therapies aimed at reducing reperfusion damage.

Pulmonary damage following hepatic I/R

The findings obtained from previous studies in liver preconditioning indicate that this surgical strategy is able of modulating local and systemic disorders associated with hepatic I/R in normothermic hepatic ischemia and liver transplantation (Peralta et al., 1996, 1999b, 2000, 2001a; Fernandez et al., 2002). In this line, preconditioning by inhibition of ET release after hepatic I/R could 1) attenuate the microvascular disorders and the inflammatory response associated with this process, 2) reduce the increase in hepatic TNF mainly released from Kupffer cells, thus attenuating the liver injury following hepatic I/R, and 3) prevent the systemic release of liver-associated TNF, thus preventing lung P-selectin up-regulation and the subsequent pulmonary damage associated with hepatic I/R (Peralta et al., 1996, 1999b, 2000, 2001a; Koti et al, 2002) (see Fig. 3). The inhibition of ET release by ischemic preconditioning is mediated by NO (Peralta et al., 1996, 1999b, 2000, 2001a, 2002b). Moreover, ischemic preconditioning is able to modulate other mechanisms involved in both local and systemic disorders including the ROS generating system xanthine/XOD (Peralta et al., 2002a; Fernandez et al, 2002). This surgical strategy, by reducing xanthine accumulation and the conversion of XDH to XOD during ischemia, protects the liver and lung damage associated with hepatic I/R. Moreover, it has been reported that preconditioning was able to increase the hepatic tolerance against reperfusion injury by attenuating the ROS production either by preserving the mitochondrial structure (Cutrin et al, 2002) or modulating Kupffer cell activation (Peralta et al., 2000, 2001a; Glanemann et al., 2003). The effect of preconditioning on antioxidant systems has been demonstrated. In this line, this surgical strategy is able to prevent the loss of glutathione content during hepatic ischemia thus decreasing the vulnerability of liver to ROS damage (Peralta et al., 2002a).

Ischemic preconditioning and fatty livers

Despite the significant improvement of outcome during the last decade, the dramatic organ shortage for transplantation forces the consideration of steatotic grafts as another option, although they have a higher vulnerability to I/R injury. The occurrence of postoperative liver failure after hepatic resection in a steatotic liver exposed to normothermic ischemia has been reported (Todo et al., 1989; D'Alessandro et al., 1991; Huguet et al., 1991). In addition, the use of steatotic livers for transplantation is associated with an increased risk for primary nonfunction or dysfunction after surgery (Behrns et al., 1989; Strasberg et al., 1994;

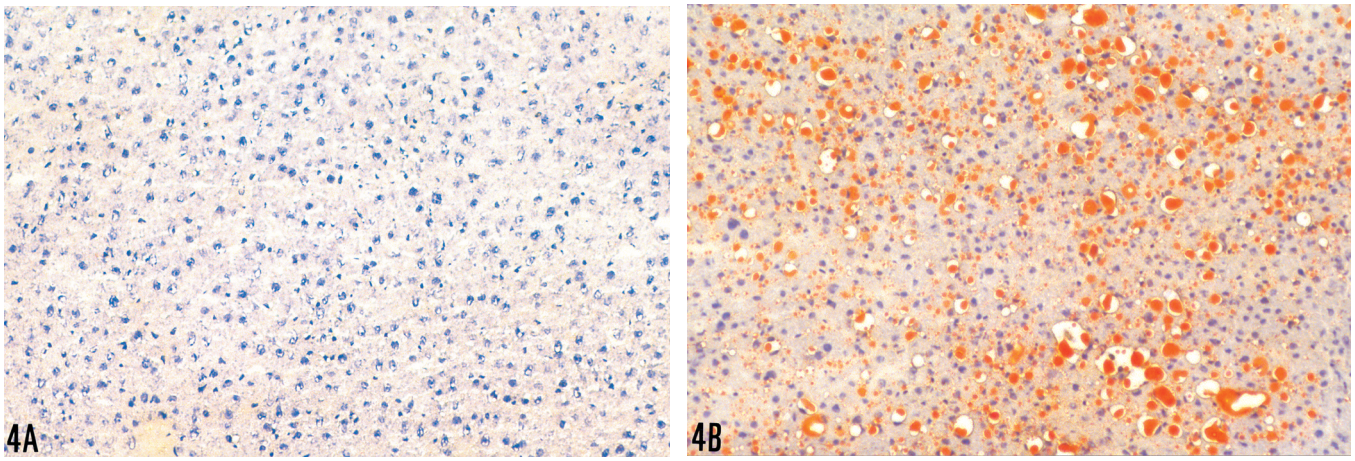

Fig. 4. Difference of steatosis in the obese vs. lean Zucker rats using red oil staining. **A.** Lean Zucker rats have no evidence of steatosis. **B.** Obese Zucker rats show fatty infiltration in hepatocytes. x 214

Trevisani et al., 1996). A recent study of normothermic ischemia conditions (Serafin et al., 2002), indicates the effectiveness of ischemic preconditioning against the increased vulnerability of fatty livers to hepatic I/R injury in Zucker rats showing severe macrovesicular and microvesicular fatty infiltration in hepatocytes (between 60% and 70% steatosis) (see Fig. 4). Preconditioning through NO generation was capable of controlling the mechanisms potentially involved in the vulnerability of fatty livers to hepatic I/R, including oxidative stress, neutrophil accumulation, and microcirculatory failure, thus reducing the subsequent hepatic injury (Serafin et al., 2002). The histological findings are shown in Fig. 5

Ischemic preconditioning and pharmacological strategies

Several studies have compared the effectiveness of ischemic preconditioning versus pharmacological strategies aimed to modulate hepatic I/R injury in normothermic ischemia and liver transplantation (Nolte et al., 1991; Downey et al., 1994; Peralta et al., 1997, 1999a,b, 2001a, 2002b; Yin et al., 1998; Howell et al., 2000; Ito et al., 2000; Kume et al., 2000; Fernandez et al, 2002; Teoh et al., 2003). In this regard, it has been reported that to minimize the liver and lung damage following liver transplantation, the addition of XOD inhibitors to the preservation solutions may be less efficient than strategies including preconditioning, which prevent the formation of xanthine/XOD in liver grafts during cold ischemia (Fernandez et al., 2002). Recent studies in human liver transplantation indicating high activities of human plasma XOD during reperfusion suggest that the use of inhibitors of XOD in preservation solutions may not be sufficient to eliminate ROS generation from XOD (Pesonen et al., 1998). According to our results, the effect of ischemic preconditioning by

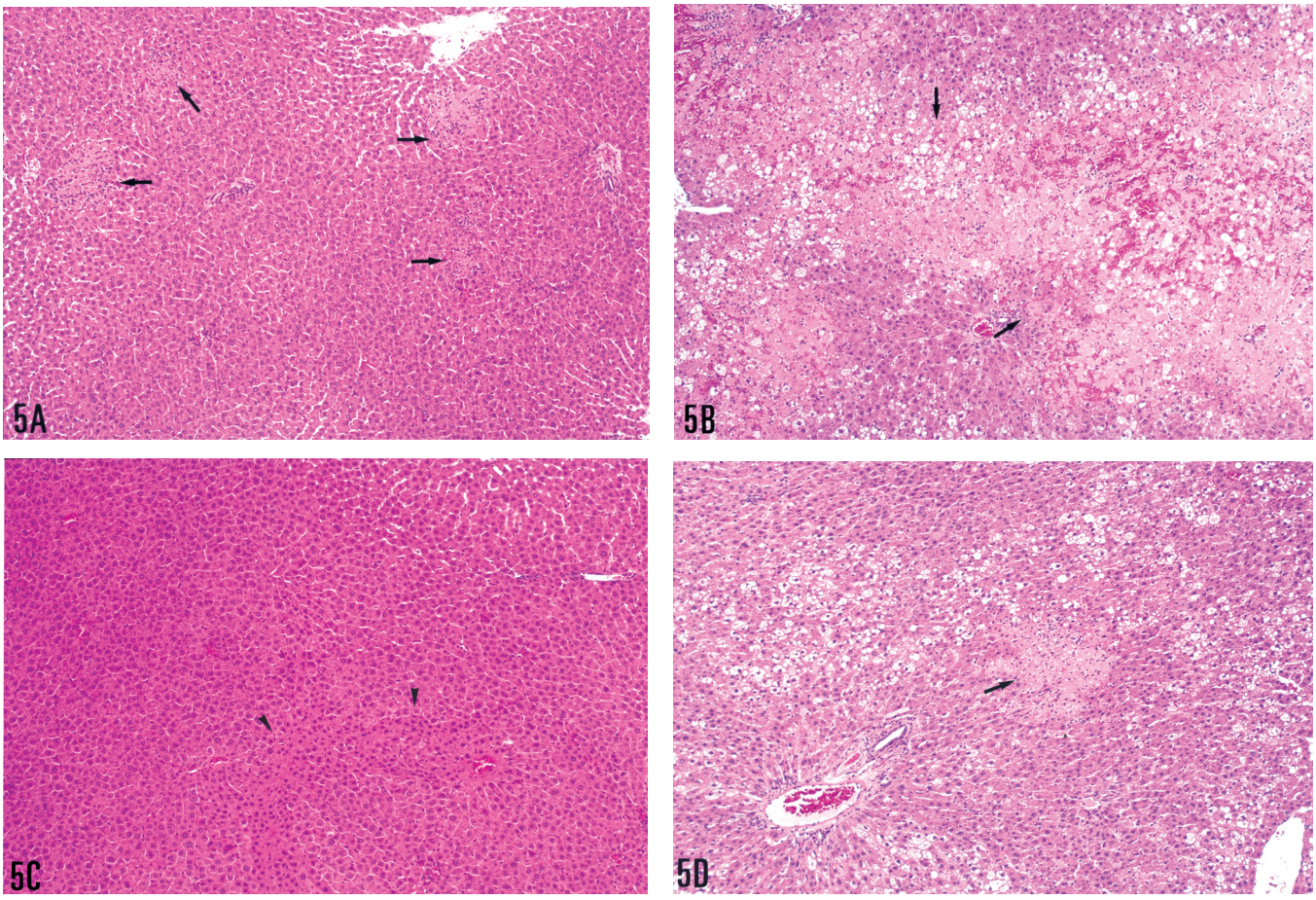

Fig. 5. Histological lesions in normal and fatty livers subjected to 60 min of normothermic hepatic ischemia. The histological study in normal livers shows moderate and multifocal areas of coagulative necrosis and neutrophil infiltration at 24 h of reperfusion, randomly distributed through the parenchyma (arrow) **(A)**, whereas severe, extensive, and confluent areas of coagulative necrosis with neutrophil infiltration (arrow) are observed in fatty livers (**B**). In contrast, when preconditioning is carried out, the extent and number of necrotic areas at 24 h after hepatic reperfusion is markedly reduced in normal and fatty livers with respect to I/R. In the case of normal livers, these areas are mainly of incipient hepatocyte necrosis (arrowhead) **(C)** whereas in fatty livers patchy areas of incipient hepatocyte necrosis and scattered multifocal areas of coagulative hepatocyte necrosis (arrow) are observed (**D**). H&E x 107

itself, and independently of the preservation solution, was more effective against lung damage (Fernandez et al., 2002). In addition, in normothermic conditions, ischemic preconditioning has been reported to be more effective than antiselectin or anti-TNF therapies in preventing hepatic I/R injury (Peralta et al., 1999b, 2001a, 2002b). The multiple mechanisms of I/R injury suggest the inherent difficulties in the effective prevention of hepatic I/R injury by using pharmacological strategies focused in the inhibition of individual mediators or factors responsible for this pathology. On the other hand, like preconditioning, different pharmacological strategies consisting in the pre-treatment with low doses of TNF, NO donors, adenosine, and the anticancer drug, doxorubicin, could simulate the benefits of preconditioning on hepatic I/R injury (Nolte et al., 1991; Downey et al., 1994; Peralta et al., 1997, 1999a; Yin et al., 1998; Howell et al., 2000; Ito et al., 2000; Kume et al., 2000; Teoh et al, 2003). It should be noted that experience with ischemic preconditioning in humans indicates that this is a simple and powerful protective strategy against hepatic I/R injury in clinical practice (Clavien et al., 2000). Preconditioning is easy to apply, inexpensive and does not require a drug with potential side effects.

Conclusion

Preconditioning could be a coming approach for preventing reperfusion injury, which appears to increase the resistance of liver cells to I/R events. Preconditioning has the greatest potential to improve the clinical outcome in liver transplantation and liver surgery associated with hepatic resections. It could afford the possibility of improving the initial conditions of the organs avaible for human liver transplantation, particularly organs considered marginal or suboptimal. Although clear benefits of ischemic preconditioning have been demonstrated in the heart for years, clinical applications have been slow to be manifested (Ricciardi et al., 2001b). Given that the benefits of ischemic preconditioning in hepatic cold preservation as well as in fatty livers subjected to normothermic ischemia have been newly discovered, further research is needed to better characterize the underlying protective mechanisms.

Acknowledgements. We thank the Ministerio de Ciencia y Tecnología (Madrid, Spain) for the Ramón y Cajal Research Contract to C. Peralta. This work was supported by the Agencia Española de Cooperación Internacional through the project 2002CN0012 and the Ministerio de Ciencia y Tecnología (BF-2002-00704 project).

References

Behrns K.E., Tsiotos G.G., DeSouza N.F., Krishna M.K., Ludwig J. and Nagorney D.M. (1998). Hepatic steatosis as a potential risk factor for major hepatic resection. J. Gastrointest. Surg. 2, 292-298.

Bilzer M. and Gerbes A.L. (2000). Preservation injury of the liver: mechanisms and novel therapeutic strategies. J. Hepatol. 32, 508-515.

Bolli R. (2000). The late phase of preconditioning. Circ. Res. 87, 972-983.

Carini R., De Cesaris M.G., Splendore R. and Albano E. (2001a). Stimulation of p38 MAP kinase reduces acidosis and Na(+) overload in preconditioned hepatocytes. FEBS Lett. 491, 180-183.

Carini R., De Cesaris M.G., Splendore R., Vay D., Domenicotti C., Nitti M.P., Paola D., Pronzato M.A. and Albano E. (2001b). Signal pathway involved in the development of hypoxic preconditioning in rat hepatocytes. Hepatology 33, 131-139.

Carini R., De Cesaris M.G., Splendore R., Domenicotti C., Nitti M.P., Pronzato M.A. and Albano E. (2003). Signal pathway responsible for hepatocyte preconditioning by nitric oxide. Free Radic. Biol. Med. 34, 1047-1055.

Carroll R. and Yellon D.M. (1999). Myocardial adaptation to ischaemia: the preconditioning phenomenon. Int. J. Cardiol. 68 (Suppl 1), S93-S101.

Clavien P.A., Harvey P.R. and Strasberg S.M. (1992). Preservation and reperfusion injuries in liver allografts. An overview and synthesis of current studies. Transplantation 53, 957-978.

Clavien P.A., Yadav S., Sindram D. and Bentley R.C. (2000). Protective effects of ischemic preconditioning for liver resection performed under inflow occlusion in humans. Ann. Surg. 232, 155-162.

Colletti L.M., Burtch G.D., Remick D.G., Kunkel S.L., Strieter R.M., Guice K.S., Oldham K.T. and Campbell D.A. Jr. (1990a). The production of tumor necrosis factor alpha and the development of a pulmonary capillary injury following hepatic ischemia/reperfusion. Transplantation 49, 268-272.

Colletti L.M., Remick D.G., Burtch G.D., Kunkel S.L., Strieter R.M. and Campbell D.A. Jr. (1990b). Role of tumor necrosis factor-alpha in the pathophysiologic alterations after hepatic ischemia/reperfusion injury in the rat. J. Clin. Invest. 85, 1936-1943.

Cutrin J.C., Perrelli M.G., Cavalieri B., Peralta C., Rosello-Catafau J. and Poli G. (2002). Microvascular dysfunction induced by reperfusion injury and protective effect of ischemic preconditioning. Free Radic. Biol. Med. 33, 1200-1208.

D'Alessandro A.M., Kalayoglu M., Sollinger H.W., Hoffmann R.M., Reed A., Knechtle S.J., Pirsch J.D., Hafez G.R., Lorentzen D. and Belzer F.O. (1991). The predictive value of donor liver biopsies on the development of primary nonfunction after orthotopic liver transplantation. Transplant. Proc. 23, 1536-1537.

Downey J.M., Cohen M.V., Ytrehus K. and Liu Y. (1994). Cellular mechanisms in ischemic preconditioning: the role of adenosine and protein kinase C. Ann. NY. Acad. Sci. 723, 82-98.

Fan C., Zwacka R.M. and Engelhardt J.F. (1999). Therapeutic approaches for ischemia/reperfusion injury in the liver. J. Mol. Med. 77, 577-592.

Fernandez L., Heredia N., Grande L., Gomez G., Rimola A., Marco A., Gelpi E., Rosello-Catafau J. and Peralta C. (2002). Preconditioning protects liver and lung damage in rat liver transplantation: role of xanthine/xanthine oxidase. Hepatology 36, 562-572.

Funaki H., Shimizu K., Harada S., Tsuyama H., Fushida S., Tani T. and Miwa K. (2002). Essential role for nuclear factor kappaB in ischemic preconditioning for ischemia-reperfusion injury of the mouse liver. Transplantation 74, 551-556.

Glanemann M., Vollmar B., Nussler A.K., Schaefer T., Neuhaus P. and Menger M.D. (2003). Ischemic preconditioning protects from hepatic

ischemia/reperfusion-injury by preservation of microcirculation and mitochondrial redox-state. J. Hepatol. 38, 59-66.

Goto M., Takei Y., Kawano S., Nagano K., Tsuji S., Masuda E., Nishimura Y., Okumura S., Kashiwagi T., Fusamoto H. and Kamada T. (1994). Endothelin-1 is involved in the pathogenesis of ischemia/reperfusion liver injury by hepatic microcirculatory disturbances. Hepatology 19, 675-681.

Horton A.A. and Fairhurst S. (1987). Lipid peroxidation and mechanisms of toxicity. Crit. Rev. Toxicol. 18, 27-79.

Howell J.G., Zibari G.B., Brown M.F., Burney D.L., Sawaya D.E., Olinde J.G., Granger D.N. and McDonald J.C. (2000). Both ischemic and pharmacological preconditioning decrease hepatic leukocyte/endothelial cell interactions. Transplantation 69, 300-303.

Huguet C., Gavelli A., Harb J., Roger-Clement R., De Millo D., Lasserre M. and McNamara M.T. (1991). L'ischemie du foie de plus d'une heure est-elle un facteur e gravité des hepatectomies? À propos de 19 cas. Chirurgie 117, 351-356.

Huguet C., Gavelli A., Chieco P.A., Bona S., Harb J., Joseph J.M., Jobard J., Gramaglia M. and Lasserre M. (1992). Liver ischemia for hepatic resection: where is the limit? Surgery 111, 251-259.

Ishii S., Abe T., Saito T., Tsuchiya T., Kanno H., Miyazawa M., Suzuki M., Motoki R. and Gotoh M. (2001). Effects of preconditioning on ischemia/reperfusion injury of hepatocytes determined by immediate early gene transcription. J. Hepatobiliary Pancreat. Surg. 8, 461-468.

Ito K., Ozasa H., Sanada K. and Horikawa S. (2000). Doxorubicin preconditioning: a protection against rat hepatic ischemia-reperfusion injury. Hepatology 31, 416-419.

Iwasaki Y., Tagaya N., Hattori Y., Yamaguchi K. and Kubota K. (2002). Protective effect of ischemic preconditioning against intermittent warm-ischemia-induced liver injury. J. Surg. Res. 107, 82-92.

Jaeschke H. (2003). Molecular mechanisms of hepatic ischemia-reperfusion injury and preconditioning. Am. J. Physiol. 284, G15-G26.

Jaeschke H., Farhood A. and Smith C.W. (1990). Neutrophils contribute to ischemia/reperfusion injury in rat liver in vivo. FASEB J. 4, 3355-3359.

Koti R.S., Yang W., Dashwood M.R., Davidson B.R. and Seifalian A.M. (2002). Effect of ischemic preconditioning on hepatic microcirculation and function in a rat model of ischemia reperfusion injury. Liver Transpl. 8, 1182-1191.

Kukan M. and Haddad P.S. (2001). Role of hepatocytes and bile duct cells in preservation-reperfusion injury of liver grafts. Liver Transpl. 7, 381-400.

Kume M., Yamamoto Y., Saad S., Gomi T., Kimoto S., Shimabukuro T., Yagi T., Nakagami M., Takada Y., Morimoto T. and Yamaoka Y. (1996). Ischemic preconditioning of the liver in rats: implications of heat shock protein induction to increase tolerance of ischemia-reperfusion injury. J. Lab. Clin. Med. 128, 251-258.

Kume M., Yamamoto Y., Yamagami K., Ishikawa Y., Uchinami H. and Yamaoka Y. (2000). Pharmacological hepatic preconditioning: involvement of 70-kDa heat shock proteins (HSP72 and HSP73) in ischaemic tolerance after intravenous administration of doxorubicin. Br. J. Surg. 87, 1168-1175.

Lentsch A.B., Kato A., Yoshidome H., McMasters K.M. and Edwards M.J. (2000). Inflammatory mechanisms and therapeutic strategies for warm hepatic ischemia/reperfusion injury. Hepatology 32, 169-173.

Li R.C., Ping P., Zhang J., Wead W.B., Cao X., Gao J., Zheng Y., Huang S., Han J. and Bolli R. (2000). PKCepsilon modulates NF-kappaB and AP-1 via mitogen-activated protein kinases in adult rabbit cardiomyocytes. Am. J. Physiol. 279, H1679-H1689.

Lichtman S.N. and Lemasters J.J. (1999). Role of cytokines and cytokine-producing cells in reperfusion injury to the liver. Semin. Liver Dis. 19, 171-187.

Matuschak G.M., Rinaldo J.E., Pinski M.R., Gavaler J.S. and Van Thiel D.H. (1987). Effect of end stage liver failure on the incidence and resolution of the adult respiratory distress syndrome. J. Crit. Care 2, 162-173.

Maulik N., Sato M., Price B.D. and Das D.K. (1998). An essential role of NFkappaB in tyrosine kinase signaling of p38 MAP kinase regulation of myocardial adaptation to ischemia. FEBS Lett. 429, 365-369.

Muller M.J., Vollmar B., Friedl H.P. and Menger M.D. (1996). Xanthine oxidase and superoxide radicals in portal triad crossclamping-induced microvascular reperfusion injury of the liver. Free Radic. Biol. Med. 21, 189-197.

Murry C.E., Jennings R.B. and Reimer K.A. (1986). Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 74, 1124-1136.

Nolte D., Lehr H.A. and Messmer K. (1991). Adenosine inhibits postischemic leukocyte-endothelium interaction in postcapillary venules of the hamster. Am. J. Physiol. 261, H651-H655.

Peralta C., Closa D., Hotter G., Gelpi E., Prats N. and Rosello-Catafau J. (1996). Liver ischemic preconditioning is mediated by the inhibitory action of nitric oxide on endothelin. Biochem. Biophys. Res. Commun. 229, 264-270.

Peralta C., Hotter G., Closa D., Gelpi E., Bulbena O. and Rosello-Catafau J. (1997). Protective effect of preconditioning on the injury associated to hepatic ischemia-reperfusion in the rat: role of nitric oxide and adenosine. Hepatology 25, 934-937.

Peralta C., Closa D., Xaus C., Gelpi E., Rosello-Catafau J. and Hotter G. (1998). Hepatic preconditioning in rats is defined by a balance of adenosine and xanthine. Hepatology 28, 768-773.

Peralta C., Hotter G., Closa D., Prats N., Xaus C., Gelpi E. and Rosello-Catafau J. (1999a). The protective role of adenosine in inducing nitric oxide synthesis in rat liver ischemia preconditioning is mediated by activation of adenosine A2 receptors. Hepatology 29, 126-132.

Peralta C., Prats N., Xaus C., Gelpi E. and Rosello-Catafau J. (1999b). Protective effect of liver ischemic preconditioning on liver and lung injury induced by hepatic ischemia-reperfusion in the rat. Hepatology 30, 1481-1489.

Peralta C., Bulbena O., Bargallo R., Prats N., Gelpi E. and Rosello-Catafau J. (2000). Strategies to modulate the deleterious effects of endothelin in hepatic ischemia-reperfusion. Transplantation 70, 1761-1770.

Peralta C., Fernandez L., Panes J., Prats N., Sans M., Pique J.M., Gelpi E. and Rosello-Catafau J. (2001a). Preconditioning protects against systemic disorders associated with hepatic ischemia-reperfusion through blockade of tumor necrosis factor-induced P-selectin up-regulation in the rat. Hepatology 33, 100-113.

Peralta C., Bartrons R., Serafin A., Blazquez C., Guzman M., Prats N., Xaus C., Cutillas B., Gelpi E. and Rosello-Catafau J. (2001b). Adenosine monophosphate-activated protein kinase mediates the protective effects of ischemic preconditioning on hepatic ischemia-reperfusion injury in the rat. Hepatology 34, 1164-1173.

Peralta C., Bulbena O., Xaus C., Prats N., Cutrin J.C., Poli G., Gelpi E. and Rosello-Catafau J. (2002a). Ischemic preconditioning: a

defense mechanism against the reactive oxygen species generated after hepatic ischemia reperfusion. Transplantation 73, 1203-1211.

Peralta C., Perales J.C., Bartrons R., Mitchell C., Gilgenkrantz H., Xaus C., Prats N., Fernandez L., Gelpi E., Panes J. and Rosello-Catafau J. (2002b). The combination of ischemic preconditioning and liver Bcl-2 overexpression is a suitable strategy to prevent liver and lung damage after hepatic ischemia-reperfusion. Am. J. Pathol. 160, 2111-2122.

Pesonen E.J., Linder N., Raivio K.O., Sarnesto A., Lapatto R., Hockerstedt K., Makisalo H. and Andersson S. (1998). Circulating xanthine oxidase and neutrophil activation during human liver transplantation. Gastroenterology 114, 1009-1015.

Ricciardi R., Chari R.S., Schaffer B.K., Kim R.D., Shah S.A., Potter M.W., Quarfordt S.H., Callery M.P. and Meyers W.C (2000). Ischemic preconditioning protects liver grafts via tyrosine kinases. Owen H. Wangensteen Surgical Forum.

Ricciardi R., Meyers W.C., Schaffer B.K., Kim R.D., Shah S.A., Wheeler S.M., Donohue S.E., Sheth K.R., Callery M.P. and Chari R.S. (2001a). Protein kinase C inhibition abrogates hepatic ischemic preconditioning responses. J. Surg. Res. 97, 144-149.

Ricciardi R., Schaffer B.K., Kim R.D., Shah S.A., Donohue S.E., Wheeler S.M., Quarfordt S.H., Callery M.P., Meyers W.C. and Chari R.S. (2001b). Protective effects of ischemic preconditioning on the cold-preserved liver are tyrosine kinase dependent. Transplantation 72, 406-412.

Ricciardi R., Shah S.A., Wheeler S.M., Quarfordt S.H., Callery M.P., Meyers W.C. and Chari R.S. (2002). Regulation of NFkappaB in hepatic ischemic preconditioning. J. Am. Coll. Surg. 195, 319-326.

Rosser B.G. and Gores G.J. (1995). Liver cell necrosis: cellular mechanisms and clinical implications. Gastroenterology 108, 252-275.

Rudiger H.A. and Clavien P.A. (2000). A cytotoxic drug against reperfusion injury?. Hepatology 31, 533-535.

Saito T., Ishii S., Abe T., Tsuchiya T., Kanno H., Miyazawa M., Suzuki M. and Gotoh M. (2001). Effect of preconditioning in the liver against ischemia/reperfusion injury, protection of sinusoidal cells and alterations of gene transcription. Transplant. Proc. 33, 849.

Sawaya D.E. Jr, Brown M., Minardi A., Bilton B., Burney D., Granger D.N., McDonald J.C. and Zibari G.B. (1999). The role of ischemic preconditioning in the recruitment of rolling and adherent leukocytes in hepatic venules after ischemia/reperfusion. J. Surg. Res. 85, 163-170.

Schmid-Schonbein G.W. (1987). Capillary plugging by granulocytes and the no-reflow phenomenon in the microcirculation. Fed. Proc. 46, 2397-2401.

Selzner M. and Clavien P.A. (1999). Resections of liver tumors: special emphasis on neoadjuvant and adjuvant therapy In: Malignant liver tumors-current and emerging therapies. Malden M.A. (ed). Oxford. Blackwell Science. pp 137-149.

Serafin A., Rosello-Catafau J., Prats N., Xaus C., Gelpi E. and Peralta C. (2002). Ischemic preconditioning increases the tolerance of Fatty liver to hepatic ischemia-reperfusion injury in the rat. Am. J. Pathol. 161, 587-601.

Serracino-Inglott F., Habib N.A. and Mathie R.T. (2001). Hepatic ischemia-reperfusion injury. Am. J. Surg. 181, 160-166.

Shinoda M., Shimazu M., Wakabayashi G., Tanabe M., Hoshino K. and Kitajima M. (2002). Tumor necrosis factor suppression and microcirculatory disturbance amelioration in ischemia/reperfusion injury of rat liver after ischemic preconditioning. J. Gastroenterol. Hepatol. 17, 1211-1219.

Simkhovich B.Z., Przyklenk K. and Kloner R.A. (1998). Role of protein kinase C as a cellular mediator of ischemic preconditioning: a critical review. Cardiovasc. Res. 40, 9-22.

Sindram D., Rudiger H.A., Upadhya A.G., Strasberg S.M. and Clavien P.A. (2002). Ischemic preconditioning protects against cold ischemic injury through an oxidative stress dependent mechanism. J. Hepatol. 36, 78-84.

Strasberg S.M., Howard T.K., Molmenti E.P. and Hertl M. (1994). Selecting the donor liver: risk factors for poor function after orthotopic liver transplantation. Hepatology 20, 829-838.

Teoh N., Dela Pena A. and Farrell G. (2002). Hepatic ischemic preconditioning in mice is associated with activation of NF-kappaB, p38 kinase, and cell cycle entry. Hepatology 36, 94-102.

Teoh N., Leclercq I., Pena A.D. and Farrell G. (2003). Low-dose TNF-alpha protects against hepatic ischemia-reperfusion injury in mice: implications for preconditioning. Hepatology 37, 118-128.

Todo S., Demetris A.J., Makowka L., Teperman L., Podesta L., Shaver T., Tzakis A. and Starzl T.E. (1989). Primary nonfunction of hepatic allografts with preexisting fatty infiltration. Transplantation 47, 903-905.

Tong H., Chen W., Steenbergen C. and Murphy E. (2000). Ischemic preconditioning activates phosphatidylinositol-3-kinase upstream of protein kinase C. Circ. Res. 87, 309-315.

Trevisani F., Colantoni A., Caraceni P. and Van Thiel D.H. (1996). The use of donor fatty liver for liver transplantation: a challenge or a quagmire? J. Hepatol. 24, 114-121.

Vajdova K., Smrekova R., Mislanova C., Kukan M. and Lutterova M. (2000). Cold-preservation-induced sensitivity of rat hepatocyte function to rewarming injury and its prevention by short-term reperfusion. Hepatology 32, 289-296.

Wyatt D.A., Edmunds M.C., Rubio R., Berne R.M., Lasley R.D. and Mentzer R.M. Jr. (1989). Adenosine stimulates glycolytic flux in isolated perfused rat hearts by A1-adenosine receptors. Am. J. Physiol. 257, H1952-H1957.

Yadav S.S., Sindram D., Perry D.K. and Clavien P.A. (1999). Ischemic preconditioning protects the mouse liver by inhibition of apoptosis through a caspase-dependent pathway. Hepatology 30, 1223-1231.

Yin D.P., Sankary H.N., Chong A.S., Ma L.L., Shen J., Foster P. and Williams J.W. (1998). Protective effect of ischemic preconditioning on liver preservation-reperfusion injury in rats. Transplantation 66, 152-157.

Zapletal C., Maksan S.M., Lehmann T., Guenther L., Fallsehr C., Mehrabi A., Weiss G., Golling M., Gebhard M.M., Herfarth C. and Klar E. (1999). Ischemic preconditioning improves liver microcirculation after ischemia/reperfusion. Transplant. Proc. 31, 3260-3262.

Accepted August 21, 2003