



since

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Microvascular dysfunction induced by reperfusion injury and protective effect of ischemic preconditioning.

This is the author's manuscript

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/81873

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



PII S0891-5849(02)01017-1

-Review Article

MICROVASCULAR DYSFUNCTION INDUCED BY REPERFUSION INJURY AND PROTECTIVE EFFECT OF ISCHEMIC PRECONDITIONING

JUAN C. CUTRÌN,* MARIA-GIULIA PERRELLI,* BARBARA CAVALIERI,* CARMEN PERALTA,[†] JOAN ROSELLÓ CATAFAU,[†] and GIUSEPPE POLI*

*Laboratory of Experimental Liver Pathology, Department of Clinical and Biological Sciences, University of Torino, Torino, Italy; and [†]Department of Medical Bioanalysis, Institute of Biomedical Investigations of Barcelona, Barcelona, Spain

(Received 29 April 2002; Revised 8 July 2002; Accepted 11 July 2002)

Abstract—Hepatic ischemia/reperfusion injury has immediate and deleterious effects on the outcome of patients after liver surgery. The precise mechanisms leading to the damage have not been completely elucidated. However, there is substantial evidence that the generation of oxygen free radicals and disturbances of the hepatic microcirculation are involved in this clinical syndrome. Microcirculatory dysfunction of the liver seems to be mediated by sinusoidal endothelial cell damage and by the imbalance of vasoconstrictor and vasodilator molecules, such as endothelin (ET), reactive oxygen species (ROS), and nitric oxide (NO). This may lead to no-reflow phenomenon with release of proinflammatory cytokines, sinusoidal plugging of neutrophils, oxidative stress, and as an ultimate consequence, hypoxic cell injury and parenchymal failure. An inducible potent endogenous mechanism against ischemia/reperfusion injury has been termed ischemic preconditioning. It has been suggested that preconditioning could inhibit the effects of different mediators involved in the microcirculatory dysfunction, including endothelin, tumor necrosis factor- α , and oxygen free radicals. In this review, we address the mechanisms of liver microcirculatory dysfunction and how ischemic preconditioning could help to provide new surgical and/or pharmacological strategies to protect the liver against reperfusion damage. © 2002 Elsevier Science Inc.

Keywords-Reperfusion injury, Microcirculatory dysfunction, Free radicals, Ischemic preconditioning

INTRODUCTION

What is hepatic microvascular dysfunction?

Hepatic injury secondary to warm ischemia and reperfusion is an important clinical issue. It has been implicated in the pathogenesis of a variety of clinical conditions including trauma, thermal injury, hypovolemic and endotoxin shock, reconstructive vascular surgery, liver transplantation, and liver resectional surgery [1–7]. A considerable number of experimental studies have indicated that ischemia/reperfusion-induced liver injury occurs in a biphasic manner [5,8,9]. Data obtained by several different research groups suggest that in both early and late phases of reperfusion injury, oxidative stress is one of the main pathogenic mechanisms [5,10,11]. The early phase, which occurs between 0.5 and 4 h from the onset of reperfusion, appears to be associated with the generation of reactive oxygen species (ROS) by the activated Kupffer cell and by the reduced

Juan C. Cutrìn, M.D., Pathologist, Ph.D. in Experimental Pathology at the School of Pharmacy and Biochemistry of the University of Buenos Aires, Argentina, and Researcher at the Department of Clinical and Biological Sciences, University of Turin, Italy.

Maria-Giulia Perrelli, B.Sc., Ph.D. student in Molecular and Experimental Pathology, University of Turin, Italy.

Barbara Cavalieri, B.Sc., Ph.D. student in Biology and Pathology of Aging, University of Genova, Italy.

Carmen Peralta, Ph.D. at the University of Barcelona, Spain and Investigator of the Superior Council of Scientific Researches, Barcelona, Spain.

Joan Roselló Catafau, Ph.D. at the University of Barcelona; Investigator of the Superior Council of Scientific Researches and Director of

the Department of Medical Bioanalysis of the Institute of Biomedical Researches of Barcelona, "August Pi i Sunyer" IDIBAPS-CSIC, Spain.

Giuseppe Poli, Professor of General Pathology at the School of Medicine of the University of Turin; M.D. at the University of Turin; Ph.D. in Biochemistry at Brunel University of West London, England; Honorary Doctor Universitatis at the University of Buenos Aires, Argentina.

Address correspondence to: Dr. Juan C. Cutrìn, Laboratorio di Patologia, Epatica Sperimentale, Dipartimento di Scienze Cliniche e Biologiche, Università di Torino, Regione Gonzole 10, 10043 Orbassano, Torino, Italy; Tel: +39 (011) 670-8122; Fax: +39 (011) 670-8124; E-Mail: juancarlos.cutrin@unito.it.



Fig. 1. Hepatic microvasculature. Cell components and their relationships under the basal condition (A) and during the course of microvascular dysfunction (B).

respiratory chains in hepatocytes and endothelial sinusoidal cells [5,8,12–14]. Furthermore, during this initial period, neutrophils are primed and activated. In the late phase of injury, between 6 and 24 h from reperfusion, an evolving inflammatory process occurs, which is mediated by oxidants of extrahepatic cellular origin [8,12,15].

Although the concept of lethal reperfusion damage of parenchymal cells is not universally accepted, it is becoming widely recognized that the hepatic microvasculature, i.e., the sinusoidal space with the lining endothelium, is particularly vulnerable to the deleterious consequences of ischemia and reperfusion [16–19].

CELLULAR AND MOLECULAR EVENTS MAINLY RESPONSIBLE FOR THE HEPATIC MICROVASCULAR DYSFUNCTION

Microvascular dysfunction results from a series of events that involve the interaction of intravascular blood cells, i.e., neutrophils, with nonparenchymal cells, endothelial and Kupffer cells, and are mediated by the synthesis and release of adhesion proteins, cytokines, reactive oxygen species (ROS), nitric oxide (NO), and endothelins (ET) [20–22] (Fig. 1A, B). It is important to note that many of the ischemia-dependent cell changes, i.e., depletion of energy stores, increase of cellular volume due to altered ion homeostasis, reduced production of certain bioactive molecules (e.g., prostacyclin, nitric oxide), and increased formation of others (e.g., endothelin, thromboxane A2), are exacerbated by reoxygenation. Changes include: increase of swelling, lifting of endothelial cells from the underlying basement membrane, vasoconstriction as a result of a net imbalance between NO and endothelin-1 production, primed-neutrophil entrapment, and platelet aggregation. The entrapment of neutrophils favors homo-type (neutrophil-neutrophil) and hetero-type (neutrophil-platelet) aggregations with further worsening of flow hindrance in the sinusoidal space and prolongation of hypoxic conditions [20,22,23].

In this scenario, Kupffer cells become activated. These resident phagocytes produce ROS and proinflammatory mediators, such as interleukins and TNF- α , enhancing the expression of endothelial cell adhesion molecules, such as ICAM-1 and P-selectin, and priming circulatory neutrophils [24–27]. On the other hand, activated Kupffer cells also polarize and protrude into the sinusoidal lumen, where they come into close contact with circulating blood cells and interfere with the movement of primed and stiffened neutrophils. Another mechanism involved in the pathogenesis of the microvascular dysfunction is the local complement activation with the formation of chemotactic and cytotoxic products. Whereas C5a contributes to cell damage by means of neutrophil recruitment and activation, platelet accumulation, and Kupffer cell activation, MAC deposition at the cell surface aggravates membrane damage by its direct cytolytic effect [28–33].

Accordingly, the ischemia/reperfusion-induced microvascular dysfunction can be substantially explained by means of: (i) imbalance between the rate of NO production and the release of endothelin-1; (ii) alteration of the ratio between NO and superoxide anion; (iii) degree of neutrophil adhesion and activation.

Imbalance between NO and endothelin levels in the endothelial sinusoidal cells

ET has been reported to provoke contraction of the stellate cells in the sinusoid, via phospholipase C activation and interactivations with membrane ion channels, and subsequently to cause sinusoidal constriction [34–36]. On the other hand, NO, especially that derived from endothelial nitric oxide synthase (eNOS) in sinusoidal endothelial cells, is able to counter the vasoconstriction effects of stellate cell activation, thus limiting perfusion deficits [37,38]. However, in the early stages of reperfusion, the increased concentration of ET reported in both plasma and liver parenchyma, as well as the low concentration of NO, most probably due to the low intracellular levels of NADPH and oxygen after the ischemic period [20], contribute both with a decrease in liver blood flow [39,40].

Imbalance between NO and superoxide anion production by the endothelial sinusoidal cells

Under normal physiological conditions, both NO and superoxide anion are produced by endothelial cells, with NO production exceeding superoxide anion generation by two/three orders of magnitude. This allows NO to (i) effectively scavenge intracellular superoxide anion, (ii) prevent platelet aggregation, and (iii) minimize adhesive interactions between neutrophils and the endothelial cell surface, increasing microvascular permeability [38].

Following ischemia and reperfusion insult, the formation of superoxide anion exceeds NO because of the overproduction of superoxide anion and/or the dramatically reduced bioavailability of NO due to (i) low intracellular levels of cofactors for the synthesis of NO (NADPH and oxygen), (ii) L-arginine breakdown by the large amount of arginase released after ischemic insult, (iii) inhibition of endothelial cell NO synthase activity, and (iv) rapid inactivation of NO by superoxide anion. Thus, under these conditions many, if not all, of NO beneficial physiological actions are lost [38,41–46].

Neutrophil activation

Activation of neutrophils has been implicated in the hepatic microvascular dysfunction and parenchymal damage associated with ischemia/reperfusion [8,9,11,12,15,47,48]. This is based on the observation that preventing neutrophil influx into tissues, either by depleting the number of circulating neutrophils or by preventing neutrophil adhesion, significantly reduces microvascular dysfunction and organ injury in animal models of ischemia/reperfusion damage [49,50].

Activated neutrophils cause endothelial and hepatocellular cell damage through the release of oxidants and proteases. The primary neutrophil oxidant-generating pathway involves NADPH oxidase. Although NADPH oxidase-derived superoxide anion and its dismutation product hydrogen peroxide are the reactive oxygen species primarily formed, the concomitant release of myeloperoxidase results in the formation of hypochlorous acid as the major oxidant. Due to the high levels of reactive oxygen species occurring in the proximity of the sinusoidal endothelial and parenchymal cells, oxidative breakdown of the membrane PUFAs is one of the main molecular mechanisms of liver cell injury during reperfusion after ischemia [51–54]

In addition to the generation of ROS, activated neutrophils release a number of proteases and hydrolytic enzymes by granule exocytosis, which may be directly cytotoxic to liver cells. It has been reported that serine proteases, such as elastase and cathepsine G, are mainly responsible for the injury [55–57].

ISCHEMIC-PRECONDITIONING AS A STRATEGY FOR ATTENUATING HEPATIC MICROVASCULAR DYSFUNCTION RESULTING FROM ISCHEMIA/REPERFUSION INJURY

Over recent years, a surgical strategy known as ischemic-preconditioning has been developed to reduce ischemia/reperfusion injury. By this procedure, an organ is made resistant to the deleterious effects of sustained ischemia and reperfusion by previous exposure to repeated short periods of ischemia, separated by intermittent reperfusion. The protective effect of ischemic-preconditioning was first described in the heart, and has since been demonstrated in many other organs [58–65].

While originally described as an immediate adaptation to brief vascular occlusion, ischemic preconditioning actually affords two types of protection, which differ in time frame and mechanisms. For this reason, distinction is made between early and delayed preconditioning. The effect of early preconditioning develops within min-



Fig. 2. The molecular basis of ischemic preconditioning. In this diagram are represented the mechanisms thought to be responsible to induce the first and second windows of protection. See text for explication.

utes of reperfusion and lasts for 2 to 3 h. Delayed preconditioning becomes apparent 12–24 h after reperfusion and the effect lasts 2 to 3 d. Further, the protective effects of early preconditioning are independent of protein synthesis, while the effects of delayed preconditioning are dependent on altered gene expression and synthesis of new proteins [66,67].

Protective mechanisms of early ischemic preconditioning

The molecular basis for ischemic preconditioning consists of an ordered series of events. In response to the *triggers* of ischemic preconditioning, a *signal* must be rapidly generated that is *transduced* into an intracellular message and amplified to influence the *effector* mechanism of protection (Fig. 2).

It seems likely that substances released from the ischemic organ act in a paracrine fashion to activate the protective mechanism. Experiments, mainly performed in the heart, suggest a number of substances as potential ligands, including adenosine, bradykinin, catecholamines, reactive oxygen species, opiods, angiotensin II, and NO [68]. With regard to the liver, adenosine has thus far been considered the major player in triggering preconditioning [62,63,65,69,70]. Adenosine is a breakdown product of ATP that is released in large quantities into the extracellular space within seconds of the onset of ischemia. Adenosine exerts its physiological effect through the interaction with four distinct types of purinergic receptors designated A_1 , A_{2a} , A_{2b} , and A_3 .

 A_1 and A_2 receptors have been chiefly implicated in the ischemic preconditioning of the myocardium and liver, respectively [62,63,65,69,71].

The mechanisms by which adenosine, released during ischemic preconditioning, can limit reperfusion injury are still uncertain. Adenosine has been shown to inhibit neutrophil oxidative metabolism and adhesion to endothelial cells, to increase membrane stability and energy production by promoting glucose transport, and to reduce Ca^{2+} influx through the activation of ATP-dependent K⁺ channels [72–75]. However, the role of all biochemical effects in adenosine-afforded protection is yet to be defined.

An increasing mass of data points to the activation of adenosine receptors (I and II) coupled to G proteins as the pathway that initiates the preconditioning response. After activation, the receptor-coupled G protein dissociates and in turn activates a membrane-bound phospholipase (phospholipase C or D). These phospholipases cleave phosphatidylinositol biphosphate into two intracellular second messengers, inositol triphosphate, which releases Ca^{2+} from nonmitochondrial intracellular stores, and diacylglycerol (DAG), which activates specific isoforms of protein kinase C (PKC) [76,77].

PKC is well placed to play a key role in cellular protection. The kinase is known to regulate a number of biological processes such as metabolism, ion transport, and gene expression. PKC is a family of at least 12 serine/threonine kinases. Each protein contains four constant regions responsible for activation and enzymatic action, as well as five variable regions responsible for translocation and substrate binding. PKC isoenzymes can be divided into three broad categories: conventional, novel, and atypical. The conventional PKCs (α , β_{I} , β_{II} , and γ) require Ca²⁺, DAG and phospholipids for activation. The novel PKC isoforms (δ , ε , η , θ) lack the calcium-binding region, so these subtypes are not dependent on Ca²⁺ for activation. Activation of isoenzymes of the atypical PKC group $(\zeta, \iota, \lambda, \mu)$ is also independent from calcium; however, atypical isoenzymes lack the Zn²⁺ finger region required for binding DAG and 3'phosphoinositides may instead be the activators of these atypical PKCs. Recent reports indicate that the novel PKC isoforms, particularly δ , ε , and η , may be implicated in the protective effect of ischemic preconditioning, whereas the classical isoforms contribute to the detrimental effects of ischemia/reperfusion [78-80]. Any way they are activated, novel PKCs afford phosphorylation of effector molecules, most probably ATPsensitive K⁺ channel (K_{ATP}), 5'-nucleotidase, and cAMP-protein kinase A activation (PKA).

It has been proposed that in the mitochondria potassium channel openers target an ATP-sensitive K^+ channel implicated in the regulation of mitochondrial ion and volume homeostasis. Under normoxic conditions, K^+ channel opening is regulated by ATP levels. Activated PKC isoforms phosphorylate K^+ channels, changing the stoichiometry of the ATP binding sites and favoring their opening to the ATP concentration seen in early ischemia. Opening of these channels leads to preservation of intramitochondrial calcium homeostasis and ATP levels, thus improving viability.

The PKC-dependent translocation of 5'-nucleotidase from the cytosol to the plasma membrane may also play an important role in the protective effects induced by ischemic preconditioning. Indeed, 5'-nucleotidase has been shown to stimulate production of adenosine from AMP. A cAMP increase is thought to reduce postischemic injury by preserving the endothelial barrier function. In addition, it has been reported to block leukocyte adhesion by reducing the expression of adhesion molecules, superoxide radical production, and phagocytic activity of neutrophils [71,77–80].

On the other hand, during the ischemic preconditioning procedure, cellular consumption of ATP leads to accumulation of AMP [81]. The involvement of AMP in the stimulation of AMP-activated protein kinase (AMPK) is well known. Once activated, AMPK responds by phosphorylating multiple downstream substrates, with the purpose of switching on catabolic pathways that generate ATP, while switching off anabolic pathways that consume ATP. Thus, through the cAMPactivated PKA, ischemic preconditioning could promote energy-saving mechanisms [81–83].

Protective mechanisms of delayed ischemic preconditioning

While the triggering and amplification of signals for protection seem to be as in early preconditioning, the effectors of this second window are probably different, but still far from certain. The time frame within which the second window confers protection lends itself to the theory that altered gene expression, with the consequent synthesis of new proteins, is the protection method. However, it is not possible to exclude posttranslational modifications or changes in compartmentalization of existing proteins as the mechanisms involved in the delayed protective response [66-68].

The transcription-dependent synthesis of proteins, which plays a significant role during the second window of protection, remains practically unknown; however, it has been demonstrated that after preconditioning, the PKC ε isomer migrates to the nucleus, inducing nuclear transcription factors. On the other hand, a cytoplasmic transcription factor, nuclear transcription factor B $(NF\kappa B)$, has also been implicated in the delayed preconditioning. Activation and nuclear translocation of these transcriptional factors govern the expression of protective genes responsible for late preconditioning [84-87]. Thus, unlike early preconditioning, late preconditioning requires increased synthesis of new proteins. The time frame of the enhanced tolerance to reperfusion damage, which requires 12 to 24 h to develop and lasts for 3-4 d is also consistent with the synthesis and degradation of protective proteins. Several proteins have been proposed as possible effectors, including nitric oxide synthase, cyclooxygenase-2, aldose reductase, antioxidant enzymes (particularly Mn-SOD), and heat shock proteins [67,68] (Fig. 2).

The precise mechanism by which ischemic preconditioning exerts its hepatoprotective effect is still under investigation. Recent studies have demonstrated that ischemic preconditioning induces activation of adenosine A_2 receptors, which in turn, by induction of NO synthesis, confer cytoprotection against postischemic damage [63,65]. Along these lines, it has been shown that inhibition of NO synthesis with *N*-nitro-L-arginine methylester, an L-arginine analogue that competitively blocks NO synthase, abolished the protective effect of preconditioning, whereas administration of a NO donor or L-arginine to ischemic and reperfused livers simulated the protective effect of preconditioning against hepatic injury [63,65].

Ischemic-preconditioning has also been observed to prevent the TNF- α release from Kupffer cells, as well as preventing generation of hepatic ET, in response to ischemia/reperfusion, thus attenuating the microvascular dysfunction of the liver; the mechanism is probably NO modulated. Moreover, in remote organs like the lung, small intestine, and pancreas, hepatic preconditioning cancelled the increase in P-selectin upregulation, preventing recruitment of circulating neutrophils and thus reducing oxidative stress and microvascular disorders in these organs, after one cycle of hepatic ischemia/reperfusion. Because administration of antibodies against Pselectin or TNF- α prior to ischemia was seen to have the same effect as preconditioning, it has been suggested that the blockade of P-selectin upregulation probably results from inhibition of systemic TNF release from Kupffer cells [88]. Moreover, recently it was reported that ischemic preconditioning was able to increase the hepatic tolerance against reperfusion injury by attenuating the release of ET [89] and the production of ROS, either by blocking the xanthine-oxidase pathway or by preserving the mitochondria structure [90,91]. In this last work it was also reported that well-preserved mitochondria were associated with an attenuated release of cytochrome c to the cytoplasm, as well as with a low *index* of caspase-3 activity [91]. Therefore, ischemic preconditioning appears to be a useful strategy to downregulate the molecular pathways involved in liver apoptosis.

Preconditioning also appears to be a useful strategy against the deleterious effects of cold storage-reperfusion injury, since it was observed that ischemic-preconditioned grafts had reduced levels of transaminases and TNF- α , as well as augmented bile flow and improved tissue blood flow [92–94]. Accordingly, in a recent publication it was observed that preconditioned rat livers were more tolerant against cold-storage-reperfusion injury, most probably due to a decreased production of $O_2^{-\bullet}$ by Kupffer cells. Moreover, the authors showed also that ischemia to half the liver confers protection to the other half, providing that heterologous preconditioning could be a tool to protect liver tissue against ischemia-reperfusion injury without imposing ischemia on the target tissue [95].

In liver surgery, the reperfusion injury is one of the most serious insults that affect the organ viability and ultimately postsurgical results. Hepatic ischemic preconditioning here described could be of clinical interest because this surgical approach appears as a potential therapeutic strategy to attenuate the postreperfusion damage. Acknowledgements — This work was supported by grants from Italian Ministry of the University (PRIN 1999, 2000, 2001), Centro Nazionale delle Ricerche (Progetto Finalizzato Biotecnologie), Turin University.

REFERENCES

- [1] Delva, E.; Camus, Y.; Nordlinger, B.; Hannoun, L.; Parc, R.; Deriaz, H.; Lienhart, A.; Huguet, C. Vascular occlusions for liver resections. Operative management and tolerance to hepatic ischemia: 142 cases. *Ann. Surg.* 209:211–218; 1989.
- [2] Huguet, C.; Addario-Chieco, P.; Gavelli, A.; Arrigo, E.; Harb, J.; Clement, R. R. Technique of hepatic vascular exclusion for extensive liver resection. *Am. J. Surg.* **163**:602–605; 1992.
- [3] Henderson, J. M. Liver transplantation and rejection: an overview. *Hepatogastroenterology* 46(Suppl. 2):1482–1484; 1999.
- [4] McCord, J. M. Oxygen derived free radicals in postischemic tissues. N. Engl. J. Med. 312:159–163; 1985.
- [5] González-Flecha, B.; Cutrìn, J. C.; Boveris, A. Time course and mechanism of oxidative stress and tissue damage in rat liver subjected to in vivo ischemia-reperfusion. J. Clin. Invest. 91:456– 464; 1993.
- [6] Lemasters, J. J.; Thurman, R. G. Reperfusion injury after liver preservation for transplantation. *Annu. Rev. Pharmacol. Toxicol.* 37:327–338; 1997.
- [7] Jaeschke, H. Mechanisms of reperfusion injury after warm ischemia to the liver. J. Hepatobiliary Pancreat. Surg. 5:402–408; 1998.
- [8] Jaeschke, H.; Farhood, A. Neutrophil and Kupffer cell-induced oxidant stress and ischemia-reperfusion injury in rat liver. Am. J. Physiol. 260:G355–G362; 1991.
- [9] Cutrìn, J. C.; Boveris, A.; Zingaro, B.; Corvetti, G.; Poli, G. *In situ* determination by surface chemiluminescence of temporal relationships between evolving warm ischemia-reperfusion injury in rat liver and phagocyte activation and recruitment. *Hepatology* 31:622–632; 2000.
- [10] Adkinson, D.; Hollwath, M. E.; Benoit, J. N.; Parks, D. A.; Mc Cord, J. M.; Granger, D. N. Role of free radicals in ischaemiareperfusion injury to the liver. *Acta Physiol. Scand.* 548(Suppl.): 101–107; 1986.
- [11] Jaeschke, H. Reactive oxygen and ischaemia/reperfusion injury to the liver. *Chem. Biol. Interact.* **79:**115–136; 1991.
- [12] Jaeschke, H.; Bautista, A. P.; Spolarics, Z.; Spitzer, J. J. Superoxide generation by neutrophils and Kupffer cells during in vivo reperfusion after hepatic ischemia in rats. *J. Leukoc. Biol.* **52**: 377–382; 1992.
- [13] Kobayashi, S.; Clemens, M. G. Kupffer cell exacerbation of hepatocyte hypoxia/reoxygenation injury. *Circ. Shock* 37:245– 252; 1992.
- [14] Cutrìn, J. C.; Llesuy, S.; Boveris, A. Primary role of Kupffer cell-hepatocyte communication in the expression of oxidative stress in the post-ischaemic liver. *Cell Biochem. Funct.* 16:65–72; 1998.
- [15] Jaeschke, H.; Farhhod, A. Neutrophils contribute to ischemia/ reperfusion injury in rat liver *in vivo*. *FASEB J.* 4:3355–3359; 1990.
- [16] Menger, M. D.; Vollmar, B.; Glasz, J.; Post, S.; Messmer, K. Microcirculatory manifestations of hepatic ischemia/reperfusion injury. *Prog. Appl. Microcirc.* **19**:106–124; 1993.
- [17] Vollmar, B.; Glasz, J.; Leiderer, R.; Post, S.; Menger, M. D. Hepatic microcirculatory perfusion failure is a determinant for liver dysfunction in warm ischemia-reperfusion. *Am. J. Pathol.* 145:1421–1431; 1994.
- [18] Vollmar, B.; Glasz, J.; Post, S.; Menger, M. D. Role of microcirculatory derangements in manifestation of portal triad crossclamping-induced hepatic reperfusion injury. J. Surg. Res. 60:49– 54; 1996.
- [19] Menger, M. D.; Richter, S.; Yamauchi, J.; Vollmar, B. Role of microcirculation in hepatic ischemia/reperfusion injury. *Hepato-gastroentrology* 46:1452–1457; 1999.

- [20] Carden, D. L.; Granger, D. N. Pathophysiology of ischaemiareperfusion injury. J. Pathol. 190:255–266; 2000.
- [21] Schauer, R. J.; Bilzer, M.; Kalmuk, S.; Gerbes, A. L.; Leiderer, R.; Schildberg, F. W.; Messmer, K. Microcirculatory failure after rat liver transplantation is related to Kupffer cell-derived oxidant stress but not involved in early graft dysfunction. *Transplantation* 72:1692–1699; 2001.
- [22] Serracino-Inglott, F.; Habib, N. A.; Mathie, R. T. Hepatic ischemia-reperfusion injury. Am. J. Surg. 181:160–166; 2001.
- [23] Kawachi, S.; Hines, I. N.; Laroux, F. S.; Hoffman, J.; Bharwani, S.; Gray, L.; Leffer, D.; Grisham, M. B. Nitric oxide syntahse and postischemic liver injury. *Biochem. Biophys. Res. Commun.* 276: 851–854; 2000.
- [24] Hisama, N.; Yamaguchi, Y.; Ishiko, T.; Miyanari, N.; Ichiguchi, O.; Goto, M.; Mori, K.; Watanabe, K.; Kawamura, K.; Tsurufuji, S.; Ogawa, M. Kupffer cell production of cytokine-induced neutrophil chemoattractant following ischemia/reperfusion injury of rat liver. *Hepatology* 26:1193–1198; 1996.
- [25] Lentsch, A. B.; Yoshidone, H.; Cheadle, W. G.; Miller, F. N.; Edwards, M. J. Chemokine involvement in hepatic ischemia/ reperfusion injury in mice: roles for macrophage inflammatory protein-2 and Kupffer cells. *Hepatology* 27:1172–1177; 1998.
- [26] Cursio, R.; Gugenheim, J.; Panaia-Ferrari, P.; Lasfar, A.; Tovey, M.; Chastanet, S.; Saint-Paul, M. C.; Ferre, C.; Mouiel, J. Improvement of normothermic rat liver ischemia/reperfusion by muramyl dipeptide. J. Surg. Res. 80:339–344; 1998.
- [27] Shigeki, A.; Masayuki, I. Physiological role of sinusoidal endothelial cells and Kupffer cells and their implication in the pathogenesis of liver injury. *J. Hepatobiliary Pancreat. Surg.* 7:40–48; 2000.
- [28] Jaeschke, H.; Farhood, A.; Bautista, A. P.; Spolarics, Z.; Spitzer, J. J. Complement activates Kupffer cells and neutrophils during reperfusion after hepatic ischemia. *Am. J. Physiol.* 264:G801– G809; 1993.
- [29] Tomasdottir, H.; Henriksson, B.-A.; Bengtson, J.-P.; Bengtsson, A.; Stenqvist, O.; Persson, H. Complement activation during liver transplantation. *Transplantation* 55:799–802; 1993.
- [30] Ronholm, E.; Tomasdottir, H.; Runeborg, J.; Bengtsson, A.; Bengtson, J.-P.; Stenqvist, O.; Friman, S. Complement system activation during orthotopic liver transplantation in man. Indications of pre-operative complement system activation in the gut. *Transplantation* 57:1594–1597; 1994.
- [31] Chavez-Cartaya, R. E.; DeSola, G. P.; Wright, L.; Jamieson, N. V.; White, D. J. G. Regulation of the complement cascade by soluble complement receptor type 1. Protective effect in experimental liver ischemia and reperfusion. *Transplantation* 59:1047– 1052; 1995.
- [32] Scoazec, J.-Y.; Borghi-Scoazec, G.; Durand, F.; Bernuau, J.; Pham, B. N.; Belghiti, J.; Feldmann, G.; Degott, C. Complement activation after ischemia-reperfusion in human liver allografts: incidence and pathophysiological relevance. *Gastroenterology* 112:908–918; 1997.
- [33] Lehmann, T. G.; Koeppel, T. A.; Münch, S.; Heger, M.; Kirschfink, M.; Klar, E.; Post, S. Impact of inhibition of complement by sCR1 on hepatic microcirculation after warm ischemia. *Microvasc. Res.* 62:284–292; 2001.
- [34] Gandhi, C. R.; Behal, R. H.; Harvey, S. A. K.; Nouchi, T. A.; Olson, M. S. Hepatic effects of endothelin. *Biochem. J.* 287:897– 904; 1992.
- [35] Kawada, N.; Tran-Thi, T.-A.; Klein, H.; Decker, K. The contraction of hepatic stellate (Ito) cells stimulated with vasoactive substances. Possible involvement of endothelia-1 and nitric oxide in the regulation of the sinusoidal tonus. *Eur. J. Biochem.* 213: 815–823; 1993.
- [36] Zhang, J. X.; Pegoli, W. J.; Clemens, M. G. Endothelin-1 induces direct constriction of hepatic sinusoids. Am. J. Physiol. 266: G624–G632; 1994.
- [37] Rockey, D. C.; Chung, J. J. Inducible nitric oxide synthase in rat hepatic lipocytes and the effect of nitric oxide on lipocyte contractility. J. Clin. Invest. 95:1199–1206; 1995.

- [38] Clemens, M. G. Nitric oxide in liver injury. *Hepatology* 30:1–5; 1999.
- [39] Kawamura, E.; Yamanaka, N.; Okamoto, E.; Tomoda, F.; Furukawa, K. Response of plasma and tissue endothelin-1 to liver ischemia and its implication in ischemia-reperfusion injury. *Hepatology* 21:1138–1143; 1995.
- [40] Nakamura, S.; Nishiyama, R.; Serizawa, A.; Yokoi, Y.; Suzuki, S.; Konno, H.; Baba, S.; Muro, H. Hepatic release of endothelin-1 after warm ischemia. Reperfusion injury and its hemodynamic effect. *Transplantation* 59:679–684; 1995.
- [41] Liu, P.; Yin, K.; Nagele, R.; Wong, P. Y. Inhibition of nitric oxide synthase attenuates peroxynitrite generation, but augments neutrophil accumulation in hepatic ischemia-reperfusion in rats. *J. Pharmacol Exp. Ther.* 284:1139–1146; 1998.
- [42] Liu, P.; Xu, B.; Hock, C. E.; Nagele, R.; Sun, F. F.; Wong, P. Y. NO modulates P-selectin and ICAM-1 mRNA expression and hemodynamic alterations in hepatic I/R. Am. J. Physiol. 275: H2191–H2198; 1998.
- [43] Farzaneth-Far, R.; Moore, K. Nitric oxide and the liver. Liver 21:161–174; 2001.
- [44] Wang, Y.; Mathews, W. R.; Guido, D. M.; Farhood, A.; Jaeschke, H. Inhibition of nitric oxide synthesis aggravates reperfusion injury after hepatic ischemia and endotoxemia. *Shock* 4:282–288; 1995.
- [45] Kawachi, S.; Hines, I. N.; Laroux, F. S.; Hoffman, J.; Bharwani, S.; Gray, L.; Leffer, D.; Grisham, M. B. Nitric oxide synthase and postischemic liver injury. *Biochem. Biophys. Res. Commun.* 276: 851–854; 2000.
- [46] Hines, I. N.; Harada, H.; Bharwani, S.; Pavlick, K. P.; Hoffman, J. M.; Grisham, M. B. Enhanced post-ischemic liver injury in iNOS-deficient mice: a cautionary note. *Biochem. Biophys. Res. Commun.* 284:972–976; 2001.
- [47] Hisama, N.; Yamaguchi, Y.; Ishiko, T.; Miyanari, N.; Ichiguchi, O.; Goto, M.; Mori, K.; Watanabe, K.; Kawamura, K.; Tsurufuji, S.; Ogawa, M. Kupffer cell production of cytokine-induced neutrophil chemoattractant following ischemia/reperfusion injury of rat liver. *Hepatology* 26:1193–1198; 1996.
- [48] Jaeschke, H.; Farhood, A.; Bautista, A. P.; Spolarics, Z.; Spitzer, J. J.; Smith, C. W. Functional inactivation of neutrophils with a Mac-1 (CD11b/CD18) monoclonal antibody protects against ischemia-reprfusion injury in rat liver. *Hepatology* **17**:915–923; 1993.
- [49] Lentsch, A. B.; Atsushi, K.; Yoshidome, H.; McMasters, K. M.; Edwards, M. J. Inflammatory mechanisms and therapeutic strategies for warm hepatic ischemia/reperfusion injury. *Hepatology* 32:169–173; 2000.
- [50] Martinez-Mier, G.; Toledo-Pereyra, L. H.; McDuffie, J. E.; Warner, R. L.; Ward, P. A. Neutrophil depletion and chemokine response after liver ischemia and reperfusion. *J. Invest. Surg.* 14:99–107; 2001.
- [51] Carlos, T. M.; Harlan, J. M. Leukocyte-endothelial adhesion molecules. *Blood* 84:2068–2101; 1994.
- [52] Babior, B. M. Oxygen-dependent microbial killing by phagocytes. N. Engl. J. Med. 298:659–668; 1978.
- [53] Mathews, W. R.; Guido, D. M.; Fisher, M. A.; Jaeschke, H. Lipid peroxidation as molecular mechanism of liver injury during reperfusion after ischemia. *Free Radic. Biol. Med.* 16:763–770; 1994.
- [54] Poli, G.; Cutrìn, J. C.; Biasi, F. Lipid peroxidation in the reperfusion injury of the liver. *Free Radic. Res.* 28:547–551; 1998.
- [55] Jaeschke, H.; Smith, C. J. W. Mechanisms of neutrophil-induced parenchymal cell injury. L. Leukoc. Biol. 61:647–653; 1997.
- [56] Harbrecht, B. G.; Billiar, T. R.; Curran, R. D.; Stadler, J.; Simmons, R. L. Hepatocyte injury by activated neutrophils in vivo is mediated by proteases. *Ann. Surg.* 218:120–128; 1993.
- [57] Fujishima, S.; Aikawa, N. Neutrophil-mediated tissue injury and its modulation. *Intensive Care Med.* 21:277–285; 1995.
- [58] Murry, C.; Jenning, R.; Reimer, K. Preconditioning with ischemia: a delay in lethal cell injury in ischemic myocardium. *Circulation* 74:1124–1136; 1986.
- [59] Glazier, S. S.; O'Rourke, D. M.; Graham, D. L.; Welsh, F. A. Induction of ischemic tolerance following brief local ischemia in rat brain. J. Cereb. Blood Flow Metab. 14:545–553; 1994.

- [60] Pang, C. Y.; Yang, R. Z.; Zhong, A.; Xu, N.; Boyd, B.; Forrest, C. R. Acute ischaemic preconditioning protects against skeletal muscle infarction in the pig. *Cardiovasc. Res.* 29:782–788; 1995.
- [61] Hotter, G.; Closa, D.; Prados, M.; Fernàndez-Cruz, L.; Prats, N.; Gelpí, E.; Roselló Catafau, J. Intestinal preconditioning is mediated by a transient increase in nitric oxide. *Biochem. Biophys. Res. Commun.* 222:27–32; 1996.
- [62] Peralta, C.; Closa, D.; Hotter, G.; Gelpí, E.; Prats, N.; Roselló Catafau, J. Liver ischemic preconditioning is mediated by the inhibitory action of nitric oxide on endothelin. *Biochem. Biophys. Res. Commun.* 229:264–270; 1996.
- [63] Peralta, C.; Hotter, G.; Closa, D.; Bulbena, O.; Roselló Catafau, J. Protective effect of preconditioning on the injury associated to hepatic ischemia-reperfusion: role of nitric oxide and adenosine. *Hepatology* 25:934–937; 1997.
- [64] Yin, D. P.; Sankary, H. N.; Chong, A. S.; Ma, L. L.; Shen, J.; Foster, P.; Williams, J. W. Protective effect of ischemic preconditioning on liver preservation-reperfusion injury in rats. *Transplantation* 66:152–157; 1998.
- [65] Peralta, C.; Hotter, G.; Closa, D.; Prats, N.; Xaus, C.; Gelpí, E.; Roselló Catafau, J. 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; 1999.
- [66] Korthius, R. J.; Gute, D. C.; Cepinska, G.; Kvietys, P. R. Cellular mechanisms of acute versus delayed preconditioning. *Pathophysiology* 5:35–48; 1998.
- [67] Bolli, R. The late phase of preconditioning. *Circ. Res.* 87:972– 983; 2000.
- [68] Carrol, R.; Yellon, D. M. Myocardial adaptation to ischaemia-the preconditioning phenomenon. *Int. J. Cardiol.* 68(Suppl. 1):S93– S101; 1999.
- [69] Nakayama, H.; Yamamoto, Y.; Kume, M.; Yamagami, K.; Yamamoto, H.; Kimoto, S.; Ishikawa, Y.; Ozaki, N.; Yasuyuki, S.; Yamaoka, Y. Pharmacological stimulation of adenosine A₂ receptors supplants ischemic preconditioning in providing ischemic tolerance in rat livers. *Surgery* **126**:945–954; 1999.
- [70] Nilsson, B.; Friman, S.; Wallin, M.; Gustafsson, B.; Delbro, D. The liver protective effect of ischemic preconditioning may be mediated by adenosine. *Transpl. Int.* 13(Suppl. 1):S558–S561; 2000.
- [71] Cohen, M. V.; Baines, C. P.; Downey, J. M. Ischemic preconditioning: from adenosine receptor to K_{ATP} channel. Annu. Rev. Physiol. 62:79–109; 2000.
- [72] Cronstein, B. N.; Levin, R. I.; Belanoff, J.; Weissman, G.; Hirschhorn, R. Adenosine: an endogenous inhibitor of neutrophil-mediated injury to endothelial cells. J. Clin. Invest. 78:760–770; 1986.
- [73] Grisham, M. B.; Hernandez, L. A.; Granger, D. N. Adenosine inhibits ischemia-reperfusion-induced leukocyte adherence and extravasation. Am. J. Physiol. 257:H1334–H1339; 1989.
- [74] Wyatt, D. A.; Edmunds, M. C.; Rubio, R.; Berne, R. M.; Lasley, R. D.; Mentzer, R. M. Jr. Adenosine stimulates glycolytic flux in isolated perfused hearts by A1-adenosine receptors. *Am. J. Physiol.* 257:H1952–H1957; 1989.
- [75] Howell, J. G.; Zibari, G. B.; Brown, M. F.; Burney, D. L.; Sawaya, D. E.; Olinde, J. G.; Granger, D. N.; McDonald, J. C. Both ischemic and pharmacological preconditioning decrease hepatic leukocyte/endothelial cell interactions. *Transplantation* 69: 300–314; 2000.
- [76] Speechly, D. M. E.; Mocanu, M. M.; Yellon, D. M. Protein kinase C. Its role in ischemic preconditioning in the rat. *Circ. Res.* 75:586–590; 1994.
- [77] Ishida, T.; Yarimizu, K.; Gute, D. C.; Korthuis, R. J. Mechanisms of ischemic preconditioning. *Shock* 8:86–94; 1997.
- [78] Simkhovich, B. Z.; Pryklenk, K.; Kloner, R. A. Role of protein kinase C as a cellular mediator of ischemic preconditioning: critical review. *Cardiovasc. Res.* 40:9–22; 1998.
- [79] Tong, H.; Chen, W.; Steenbergen, C.; Murphy, E. Ischemic preconditioning activates phosphatidylinositol-3-kinase upstream of protein kinase C. Circ. Res. 87:309–315; 2000.
- [80] 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.; Chari, R. S. Protein kinase C inhibition abrogates hepatic ischemic preconditioning responses. *J. Surg. Res.* **97**:144–149; 2001.

- [81] Peralta, C.; Bartrons, R.; Riera, R.; Manzano, A.; Xaus, C.; Gelpí, E.; Roselló Catafau, J. Hepatic preconditioning preserves energy metabolism during sustained ischemia. *Am. J. Physiol.* 279: G163–G171; 2000.
- [82] Stein, S. C.; Woods, A.; Jones, N. A.; Davison, M. D.; Carling, D. The regulation of AMP-activated protein kinase by phosphorylation. *Biochem. J.* 345:437–443; 2000.
- [83] Peralta, C.; Bartrons, R.; Serafin, A.; Blazquez, C.; Guzman, M.; Prats, N.; Xaus, C.; Cutillas, B.; Gelpí, E.; Roselló Catafau, J. Adenosine monophosphate-activated protein kinase mediates the protective effects of ischemic preconditioning on hepatic ischemia-reperfusion in the rat. *Hepatology* 34:1164–1173; 2001.
- [84] Maulik, N.; Sato, M.; Price, B. D.; Das, D. K. An essential role of Nf kappa B in tyrosine kinase signaling of p38 MAP kinase regulation of myocardial adaptation to ischemia. *FEBS Lett.* **429**: 365–369; 1998.
- [85] Xuan, Y. T.; Tang, X. L.; Banerjee, S.; Takano, H.; Li, R. C.; Han, H.; Qiu, Y.; Li, J. J.; Bolli, R. Nuclear factor-kappa B plays an essential role in the late phase of ischemic preconditioning in conscious rabbits. *Circ. Res.* 84:1095–1109; 1999.
- [86] Li, R. C.; Ping, P.; Zhang, J.; Wead, W. B.; Cao, X.; Gao, J.; Zheng, Y.; Huang, S.; Han, J.; Bolli, R. PKC epsilon modulates NF-kappa B and AP-1 via mitogen-activated protein kinases in adult rabbit cardiomyocytes. *Am. J. Physiol. Heart Circ. Physiol.* 279:H1679–H1689; 2000.
- [87] Valen, G.; Yan, Z. Q.; Hansson, G. K. Nuclear factor kappa-B and the heart. J. Am. Coll. Cardiol. 38:307–314; 2001.
- [88] Peralta, C.; Fernández, L.; Panés, J.; Prats, N.; Sans, M.; Piqué, J. M.; Gelpí, E.; Roselló Catafau, J. 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; 2001.
- [89] Peralta, C.; Bulbena, O.; Bargalló, R.; Prats, N.; Gelpí, E.; Roselló Catafau, J. Strategies to modulate the deleterious effects of endothelin in hepatic ischemia-reperfusion. *Transplantation* 70:1–10; 2000.
- [90] Peralta, C.; Bulbena, O.; Xaus, C.; Prats, N.; Cutrìn, J. C.; Poli, G.; Gelpí, E.; Roselló Catafau, J. Ischemic preconditioning: defense mechanism against the reactive oxygen species generated after hepatic ischemia reperfusion. *Transplantation* 73:1–9; 2002.
- [91] Cavalieri, B.; Perrelli, M.-G.; Aragno, M.; Mastrocola, R.; Corvetti, G.; Durazzo, M.; Poli, G.; Cutrìn, J. C. Ischemic preconditioning attenuates the oxidant-dependent mechanisms of reperfusion cell damage and death in rat liver. *Liver Transpl.* In press.
- [92] Yin, D. P.; Sankary, H. N.; Chong, A. S.; Ma, L. L.; Shen, J.; Foster, P.; Williams, J. W. Protective effects of ischemic preconditioning on liver preservation-reperfusion injury in rats. *Transplantation* **66**:152–157; 1998.
- [93] Arai, M.; Thurman, R. G.; Lemasters, J. J. Involvement of Kupffer cells and sinusoidal endothelial cells in ischemic preconditioning to rat livers stored for transplantation. *Transplant. Proc.* 31:425–427; 1999.
- [94] 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.; Chari, R. S. Protective effects of ischemic preconditioning on the cold-preserved liver are tyrosine kinase dependent. *Transplantation* **72**:406–412; 2001.
- [95] Arai, M.; Thurman, R. G.; Lemasters, J. J. Ischemic preconditioning of rat livers against cold storage-reperfusion injury: role of nonparenchymal cells and the phenomenon of heterologous preconditioning. *Liver Transpl.* 7:292–299; 2001.

ABBREVIATIONS

AMPK—AMP-activated protein kinase DAG—diacylglycerol

eNOS-endothelial nitric oxide synthase

ET—endothelinMHSP—heat shock proteinsM H_2O_2 —hydrogen peroxideNiNOS—inducible nitric oxyde synthaseNI κ B—inhibitor of kappa BPIICAM-1—intercellular adhesion molecule 1PIIL-1—interleukin-1PI $O_2^{-\bullet}$ —superoxide anionPIPi-K $_{ATP}$ channel—phosphorylated ATP-sensitive potassium channelT

Mn-SOD—manganase superoxide dismutase MAC—membrane attack complex NO—nitric oxide NF- κ B—nuclear factor kappa B PLC—phospholipase C PLD—phospholipase D PKC—protein kinase C PUFAs—polyunsatured fatty acids ROS—reactive oxygen species TNF- α —tumor necrosis factor alpha