Molecular mechanisms of hepatic ischemia-reperfusion injury and preconditioning

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Jaeschke, Hartmut. Molecular mechanisms of hepatic ischemia-reperfusion injury and preconditioning. Am J Physiol Gastrointest Liver Physiol 284: G15–G26, 2003; 10.1152/ajpgi.00342.2002.—Ischemia-reperfusion injury is, at least in part, responsible for the morbidity associated with liver surgery under total vascular exclusion or after liver transplantation. The pathophysiology of hepatic ischemia-reperfusion includes a number of mechanisms that contribute to various degrees in the overall injury. Some of the topics discussed in this review include cellular mechanisms of injury, formation of pro- and anti-inflammatory mediators, expression of adhesion molecules, and the role of oxidant stress during the inflammatory response. Furthermore, the roles of nitric oxide in preventing microcirculatory disturbances and as a substrate for peroxynitrite formation are reviewed. In addition, emerging mechanisms of protection by ischemic preconditioning are discussed. On the basis of current knowledge, preconditioning or pharmacological interventions that mimic these effects have the greatest potential to improve clinical outcome in liver surgery involving ischemic stress and reperfusion.

Kupffer cells; neutrophils; complement; adhesion molecules; reactive oxygen; nitric oxide; apoptosis; cytokines; chemokines

LIVER DYSFUNCTION OR FAILURE is still a significant clinical problem after transplantation surgery, tissue resections (Pringle maneuver), and hemorrhagic shock. Despite the significant improvement of clinical outcome during the last decade, the dramatic organ shortage for transplantation forces consideration of cadaveric or steatotic grafts, which have a higher susceptibility to ischemia-reperfusion injury. Although substantial progress has been made in elucidating mechanisms of ischemia-reperfusion injury, there is still a need to better understand the pathophysiology. The current review will summarize established basic concepts of reperfusion injury in the liver together with recent new insights into injury mechanisms and novel therapeutic strategies. Due to the complexity of the overall process, this review can only provide an in-depth discussion on selected aspects of the pathophysiology. For areas not sufficiently covered, the reader is referred to other, excellent reviews (11, 93, 99, 116, 147).

THE INFLAMMATORY RESPONSE DURING REPERFUSION: CELLULAR MECHANISMS AND OXIDANT STRESS

An excessive inflammatory response is clearly recognized as a key mechanism of injury during reperfusion (53, 54). Ischemia activates Kupffer cells (Fig. 1), which are the main sources of vascular reactive oxygen formation during the initial reperfusion period (59, 63, 136). Interestingly, this effect is only observed after no-flow ischemia (Pringle, transplantation) but not after hemorrhagic shock, i.e., low-flow ischemia (62). In addition to Kupffer cell-induced oxidant stress, with increasing length of the ischemic episode, intracellular generation of reactive oxygen by xanthine oxidase and in particular mitochondria (39, 42, 71) may also contribute to liver dysfunction and cell injury during reperfusion (39, 83). In addition, the presence of a phagocyte-type NADPH oxidase was recently recog-
nized as a major source of superoxide formation in endothelial cells (97) and hepatocytes (126). Rac1, a member of the Rho family of small GTPases, regulates this enzyme. Inhibition of Rac1 attenuated the intracellular oxidant stress during the early reperfusion phase and protected against liver injury (126). Proinflammatory cytokines, chemokines, and activated complement factors are responsible for neutrophil recruitment and the subsequent neutrophil-induced oxidant stress during the later reperfusion phase (60). Stimulation of primed Kupffer cells by complement factors causes the continuous activation of these macrophages (60, 64). The Kupffer cell- and neutrophil-induced oxidant stress is an important factor in vascular and parenchymal cell injury during reperfusion (63, 65, 67). The relevance of this postischemic vascular oxidant stress was demonstrated by the protective effect of extracellular glutathione (GSH) (14, 58, 100), which can scavenge hydrogen peroxide, hypochlorous acid, and peroxynitrite (13, 77, 100). Despite the mainly vascular origin of the oxidant stress, reactive oxygen generated by Kupffer cells (12) or adherent neutrophils (70) causes a substantial intracellular oxidant stress in hepatocytes. Animals deficient in glutathione peroxidase are significantly more susceptible to neutrophil-induced oxidant stress than wild-type animals (70). This suggests that intracellular defense mechanisms are critical for detoxification of reactive oxygen species generated by intracellular as well as extracellular sources. This mechanism explains why antioxidants targeted to either extracellular or intracellular sites attenuated reperfusion injury in the liver (12, 55, 100, 164, 168, 178).

A topic of substantial controversy during the last two decades was the discussion about the molecular mechanism of injury (Fig. 2). Initially, it was assumed that any postischemic oxidant stress leads to cell death by lipid peroxidation. However, lipid peroxidation is quantitatively insufficient to explain the severe cell injury during reperfusion (109). Inflammatory cells also release proteases, which may be the actual cytotoxic mediators of neutrophils (110). The beneficial effect of protease inhibitors supported a role of proteases in the pathophysiology of reperfusion injury in experimental models (86, 98) and in humans (76). In fact, it was hypothesized that the role of reactive oxygen species in an inflammatory injury in vivo is actually not to cause cell injury but to inactivate anti-
proteases of the plasma by oxidation in the vicinity of the neutrophil (163). This would allow neutrophil-derived proteases to act locally without interference of anti-proteases. On the other hand, proteases, which escape into the circulation, can still be inactivated to prevent systemic vascular injury (163). However, more recent data clearly indicate that Kupffer cells (12) and neutrophils (70) can kill hepatocytes in vivo by reactive oxygen species. In general, oxidant stress-induced cell killing involves oxidation of pyridine nucleotides, accumulation of calcium in mitochondria, and superoxide formation by mitochondria, which ultimately leads to formation of membrane permeability transition pores and breakdown of the mitochondrial membrane potential (121, 122). In support of this intracellular signaling mechanism, the mitochondrial membrane permeability transition was observed during hepatic ischemia-reperfusion (29, 88, 130). Pharmacological inhibition of the mitochondrial membrane permeability transition protected against reperfusion injury (29, 42, 73, 88). Thus in an acute attack by Kupffer cells and neutrophils, proteases may not be necessary to cause hepatocellular necrosis. On the other hand, during a prolonged neutrophil response over several days, the injury may be caused by a combination of reactive oxygen and proteases (Fig. 2).

In addition to the inactivation of anti-proteases and the direct cytotoxic effects, reactive oxygen can promote reperfusion injury through stimulation of the transcription factors NF-κB and activator protein-1 (AP-1) (34, 56). The postischemic oxidant stress can enhance the expression of genes, such as TNF-α, inducible nitric oxide (NO) synthase (iNOS), heme oxygenase-1, CXC chemokines, and adhesion molecules. On the other hand, antioxidants attenuate proinflammatory gene expression through inhibition of NF-κB and AP-1 activation (8, 32, 70, 132, 181).

Microcirculatory disturbances and nonperfused sinusoids are well-recognized phenomena that contribute to reperfusion injury after hepatic ischemia (21, 81, 155). Although vascular lining cell injury and intravascular coagulation can reduce or block blood flow in sinusoids and cause liver injury, active vasoconstriction is also a major contributing factor (112, 113). In particular, endothelins have been identified as the most potent vasoconstrictors generated during reperfusion (40, 119). In addition, increased expression of the ETB receptor during reperfusion confers an increased responsiveness of the hepatic vasculature to endothelins (171). Therefore, endothelin receptor antagonists were shown to attenuate reperfusion injury in the liver (40, 79). These observations indicate that insufficient amounts of NO are produced to effectively counteract the enhanced vasoconstrictive state during reperfusion. In support of this hypothesis, it has been shown that any NOS inhibitor that affects eNOS reduces microvascular perfusion and aggravates liver injury during endotoxemia (123) and ischemia-reperfusion (44, 161). These data were recently confirmed in eNOS gene knockout mice (46, 89). All detrimental effects of eNOS inhibition can be completely reversed by exogenous NO-donors (134, 135, 161). Interestingly, NO protects under these conditions despite the increased formation of peroxynitrite (103, 161). This would suggest that the beneficial effects of maintaining liver blood flow by far outweigh the potential for cell damage by peroxynitrite. On the other hand, excessive NO formation after endotoxin-induced iNOS gene expression causes increased injury by peroxynitrite formation and systemic hypotension, which reduces liver blood flow (152). Although we could confirm the reported beneficial effects of a selective iNOS inhibitor in endotoxemia, the same inhibitor reduced microvascular blood flow and enhanced liver injury during hepatic ischemia-reperfusion (160). These findings, which were recently confirmed (50), suggest a potential role of iNOS in maintaining liver blood flow during reperfusion. However, others reported no effect of iNOS-derived NO on reperfusion injury (47, 135) or even a beneficial effect of a novel iNOS inhibitor (115). Similarly, mice deficient in iNOS showed a moderate reduction of reperfusion injury (89). However, some of the protective effects of iNOS inhibition were observed well before iNOS induction (89, 115). This raises the possibility that other effects of the drugs or compensatory mechanism for iNOS deficiency have to be considered (46). Independent of the source of NO, the available data in the literature indicate that in the postischemic liver, a new equilibrium is established between the increased vasoconstrictor formation and enhanced responsiveness on the one hand and the formation of NO as a countermeasure to maintain microvascular blood flow.

Despite the increased superoxide and NO formation during reperfusion, there is only little evidence for peroxynitrite generation with no obvious pathophysiological relevance (103, 161). The reason for the limited importance of peroxynitrite in hepatic ischemia-reperfusion injury might be the critical role of NO in
preventing excessive vasoconstriction and the fact that glutathione, a potent scavenger of peroxynitrite, is available in sufficient concentrations intra- and extracellularly to prevent tissue damage (77). In addition, other beneficial effects of NO, such as protection of mitochondria and induction of heat shock proteins, may also be involved (162).

NEUTROPHIL RESPONSE DURING REPERFUSION: CELLULAR ADHESION MOLECULES

During the development of the early reperfusion injury, neutrophils are recruited into the liver vasculature, are activated, and then cause aggravation of reperfusion injury (67). For neutrophil accumulation at a site of inflammation, several families of CAMs are involved (41). Initial tethering of neutrophils in post-capillary venules requires expression of selectins on endothelial cells and interaction with their counter-receptors on neutrophils. Subsequent activation of β2 integrins on neutrophils by chemotactic factors and upregulation of ICAM-1 on endothelial cells leads to the firm adhesion of neutrophils on the endothelial surface followed by extravasation and migration to the inflammatory site (41). However, this general scheme is only partially applicable to the postischemic liver (Figs. 1 and 2). The liver has two principal vascular beds for neutrophil accumulation during reperfusion: sinusoids and postsinusoidal venules (157). Venular endothelial cells can upregulate P-selectin expression by mobilizing preformed molecules from Weibel Palade bodies (137). In addition, P- and E-selectin as well as ICAM-1 and VCAM-1 can be transcriptionally upregulated (52). Sinusoidal endothelial cells do not contain Weibel Palade bodies and do not form relevant amounts of P-selectin but are able to express all other CAMs (52). The expression patterns of CAMs in experimental models are in agreement with observations in human livers (140, 145).

Selectins (137) and β2 integrins-ICAM-1 interactions (133, 156) are involved in neutrophil rolling and adhesion, respectively, in postsinusoidal venules. Recently, β1 integrins, such as α4β1 have also been implicated in leukocyte rolling/adhesion (37). However, evidence for transmigration from postsinusoidal venules is limited (157), and the relevance of this location for neutrophil migration into the parenchyma has been questioned (20, 133). In contrast, sinusoids were identified as the dominant sites for neutrophil extravasation (20) (Fig. 2). However, multiple experimental approaches could not establish a role of any adhesion molecule in neutrophil accumulation in hepatic sinusoids (31, 35, 37, 66, 133, 156, 167). Thus it was repeatedly postulated that a combination of factors, such as active vasoconstriction, vascular lining cell swelling and injury, and reduced membrane flexibility after activation of the neutrophil, contribute to the mechanical trapping of these leukocytes in sinusoids (66, 72, 113). Neutrophil sequestration in sinusoids may increase flow resistance but does not cause perfusion failure and injury (21, 158). Extravasation is a prerequisite for cell damage by neutrophils to occur (20). For this process, the neutrophil requires β2 integrin-ICAM-1 (31) and β1 integrin-VCAM-1 (30) interactions. Engagement of these adhesion molecules and E-selectin leads to further activation of the transmigrating leukocyte (87). Once extravasated, the neutrophil uses, in part, the β2 integrin lymphocyte function-associated antigen-1 (LFA-1; CD11a/CD18) to adhere to ICAM-1 expressed on hepatocytes (118). More importantly, engagement of the β2 integrin Mac-1 (CD11b/CD18) during neutrophil adhesion to its target causes degranulation (protease release) and a long-lasting, adhesion-dependent oxidant stress (107, 118, 141). During hepatic ischemia-reperfusion, ICAM-1 is transcriptionally induced on hepatocytes and sinusoidal endothelial cells (10, 35). However, the extensive vascular injury during reperfusion (15, 36, 114) eliminates, in part, the sinusoidal endothelial cell barrier and the neutrophil has direct access to hepatocytes. This leaves the adherence to hepatocytes, which is only partially dependent on LFA-1-ICAM-1 interactions (118), as the only role for ICAM-1. As a consequence, ischemia-reperfusion injury is only moderately or not at all attenuated by anti-ICAM-1 antibodies or in ICAM-1 gene knockout mice (35, 124, 133, 156). In contrast, blocking neutrophil transmigration with anti-ICAM-1 antibodies during endotoxemia prevented the neutrophil-induced injury (31). For similar reasons, blocking LFA-1 during endotoxemia was much more protective than the same intervention in hepatic ischemia-reperfusion (61). In contrast, blocking Mac-1 (CD11b) or the common β2 subunit CD18 was highly effective in preventing hepatic injury during ischemia-reperfusion (65, 101) and endotoxemia (68). In both cases, antibodies functionally inactivated these neutrophils and prevented the neutrophil-induced oxidant stress (65, 68), which led to a significant reduction in injury.

The role of selectins in liver inflammation, in particular P-selectin, is controversial. Sinusoidal endothelial cells neither contain Weibel Palade bodies nor do they transcriptionally upregulate relevant levels of P-selectin (33). No selectin-dependent rolling of leukocytes has been observed in sinusoids (167). However, most neutrophils extravasate from sinusoids (20). Consistent with these findings, no protective effect was found in a model of neutrophil-mediated liver injury when P-selectin was blocked with antibodies or in gene knockout mice (33). In contrast, during ischemia-reperfusion, a number of interventions directed against selectins reduced hepatic neutrophil accumulation and cell injury (e.g., 3, 38, 108, 169). Because these findings cannot be explained by the prevention of P- or L-selectin-dependent rolling in sinusoids, alternative explanations must be considered. The severe vascular injury during reperfusion induces aggregation of platelets, which can adhere through a selectin-dependent mechanism (169). Neutrophils may adhere to platelets rather than endothelial cells through P-selectin. Kubes et al. (82) suggested recently that most liver ischemia-reperfusion models include some degree of intestinal ischemia, which leads to neutrophil accumulation in remote or-
gans including the liver (49). Thus the lower number of neutrophils in the liver when selectins are blocked may be a secondary effect due to the protection of antiselectin therapy against intestinal reperfusion injury (82).

NETWORK OF PRO- AND ANTI-INFLAMMATORY MEDIATORS

Complement. Activation of complement is a critical event during reperfusion in experimental animals (64) and humans (146) (Figs. 1 and 2). The complement cascade can be rapidly activated by the extensive release of cellular proteins during the early reperfusion period. Complement factors, such as C5a, upregulate the Mac-1 receptor on circulating neutrophils (166) and cause neutrophil recruitment into sinusoids (7). C5a primes and activates neutrophils and Kupffer cells for reactive oxygen formation (64). However, complement activation has no effect on NF-κB activation and the expression of adhesion molecules on endothelial cells and hepatocytes (7). In addition to the proinflammatory effect, the assembled membrane attack complex can directly cause cell injury. Evidence for complement deposition was found in rat (19) as well as human livers (139) during reperfusion. Thus blocking complement activation effectively reduced the inflammatory response (64), microcirculatory disturbances (90), and cell injury (19, 64, 90).

Proinflammatory cytokines. Primary cytokines, such as TNF-α and IL-1, are generated mainly by Kupffer cells (25, 148) but also by extrahepatic macrophages (125) during reperfusion (Figs. 1 and 2). Both TNF-α and IL-1 can upregulate Mac-1 (CD11b/CD18) on neutrophils and recruit these cells into the liver vasculature (7, 166). In addition, these cytokines recruit and activate CD4+ T-lymphocytes in the liver during the early reperfusion period (180). Resident (92) and newly accumulated (180) CD4+ T-lymphocytes can produce mediators, such as TNF-β, IFN-γ, and granulocyte colony stimulating factor, which amplify Kupffer cell activation and promote neutrophil recruitment into the liver. In fact, CD4+ T-lymphocyte-deficient nude mice accumulate less neutrophils in the postischemic liver and sustain less injury during the later reperfusion phase (180). Adoptive transfer of wild-type T-cells into the nude mice restored neutrophil response and injury during reperfusion (180). In a model of cold storage and ex vivo, blood-free reperfusion, livers from nude mice produced less TNF-α and IFN-γ and had less injury (92). These data suggest that resident CD4+ T lymphocytes are activated during hepatic ischemia-reperfusion. The fact that inactivation of Kupffer cells with gadolinium chloride had similar effects indicates an interaction and cross-activation between these cells (92). However, despite the increased hepatic CD40 expression during reperfusion, no effect on cytokine formation or injury was found in livers from CD40-deficient mice (92). In contrast, recent observations in a warm ischemia-reperfusion model indicate that CD154-CD40 T-cell co-stimulation is important for injury and neutrophil recruitment in vivo (142). These data are consistent with previous reports (75, 85, 149), which described reduced hepatic neutrophil infiltration and injury in animals treated with T-cell-deactivating drugs, such as cyclosporine and FK506. Together, these newer findings clearly demonstrate a key role for CD4+ T-lymphocytes in cytokine formation, Kupffer cell activation and hepatic neutrophil recruitment. In contrast to activated complement factors, cytokines cause neutrophil sequestration in sinusoids as well as adherence in postsinusoidal venules (7). The latter effect is mediated by the transcriptional upregulation of adhesion molecules on endothelial cells due to the activation of the transcription factor NF-κB (10, 23, 31, 32). In addition, TNF-α and IL-1 are potent inducers of hepatic CXC chemokine synthesis (24), and under certain circumstances TNF-α can directly trigger apoptotic cell death (91). Because of the central role of TNF-α in promoting the inflammatory response at different levels, suppressing the formation of TNF-α or neutralizing it with antibodies proved to be highly effective in attenuating acute posts ischemic inflammation and injury (23, 25, 32). However, it must be considered that TNF-α is also involved in liver regeneration (1), which is vital for the long-term recovery from the ischemic insult and survival (16).

Chemokines. Several studies demonstrated the importance of chemokines, especially the neutrophil chemoattractant CXC chemokines MIP-2, KC, and cytokine-induced neutrophil chemoattractant-1 (CINC-1), in hepatic ischemia-reperfusion injury (24, 48, 94) (Figs. 1 and 2). Cytokines induce chemokine formation in Kupffer cells and hepatocytes (24, 48). Because of their potent chemotactic activity for neutrophils, it is generally assumed that CXC chemokines recruit neutrophils into the posts ischemic liver. Transgenic mice, which overexpress the human IL-8 gene, had neutrophil accumulation in liver sinusoids without injury (143). However, selective overexpression of CINC-1 in hepatocytes with a viral vector induced a chemotactic gradient, which not only caused neutrophil sequestration in sinusoids but also transmigration and injury (105). The capacity to upregulate Mac-1 on neutrophils was demonstrated for IL-8 (28) and MIP-2 (7) but not for CINC-1 (176) and KC (7). Compared with TNF-α, IL-1, and complement factors, recombinant CXC chemokines proved to be not very potent systemic activators of neutrophils (7). Thus the capacity of CXC chemokines to recruit neutrophils into sinusoids or even venules was limited compared with the other mediators (7). This raises questions regarding the actual mechanism of CXC chemokine involvement in a complex pathophysiology, such as ischemia-reperfusion injury.

Lipid mediators. Several lipid-derived inflammatory mediators have been implicated in the pathophysiology of reperfusion injury (Figs. 1 and 2). Platelet activating factor (PAF) is formed mainly by endothelial cells in experimental models and in humans during ischemia-reperfusion (45, 177). PAF can prime neutrophils for generation of superoxide (9). In addition, PAF is a potent activator of the β2 integrin Mac-1 and of adher-
ence-dependent reactive oxygen formation (141). PAF receptor antagonists protected against reperfusion injury (150, 177). The beneficial effect is, at least in part, due to reduced neutrophil activation and reduced microvascular damage (159). Leukotriene B4 (LTB4) is a potent chemotactic factor for human neutrophils (138). It is generated in large quantities presumably by neutrophils during the neutrophil-induced injury phase after hepatic ischemia (51). As such, it may contribute to the amplification of the neutrophil response during reperfusion (51). Lipid peroxidation products are chemotactic factors for neutrophils (27). This mechanism may be responsible for the propagation of the inflammatory injury during reperfusion especially at times when many peptide mediators are no longer generated (102). Lipid-soluble antioxidants and iron chelators can reduce the inflammatory response and reperfusion injury by reducing the signal (lipid peroxidation products) for continued neutrophil recruitment (55, 102).

**Anti-inflammatory cytokines.** Inflammation is a complex process, which requires not only mediators that promote but others that downregulate the inflammatory response (Fig. 1). IL-6 is formed during reperfusion after hepatic ischemia (16, 111). Reperfusion injury can be attenuated by the administration of recombinant IL-6 or is aggravated in IL-6-deficient mice (16). IL-6 is thought to act through several different mechanisms. IL-6 can downregulate TNF-α mRNA during reperfusion, and it promotes hepatocyte regeneration (16). The administration of IL-10 to rats after liver transplantation reduced formation of CXC chemokines and improved reperfusion injury and survival (179). The protective effect of IL-10 may be related to its ability to suppress the transcriptional activation of TNF formation, which may be responsible for the subsequent effects, such as reduced chemokine formation, reduced accumulation of neutrophils, and less ICAM-1 expression (173). A similar anti-inflammatory mechanism was described for IL-13, which attenuates reperfusion injury (174). The difference between IL-10 and IL-13 appears to be that IL-10 prevents activation of NF-κB and IL-13 activates the transcription factor STAT-6 (174). Secretory leukocyte protease inhibitor (SLPI) is a protein that can reduce TNF formation by macrophages (74) and can inhibit serine proteases including proteases released by neutrophils (e.g., elastase, cathespin G, etc.) (153). Recently, Lentzsch et al. (96) demonstrated that SLPI protected against reperfusion injury by reducing TNF formation and attenuation of the TNF-dependent inflammatory response. In contrast to IL-6, IL-10, IL-13, and SLPI, IL-12 appears to be supporting the inflammatory response and postischemic injury (95). Studies with neutralizing anti-IL-12 antibodies or with IL-12-deficient mice showed that IL-12 formation is important for prolonged TNF-α and IFN-γ formation, both of which promote a neutrophil-dependent injury mechanism during reperfusion (95). Thus a complex network of regulatory cytokines and other mediators modulates the inflammatory response after hepatic ischemia (Figs. 1 and 2).

**MODE OF CELL DEATH DURING ISCHEMIA-REPERFUSION**

Comments regarding this controversial topic will be limited. For an in-depth discussion, the reader is referred to a more specialized review (57). During reperfusion, cells are killed by a combination of several mechanisms including intracellular oxidant stress, exposure to external cytotoxic mediators, and prolonged ischemia. Cell death of hepatocytes and endothelial cells during reperfusion is characterized by swelling of cells and their organelles, release of cell contents, eosinophilia, karyolysis, and induction of inflammation (43). These morphological features are characteristic for oncotic necrosis. However, in recent years it was postulated that most liver cells actually die by apoptosis (26, 80), which is morphologically characterized by cell shrinkage, formation of apoptotic bodies with intact cell organelles, and the absence of inflammation (106). On the basis of the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay, it was suggested that 50–80% of all liver endothelial cells and hepatocytes die through apoptosis during the first 3–6 h of reperfusion (26, 80). However, on closer scrutiny of these data, a number of serious concerns emerged. First, the TUNEL assay alone is not suitable to identify apoptosis, because DNA strand breaks also occur during oncotic necrosis (57). Second, the minimal amount of caspase activation does not correlate with the alleged large number of apoptotic cells (43, 57). Third, immediate cell contents release and inflammation are not consistent with apoptosis as the only mode of cell death (43, 57). Fourth, interventions such as overexpression of Bcl-2 can prevent both apoptotic and necrotic cell death (57). Fifth, on the basis of morphological criteria, the number of apoptotic cells never exceeds 1–2% of all endothelial cells and hepatocytes at any time during reperfusion after 60 min of warm ischemia (43). Similar observations were made after cold storage and reperfusion (131). Thus even if the turnover of apoptotic cells is considered, >90% of cells die by oncotic necrosis during ischemia-reperfusion. In contrast to previous assumptions that apoptosis does not cause inflammation, we demonstrated that apoptotic cell death can trigger neutrophil transmigration with massive aggravation of the apoptotic cell injury (69). This mechanism may explain why caspase inhibitors can have a significant overall protective effect on hepatic ischemia-reperfusion injury (78).

**HEAT SHOCK AND ISCHEMIC PRECONDITIONING**

Heat shock (i.e., increasing the body temperature to 42°C for 15 min) or ischemic preconditioning [i.e., exposure of the liver to a brief period of ischemia (5–10 min)] and reperfusion effectively protect the liver against warm ischemia (84, 104) and cold storage injury (170). Preliminary studies in humans confirmed the efficacy of ischemic preconditioning (22). There is strong evidence that adenosine is a key mediator in ischemic preconditioning (128). Adenosine stimulates the adenosine A2 receptor (5, 120, 129), which initiates...
NO formation (128, 129), and causes activation of protein kinase C, adenosine monophosphate-activated protein kinase, and p38 MAPK (17, 18, 127, 151). Activation of these intracellular signaling pathways 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 initiate a regenerative response (151). Stimulation of regeneration with recombinant IL-6 was shown to attenuate reperfusion injury after warm ischemia (16). Induction of heat shock proteins (HSP), especially HSP70 (84) and heme oxygenase-1 (HSP32) (2, 131), has also been implicated in the mechanism of preconditioning. HSP induction can reduce the nuclear binding of proinflammatory transcription factors (154) and increase the antioxidant capacity of cells (8). Both effects may contribute to the reduced formation of TNF-α and an attenuated inflammatory response in preconditioned livers (6, 172). In addition, carbon monoxide, a by-product of heme oxygenase-1 activity, was shown to activate p38 MAPK as a key mechanism of carbon monoxide-mediated protection against ischemia-reperfusion injury (4). Thus a combination of factors may contribute to the reduced injury and the improved long-term survival in animals subjected to preconditioning including the increased tolerance to ischemic injury and oxidant stress, a reduced inflammatory response, and enhanced regeneration.

In summary, ischemia-reperfusion injury is a complex pathophysiology with a number of contributing factors (Figs. 1 and 2). The ischemic insult can lead to sublethal cell injury, which is aggravated by the formation of reactive oxygen from various intracellular sources during reperfusion. In addition, formation of proinflammatory mediators and the recruitment and activation of macrophages, neutrophils, and lymphocytes can further enhance the injury. Microcirculatory disturbances lead to underperfused areas in the liver and may cause ischemic injury. All mechanisms contribute to various degrees in the overall pathophysiology. Therefore, it is difficult to achieve effective protection by targeting individual mediators or mechanisms. In contrast, the most promising protective strategy against ischemia-reperfusion injury explored during the last few years is preconditioning, which appears to increase the resistance of liver cells to ischemia and reperfusion events. Preconditioning or pharmacological interventions that mimic these effects have the greatest potential to improve clinical outcome in liver transplantation and liver surgery with vascular exclusion.

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