Protection of the Liver by Ischemic Preconditioning: A Review of Mechanisms and Clinical Applications

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Key Words
Liver • Preconditioning • Ischemia • Reperfusion •
Liver injury • Hepatoprotection • Liver transplantation •
Liver resection • Nitric oxide • Adenosine •
Heat shock proteins • Protein kinase C

Abstract
Ischemic preconditioning refers to the endogenous mechanism of protection against a sustained ischemic insult following an initial, brief ischemic stimulus. Ischemia-reperfusion injury of the liver is a major cause of morbidity and mortality in liver surgery and transplantation and ischemic preconditioning is a promising strategy for improving the outcome of liver surgery. The preconditioning phenomenon was first described in a canine model of myocardial ischemia-reperfusion injury in 1986 and since then has been shown to exist in other organs including skeletal muscle, brain, kidneys, retina and liver. In the liver, the preconditioning effect has been demonstrated in rodents and a recent study has demonstrated human clinical benefits of preconditioning during hemihepatectomies. Ischemic preconditioning has been described as an adaptive response and although the precise mechanism of hepatoprotection from preconditioning is unknown it is likely to be a receptor-mediated process. Several hypotheses have been proposed and this review assesses possible mechanisms of ischemic preconditioning and its role in hepatic surgery and liver transplantation. The future lies in defining the mechanisms of the ischemic preconditioning effect to allow drug targeting to induce the preconditioning response.

Introduction
Ischemia-reperfusion injury is a major cause of morbidity and mortality following liver surgery and transplantation. Ischemia-reperfusion injury after prolonged ischemia has been shown to occur in virtually all organ systems. Ischemic (and reperfusion) injury to the liver occurs during liver resections performed under temporary inflow occlusion (Pringle manoeuvre) or inflow and outflow occlusion commonly used to reduce intraoperative blood loss, and during storage and implantation of livers for transplantation. The liver tolerates prolonged ischemia poorly and safe ischemic times particularly for the diseased liver are not known. Both warm and cold ischemias result in significant liver injury [1], and ischemia reperfusion injury of the liver can result in multiple system organ failure and systemic inflammatory response syndrome (fig. 1).
Brief episodes of ischemia followed by a period of reperfusion called ischemic preconditioning (IPC) have been shown to protect organs against subsequent sustained ischemia. IPC was first described by Murry et al. [2] in 1986. In a canine model, they demonstrated that multiple brief ischemic episodes protected the heart from a subsequent sustained ischemic insult. Since then myocardial IPC has been shown to occur in many animal species [3] and in humans [4]. Subsequently, IPC has been demonstrated in other organ systems including skeletal muscle [5], brain [6], spinal cord [7], kidney [8], intestine [9] and liver [10]. Although these studies suggested a preconditioning response in most organ systems the mechanism of the preconditioning effect remains uncertain.

IPC has been described as an endogenous adaptive mechanism for prevention of injury resulting from ischemia reperfusion [2]. The phenomenon is fascinating, as it is easily reproducible and potentially readily applicable in clinical situations. In the liver the preconditioning effect is a promising strategy in assisting preservation of the liver in clinical situations of anticipated hepatic ischemia such as transplantation and during resection for tumors using hepatic vascular occlusion. Increase in ischemic hepatic tissue tolerance may protect against ischemia-reperfusion injury and assist preservation of livers for transplantation as well improve outcome after surgical procedures. Clearly, identifying the mechanism of preconditioning may allow recognition of a pharmacological agent, to protect the liver from ischemic injury.

The mechanisms underlying the preconditioning effect have not been defined. In contrast, various potential mediators have been proposed and investigated. Most of the data on mediators of preconditioning in organs including the liver has been extrapolated from information gathered in the heart. This article reviews the major developments in characterization of mechanisms of IPC in the liver. In addition, clinical applications of IPC to minimize ischemic injury to the liver have been discussed.

**Methods of Search**

All the studies were identified by PubMed, ISIS and CAS searches between the years 1966 and September 2002 with the following key words: ischemia, ischemia-reperfusion injury, preconditioning, IPC, hepatoprotection. Other sources include review articles and textbooks.

**Evidence that IPC Occurs in the Liver**

**Studies on Warm Ischemia**

Over the last decade many investigators have studied the effects of IPC on regional and global ischemia in the liver, and the evidence is encouraging (see table 1). Lloris-Carsi et al. [10] in 1993 first demonstrated in the rat liver that a single episode of preconditioning with 5 min portal triad clamping followed by 10 min reperfusion showed improved survival and decreased liver enzyme levels after subsequent 90 min ischemia. Hardy et al. [11] showed improved survival in rats undergoing liver resection during 45 min ischemia after prior 5 min ischemia with 10 min reperfusion. Similarly Yoshizumi et al. [12] have demonstrated improved survival and increased tissue ATP with preconditioning in a rat liver resection model. Subsequently the IPC effect in the liver has been reproduced in several in vivo rodent models of partial and global liver ischemia [13–38]. These studies have demonstrated that liver IPC for warm ischemia resulted in...
Table 1. Current data on hepatic IPC: published studies

<table>
<thead>
<tr>
<th>Study group</th>
<th>Year</th>
<th>Species</th>
<th>IPC time</th>
<th>Ischemia time</th>
<th>Reperfusion time</th>
<th>Hepatic ischemia</th>
<th>Pharmacological manipulations</th>
<th>Parameters assessed</th>
<th>Outcome of IPC</th>
<th>Proposed mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lloris-Carsi et al. [10]</td>
<td>1993</td>
<td>Rat</td>
<td>1 × 3 (5I + 10R)</td>
<td>90</td>
<td>3 days</td>
<td>Total</td>
<td>Nil</td>
<td>LFTs and survival</td>
<td>70% 3 day survival + ↓ liver enzyme levels</td>
<td>Not addressed</td>
</tr>
<tr>
<td>Hardy et al. [11]</td>
<td>1996</td>
<td>Rat</td>
<td>5I + 10R</td>
<td>0–45</td>
<td>1–8 days</td>
<td>Partial</td>
<td>Nil</td>
<td>LFTs, histology and survival</td>
<td>90% 1 day survival, ↑ prothrombin time</td>
<td>Not addressed</td>
</tr>
<tr>
<td>Peralta et al. [13]</td>
<td>1996</td>
<td>Rat</td>
<td>10I + 10R</td>
<td>90</td>
<td>90</td>
<td>Partial</td>
<td>Spermine NONOate, L-NAME/bosentan</td>
<td>LFTs, tissue endothelin and NOS activity, histology</td>
<td>↓ GPT and endothelin ↑ cNOS</td>
<td>NO</td>
</tr>
<tr>
<td>Kume et al. [29]</td>
<td>1996</td>
<td>Rat</td>
<td>15I</td>
<td>30</td>
<td>10 and 40</td>
<td>Total</td>
<td>Hyperthermia: 42°C for 15 min</td>
<td>ATP, transaminase, LDH, HSP72, survival</td>
<td>100% survival, ↑ ATP, ↓ transaminase and LDH</td>
<td>HSP72</td>
</tr>
<tr>
<td>Peralta et al. [17]</td>
<td>1998</td>
<td>Rat</td>
<td>10I + 10R</td>
<td>90</td>
<td>90</td>
<td>Partial</td>
<td>Spermine NONOate; adenosine, L-NAME</td>
<td>LFTs, HM</td>
<td>↓ transaminases and LDH</td>
<td>Adenosine and NO</td>
</tr>
<tr>
<td>Peralta et al. [18]</td>
<td>1998</td>
<td>Rat</td>
<td>2–30I + 10R</td>
<td>90</td>
<td>90</td>
<td>Partial</td>
<td>Adenosine deaminase/xanthine; spermine NONOate</td>
<td>LFTs</td>
<td>↓ transaminases</td>
<td>Adenosine and NO</td>
</tr>
<tr>
<td>Yoshizumi et al. [12]</td>
<td>1998</td>
<td>Rat</td>
<td>5I + 10R</td>
<td>40</td>
<td>120</td>
<td>Partial</td>
<td>Nil</td>
<td>LFTs, bile flow, tissue ATP</td>
<td>↓ transaminase, LDH and tissue necrosis, ↑ ATP</td>
<td>Not addressed</td>
</tr>
<tr>
<td>Yin et al. [26]</td>
<td>1998</td>
<td>Rat</td>
<td>5I, 10I or 20I + 10R</td>
<td>60 min</td>
<td>6 min to 5 hours</td>
<td>Cold storage</td>
<td>L-arginine/adenosine; L-NAME</td>
<td>LFTs, bile flow, TNF-a, survival</td>
<td>87.5% 1 day and 75% 3 days graft survival</td>
<td>NO</td>
</tr>
<tr>
<td>Adam et al. [57]</td>
<td>1998</td>
<td>Rat</td>
<td>5 or 10I + 10R</td>
<td>24 h</td>
<td>180</td>
<td>Cold storage (UW)</td>
<td>Nil</td>
<td>Bile, transaminases, LDH release, vascular resistance</td>
<td>↑ transaminases, LDH and vascular resistance</td>
<td>Not addressed</td>
</tr>
<tr>
<td>Peralta et al. [19]</td>
<td>1999</td>
<td>Rat</td>
<td>10I + 10R</td>
<td>90</td>
<td>90</td>
<td>Partial</td>
<td>Adenosine; adenosine deaminase; DPPC; DMPX</td>
<td>Transaminases, hepatic perfusion, nitrite/nitrate</td>
<td>Adenosine A2 receptor antagonist abolished</td>
<td>Adenosine A2 receptors and NO</td>
</tr>
<tr>
<td>Peralta et al. [20]</td>
<td>1999</td>
<td>Rat</td>
<td>10I + 10R</td>
<td>90</td>
<td>90</td>
<td>Partial</td>
<td>Gadolinium chloride, TNF, L-NAME, spermine NONOate</td>
<td>TNF, transaminases, vascular permeability, edema, MPO, histology</td>
<td>↑ TNF and tissue injury</td>
<td>NO</td>
</tr>
<tr>
<td>Yadav et al. [27]</td>
<td>1999</td>
<td>Mouse</td>
<td>10I + 10R</td>
<td>75–90</td>
<td>60–180</td>
<td>Partial and total</td>
<td>Nil</td>
<td>LFTs, hepatocellular apoptosis, survival</td>
<td>↓ apoptosis of hepatocytes and SEC</td>
<td>Modulation of apoptosis cascade</td>
</tr>
<tr>
<td>Nakayama et al. [28]</td>
<td>1999</td>
<td>Rat</td>
<td>10I + 10R</td>
<td>45</td>
<td>40 min to 24 h</td>
<td>Total</td>
<td>AdoRA1, A2 antagonists and agonists</td>
<td>LFTs, tissue ATP, histology, survival</td>
<td>↑ adenosine ↓ tissue damage</td>
<td>Adenosine A2 receptors</td>
</tr>
<tr>
<td>Zapletal et al. [40]</td>
<td>1999</td>
<td>Rat</td>
<td>5I + 10R</td>
<td>70</td>
<td>30</td>
<td>Partial</td>
<td>Nil</td>
<td>Intravital microscopy</td>
<td>↑ perfusion parameters, ↑ leucocyte adherence</td>
<td>Adenosine</td>
</tr>
<tr>
<td>Nilsson et al. [24]</td>
<td>2000</td>
<td>Rat</td>
<td>10I + 15R</td>
<td>60</td>
<td>60</td>
<td>Total</td>
<td>Dipyriridamole</td>
<td>LFTs, HM</td>
<td>↑ peripheral liver blood flow and ↓ transaminase</td>
<td>Adenosine</td>
</tr>
<tr>
<td>Howell et al. [25]</td>
<td>2000</td>
<td>Mouse</td>
<td>5I + 10R</td>
<td>30</td>
<td>30 min to 24 h</td>
<td>Partial</td>
<td>Dipyriridamole</td>
<td>LFTs, leucocyte/endothelial cell adhesion</td>
<td>↑ endothelial/leucocyte interaction and transaminase</td>
<td>Adenosine</td>
</tr>
<tr>
<td>Clavien et al. [43]</td>
<td>2000</td>
<td>Human</td>
<td>10I + 10R</td>
<td>30</td>
<td>30</td>
<td>Total</td>
<td>Nil</td>
<td>LFTs, hepatocellular apoptosis</td>
<td>↓ transaminases and apoptotic sinusoidal lining cells</td>
<td>Modulation of apoptosis cascade</td>
</tr>
<tr>
<td>Peralta et al. [21]</td>
<td>2000</td>
<td>Rat</td>
<td>10I + 10R</td>
<td>0–10</td>
<td>90</td>
<td>Partial</td>
<td>SQ-22536, forskolin</td>
<td>Adenine nucleotides, glycoprotein, glucose-6-P, fructose-6-P, transaminases</td>
<td>Preserved energy metabolism during sustained ischemia</td>
<td>cAMP dependent PKC</td>
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<tr>
<td>Tsunaya et al. [31]</td>
<td>2000</td>
<td>Mice</td>
<td>10I/15I</td>
<td>70</td>
<td>1–48 h</td>
<td>Total</td>
<td>Nil</td>
<td>TNF, IL-6, transaminase, histology, survival</td>
<td>↑ survival, ↓ transaminase, TNF, IL-6 and liver necrosis</td>
<td>Not addressed</td>
</tr>
<tr>
<td>Peralta et al. [22]</td>
<td>2001</td>
<td>Rat</td>
<td>10I + 10R</td>
<td>90</td>
<td>0–360</td>
<td>Partial</td>
<td>AICAR/8-bromo-AMP-AraA/ZVAD-spermine NONOate/L-NAME</td>
<td>AMPK activity, nucleotides, lactate, transaminases, apoptosis, histology</td>
<td>AMPK activation, ↑ ATP, ↓ lactate and hepatic injury</td>
<td>AMP via PKC</td>
</tr>
<tr>
<td>Peralta et al. [23]</td>
<td>2001</td>
<td>Rat</td>
<td>10I + 10R</td>
<td>90</td>
<td>90</td>
<td>Partial</td>
<td>ICAM, P-selectin and TNF blockade; TNF/gadolinium</td>
<td>MPO and lipid peroxidation, vascular permeability, TNF, transaminases, hepatic perfusion, histology, ICAM and P-selectin expression</td>
<td>Remote organ protection by hepatic preconditioning</td>
<td>TNF and P-selectin</td>
</tr>
<tr>
<td>Schulz et al. [58]</td>
<td>2001</td>
<td>Pig</td>
<td>10I + 10R</td>
<td>200</td>
<td>480 or 300</td>
<td>Total</td>
<td>Nil</td>
<td>ICG clearance, bile flow, transaminases, ATP, glycogen and lactate contents, histology</td>
<td>No protection against prolonged ischemia</td>
<td>Not addressed</td>
</tr>
</tbody>
</table>

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| Study group          | Year | Species | IPC time min | Ischemia time min | Reperfusion time min | Hepatic ischemia | Pharmacological manipulations | Parameters assessed                                                                 | Outcome of IPC                                                                 | Proposed mechanism                                                                 |
|---------------------|------|---------|--------------|------------------|---------------------|-----------------|-----------------------------|---------------------------------|-----------------------------------------------------------------------------------|
|                     |      |         |            |                  |                     |                 |                             |                                  |                                    |                                                                                   |
| Arai et al. [51]    | 2001 | Rat     | 5 or 10I+10R| 30 b-           | 15 or 240           | Cold storage     | Nil                          | SEC injury, superoxide formation in Kupffer cells, graft survival and TNF after OLT | ↑ graft survival and protection contralateral liver                           | Not addressed                                                                     |
| Ricciardi et al. [52]| 2001 | Pig     | 15I+15R     | 120 b-          | 240                 | PKC inhibitor chelerythrine | Graft function and circulation, LDH, endothelial cell damage, PKC levels | ↑ graft function                   | PKC                                                                               |
| Ricciardi et al. [53]| 2001 | Pig     | 15I+15R     | 120 b-          | 240                 | Tyrosine kinase inhibitor, genistein | Graft function and circulation, Tyrosine kinase activity | ↑ graft function                   | Tyrosine kinases                                                                  |
| Saito et al. [33]   | 2001 | Rat     | 10I+10R     | 40               | 6–48 h              | Partial          | Nil                          | Transaminases, endothelial cell injury, apoptosis, transcription of IEG's          | transaminases, endothelial cell injury, necrosis, apoptosis and IEG transcription | Not addressed                                                                     |
| Serafin et al. [47] | 2002 | Rat     | 10I+10R     | 40–120           | Up to 7 days        | Partial          | Nil                          | Transaminases, LDH, necrosis, hepatocyte regeneration                          | ↓ transaminases, LDH and necrosis                                              | Not addressed                                                                     |
| Zhang et al. [34]   | 2001 | Mice    |              |                  |                     |                 |                             | Transaminases, LDH, LPO, SOD                                                   | ↓ transaminases, LDH, LPO and ↑ SOD                                           | Not addressed                                                                     |
| Inhui et al. [39]   | 2001 | Rat     | 10I+10R     | 40               | 6–48 h              | Nil              | Transaminases, LDH, necrosis, apoptosis, IEG transcription alterations          | ↓ transaminases, LDH, necrosis, apoptosis, IEG transcription alterations          | Not addressed                                                                     |
| Peralta et al. [35] | 2002 | Rat     | 10I+10R     | 90               | 90                  | Partial          | Xanthine, xanthine oxidase, allopurinol, GSH ester                           | Xanthine, glutathione, superoxide dismutase, lipid peroxidation, transaminases | ↓ xanthine, XO in liver with ↑ neutrophil accumulation, oxidative stress, and microvascular disorders in lung | Xanthine/XOD pathway for ROS generation                                           |
| Sindramp et al. [50]| 2002 | Rat     | 10I+15R     | 30 b-           | 60                  | Cold storage (UW) | N-acetyl-cysteine              | SEC detachment, apoptosis, peroxide, gelatinolytic and gelatinase activity       | ↓ transaminases, apoptosis markers, histology, survival                          | ↓ transaminase, no apoptosis or necrosis, 100% survival for ischemic period up to 75 min but not 120 min | Modulation of apoptosis cascade                                                   |
| Rodiger et al. [36] | 2002 | Mice    | 10I+15R     | 75-120           | 180                 | Partial          | Nil                          | Transaminases, LDH, necrosis, histology                                          | ↓ transaminases, LDH and necrosis                                              | Not addressed                                                                     |
| Ajamieh et al. [37] | 2002 | Rat     | 10I+10R     | 90               | 90                  | Partial          | Ozone                        | Transaminases, 5'-NT, oxidative stress, histology                                | ↓ hepatocellular injury and oxidative stress                                     | Not addressed                                                                     |
| Peralta et al. [14] | 2002 | Mice    | 10I+15R     | 90               | 6–24 h              | Partial          | Gadolinium chloride, TNF, MIP-2 antibodies against TNF and MIP-2             | Transaminases, TNF, MIP-2, MDA, MPO, P-selectin expression                     | Preconditioning and Bcl-2 overexpression together abolished liver injury       | Via TNF and MIP-2 inhibition                                                    |
| Serafin et al. [47] | 2002 | Rat     | 10I+10R, 10I+15R or 5I+10R | 60 | 2–24 h | Partial | NO donors and inhibitors, glutathione ester | Microcirculation, neutrophil activity, lipid peroxidation | ↓ hepatic injury in normal and fatty livers | NO                                                                            |
| Teoh et al. [15]    | 2002 | Mice    | 2–20I+10R   | 90               | 24 h                | Partial          | Nil                          | Transaminases, histology, microcirculation, neutrophil activity, lipid peroxidation | ↓ hepatic injury in normal and fatty livers | NF-κB and SAPKs                                                               |
| Fernandez et al. [16]| 2002 | Rat     | 10I+10R     | 90               | 16 h                | Total            | Xanthine, XOD                | Transaminases, ROS                                                              | ↓ liver and lung injury                                                        | Via xanthine/XOD blockade                                                   |
| Kori et al. [42]    | 2002 | Rat     | 5I+10R     | 45               | 120                 | Partial          | L-arginine, L-NAMe | Transaminases, NOx, hepatic oxygenation                                      | ↓ hepato-cellular injury, intracellular oxygenation | NO                                                                    |
| Kori et al. [41]    | 2002 | Rat     | 5I+10R     | 45               | 120                 | Partial          | L-arginine, L-NAMe | Transaminases, NOx, cGMP, microcirculation                                     | ↓ hepato-cellular injury, microcirculation                                       | NO                                                                    |
| Iwashita et al. [38]| 2002 | Rat     | 10I+10R    | 15 × 3 or 45    | Up to 180 min       | Total            | Nil                          | Transaminases, TNF, histology                                                 | ↑ protective effect for intermittent than continuous l                         | Not addressed                                                                     |
| Funaki et al. [62]  | 2002 | Mice    | 15I+20R    | 70               | 0–24 h              | Total            | Nil                          | NF-κB activity                                                                | ↓ NF-κB activation                                                              | NF-κB                                                                         |
| Ricciardi et al. [63]| 2002 | Pig     | 15I+15R    | Cold storage  | Genistein, chelerythrine | TK, PKC, NF-κB   | TK, PKC, NF-κB activation                                                     | PKC and TK, NF-κB                                                               |                                                                                   |
Table 1 (continued)

<table>
<thead>
<tr>
<th>Study group</th>
<th>Year</th>
<th>Species</th>
<th>IPC time min</th>
<th>Ischemia time min</th>
<th>Reperfusion time, min</th>
<th>Hepatic ischemia</th>
<th>Pharmacological manipulations</th>
<th>Parameters assessed</th>
<th>Outcome of IPC</th>
<th>Proposed mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arai et al. [48]</td>
<td>1999</td>
<td>Rat</td>
<td>5I+5R</td>
<td>30 b)</td>
<td>15</td>
<td>Cold storage (UW)</td>
<td>Nil</td>
<td>SEC killing, Kupffer cell activation</td>
<td>↓ SEC death and KC activation</td>
<td>Not addressed</td>
</tr>
<tr>
<td>Arai et al. [49]</td>
<td>2000</td>
<td>Rat</td>
<td>5I+5R</td>
<td>30 b)</td>
<td></td>
<td>Cold storage (UW)</td>
<td>AdoR A1, A2 agonists and antagonists</td>
<td>SEC killing, SEC cAMP</td>
<td>↓ SEC death and cAMP</td>
<td>Adenosine A2 receptors via cAMP</td>
</tr>
<tr>
<td>Carini et al. [54]</td>
<td>2000</td>
<td>Rat</td>
<td>10I+10R</td>
<td>90</td>
<td></td>
<td>Hypoxia</td>
<td>PKC stimulators and inhibitors</td>
<td>Hepatocyte viability, pH, Na+, ATP</td>
<td>↓ hepatocyte cell death</td>
<td>PKC</td>
</tr>
<tr>
<td>Carini et al. [55]</td>
<td>2001</td>
<td>Rat</td>
<td>10I+10R</td>
<td>90</td>
<td></td>
<td>Hypoxia</td>
<td>AdoR A1, A2 agonists and antagonists, PKC, MEK inhibitors</td>
<td>Cell viability, PKC isoenzyme activity, P38 MAPK activity</td>
<td>hepatocyte killing ↓ reduced by 35%</td>
<td>Adenosine A2 receptors, Gi proteins, phospholipase C, PKC, P38 MAPK</td>
</tr>
<tr>
<td>Compagnon et al. [56]</td>
<td>2002</td>
<td>Rat</td>
<td>10 anoxia + 10 reoxygenation</td>
<td>30 + 24 -48 h</td>
<td>60</td>
<td>Warm ischaemia + cold storage</td>
<td>Nil</td>
<td>LDH, ATP, oxygen uptake, protein synthesis</td>
<td>hepatocyte viability, ↑ ATP and protein synthesis</td>
<td>Not addressed</td>
</tr>
</tbody>
</table>

1 = Ischemia; R = reperfusion; LFTs = liver function tests; NOS = nitric oxide synthase; cNOS = constitutive nitric oxide synthase; GPT = glutamate pyruvic transaminase; ATP = adenosine triphosphate; LDH = lactate dehydrogenase; HM = hepatic microcirculation; MPO = myeloperoxidase; IL-6 = interleukin-6; AMPK = adenosine monophosphate-activated protein kinase; ICAM = intercellular adhesion molecule; ICG = indocyanine green; OLT = orthotopic liver transplantation; iEGF = immediate early genes; LPO = lipid peroxidation; SOD = superoxide dismutase; XOD = xanthine oxidase; 5'-NT = 5'-nucleotidase; MIP-2 = macrophage inflammatory protein-2; MDA = malondialdehyde; ROS = reactive oxygen species; cAMP = cyclic adenosine monophosphate; SAPKs = stress-activated protein kinases; p38 MAPK = p38 mitogen-activated protein kinase; ↑ = increased; ↓ = decreased.

Cold ischemia.

Decreased hepatocellular injury [17, 18], increased tissue ATP [12, 22], decreased tumor necrosis factor-α (TNF-α) [19, 31] and IL-6 [31] release, decreased leukocyte/endothelial cell interactions [25], decreased endothelial cell injury [39], increased peripheral liver blood flow [24], increased microcirculation [40, 41], decreased hepatocellular apoptosis [27], preserved energy metabolism [21], increased hepatic intracellular oxygenation [42] and remote organ protection [23]. These studies provide considerable evidence that preconditioning ameliorates ischemia-reperfusion-induced liver injury in the rodent liver. Encouragingly, recent investigations by Clavien et al. [43] have shown that IPC exists in the human liver. In this study, patients undergoing hemihepatectomies under in-flow occlusion showed inhibition of sinusoidal endothelial cell (SEC) apoptosis within 30 min of reperfusion in the preconditioned livers. In a murine model of partial hepatic ischemia, the same group showed that IPC also inhibits apoptosis of hepatocytes at later stages of reperfusion [27].

The steatotic liver is particularly susceptible to ischemia-reperfusion injury resulting in poor outcome following liver surgery [44] and transplantation [45, 46]. There is, therefore, an urgent need for strategies against ischemia-reperfusion injury to increase the number of organs available for liver transplantation and, moreover, improve the outcomes after transplantation of fatty livers and after hepatic resections. The recent report by Serafin et al. [47] shows IPC increases the tolerance of fatty livers to ischemia-reperfusion injury in rats. In this study obese Zucker rats subjected to 60 min of lobar liver ischemia had 70% survivors at 30 days in the ischchemically preconditioned group as compared to no survivors in the ischemic controls.

Studies on Cold Ischemia

The protective effect of IPC is not restricted to warm ischemia and decreased tissue damage in cold preserved livers (cold storage-reperfusion injury) after IPC has been demonstrated in small and large animal models. In rat livers, IPC prior to storage in cold University of Wisconsin (UW) solution for 30 h decreased SEC death and Kupffer cell (KC) activation [48, 49]. In another study combining two sets of experiments, IPC prior to preservation of rat livers in cold UW solution for 30 h decreased SEC detachment and activities of matrix metalloproteinases, and also decreased SEC apoptosis after 1 h of reperfusion in an isolated perfused rat liver model [50]. In a rat liver transplant model IPC protected liver grafts from ischemia-reperfusion injury [26]. Furthermore, in a recent study in...
cold-preserved rat livers Arai et al. [51] have observed that the benefit of IPC extends not only to the ipsilateral lobe, but also to the contralateral lobe resulting in an improved graft survival after orthotopic liver transplantation. In this study the authors observe that ‘such heterologous preconditioning provides a new means to protect liver tissue against ischaemia reperfusion injury without imposing ischaemia on the target tissue’ [51]. IPC also increased resistance to cold ischemic liver injury in pigs [52, 53].

Studies on Isolated Hepatocytes

The hepatoprotective response of IPC has also been shown in isolated hepatocytes. In in vitro studies on freshly isolated hepatocytes, preconditioned hepatocytes showed increased resistance to cell killing during hypoxic incubation [54, 55]. Another study has shown IPC-improved hepatocyte viability and energy metabolism in a model of isolated rat hepatocytes subjected to hypothermic preservation injury preceded by normothermic ischemia [56].

The above studies mostly in rodent livers have shown liver protection by IPC to warm and cold ischemia. However, there are a few published studies which have suggested that hepatic IPC may have only limited benefit. A study by Adam et al. [57] in fact suggested that preconditioning had a deleterious effect on hepatic tolerance to cold ischemia. This study used a model of isolated perfused livers from Wistar rats [57]. Preconditioning protocol of 5 or 10 min ischemia followed by 10 min reperfusion before liver harvesting, prior to extended cold ischemia of 24 h resulted in extensive reperfusion injury, increased vascular resistance and increased transaminases and LDH release. In a larger animal model using pigs, a preconditioning protocol of repeated 10 min ischemia followed by 10 min reperfusion, prior to 120 min or 200 min sustained ischemia was tested [58]. In the 120-min ischemia group IPC increased bile flow and ATP, but the degree of necrosis and apoptosis was not different from the control group. With 200 min ischemia IPC resulted in no significant differences in bile flow, ATP and liver enzymes from control group, and the degree of necrosis and apoptosis was in fact greater in preconditioned livers. This study suggested that IPC conferred some functional protection against reversible ischemia but no protection from prolonged ischemia in pigs [58]. The major difference between this study and those showing benefits with IPC is the use of three cycles of preconditioning in comparison with a single episode. In a more recent study Rudiger et al. [36] noted that in mice IPC resulted in 100% animal survival with no morphological parenchymal injury after 75 min sustained ischemia as against 14% survival with significant parenchymal injury after 120 min ischemia.

Thus, a large body of evidence favors liver protection by IPC from injury in both warm and cold ischemia. The existence of IPC in the liver has been demonstrated in rodents, pig and humans. Although most of the data on hepatic IPC has been gathered in rodents and it is recognized that information on preconditioning in rats may not always be extrapolated to larger species and humans, the recent report by Clavien et al. [43] of the first human study is a thoughtful example of potential clinical application of the preconditioning effect.

Possible Mechanisms of Preconditioning

The precise mechanism of the IPC response is unknown. From studies on preconditioned myocardium, it is widely accepted that IPC is mediated via a receptor-targeting mechanism [59, 60]. Molecules released during ischemia attach to cellular receptors and contribute to preconditioning response. The candidate compounds implicated in liver IPC include adenosine [17, 20, 25, 48, 49], protein kinase C (PKC) [53–55], nitric oxide (NO) [13, 17, 20, 26, 41, 42], heat shock proteins (HSPs) [29, 61], tyrosine kinases [52], mitogen-activated protein kinases [55], oxidative stress [35, 50], nuclear factor κB (NF-κB) [62, 63], and modulation of apoptosis cascade [14, 27]. However the characterizations of these candidate compounds into different processes in the preconditioning cascade such as initiating trigger, signalling pathway and end effector are not defined and the interrelationship between these processes is unknown. In the liver, the most investigated molecules are NO [13], adenosine [49], PKC [54] and HSPs [29]. This article reviews the major developments in the characterization of these proposed mechanisms of preconditioning (fig. 2). Other mechanisms will not be discussed in further detail.

The Role of Adenosine

Adenosine is an extracellular molecule proposed both as ‘trigger’ and ‘mediator’ of IPC [3]. During ischemia, adenosine triphosphate is degraded to adenosine. The extracellular adenosine released in large quantities during ischemia is believed to play a role in the protective effect of IPC during reperfusion of ischemic tissue. Ischemia-reperfusion injury is associated with neutrophil and leukocyte activation and primary microvascular failure.
Adenosine inhibits leukocyte adhesion, decreases expression of adhesion molecules and inhibits neutrophil and platelet function [64, 65]. Adenosine also inhibits free radical production [66, 67], which are important mediators of cellular damage in the early phase of ischemia-reperfusion injury, and is a potent vasodilator [68]. The above would suggest adenosine may be protective against ischemia-reperfusion injury and the effects of adenosine in IPC are likely to be multifactorial. Most of the data on the role of adenosine in IPC has been gathered in cardiac muscle [69–71] and extrapolated to skeletal muscle [5] and kidneys [8].
The Role of adenosine in myocardial preconditioning is supported indirectly by demonstrating a dose-dependent increase in cAMP by adenosine and selective A2 receptor agonist CGS-21680 [49]. In this study by Arai et al. [49], adenosine A2 receptor blockade prevented the protective effect of IPC in rat livers preserved in cold UW solution. IPC and administration of adenosine A2 receptor agonist, in this study, decreased SEC death and increased cAMP level [49]. The authors have proposed that SEC protection by IPC is mediated by activation of adenosine A2 receptors producing an increase in cAMP levels in SEC, but the mechanism downstream to increased cAMP, by which adenosine decreases SEC injury, is not explained. The same authors have previously shown that IPC suppressed KC activation and have stipulated the involvement of adenosine A2 receptors in this response [48]. IPC-induced protection of SECs can have profound implications for preservation of livers for transplantation, since SECs are more susceptible to cold preservation injury [73, 74] in contrast to hepatocytes which are vulnerable to warm ischemia-reperfusion injury [75]. SEC injury rather than hepatocellular injury has been shown to be responsible for graft failure from cold ischemia-reperfusion injury [73, 74, 76]. Peralta et al. [17] have postulated activation of adenosine A2 receptors with subsequent formation of NO to play a role in mediating IPC against warm ischemia-reperfusion injury. In this study adenosine administration in the presence of a NO donor reproduced the protective effect of IPC on hepatic parenchymal cells. In another study, a 3-fold increase in adenosine after IPC was associated with decreased parenchymal tissue damage [20]. Both IPC and increasing endogenous adenosine concentrations with the adenosine uptake inhibitor dipyridamole decreased hepatic leukocyte/endothelial cell interactions after ischemia-reperfusion injury [25]. All of the above studies have been carried out in rats and although the evidence is limited, suggest that adenosine modulates IPC-induced protection of non-parenchymal and parenchymal cells against cold and warm hepatic ischemia-reperfusion injury in the rat liver. There are no studies challenging the involvement of adenosine in the rat liver.

The data on adenosine from IPC studies in rat livers contradicts the information gathered in the rat heart. The role of adenosine in myocardial preconditioning is supported indirectly by studies in rabbits [77], pigs [78], dogs [79], and humans [80] demonstrating abolition of preconditioning by adenosine receptor blockade. However in rats, it is evident that adenosine has no role in IPC of the myocardium [81]. IPC is effective in the absence of extra-cellular accumulation of adenosine in the rat heart [82]. Thus, adenosine does not appear to be an endogenous trigger or obligatory mediator of preconditioning in rat hearts. Thus, in the rat species the adenosine concept does not seem to apply consistently to different tissues. It therefore seems likely that adenosine may only be a mediator to IPC of the liver but not a sole mechanism.

The Role of PKC

The PKC-mediated signalling pathway of myocardial preconditioning was proposed by Downey and colleagues [60, 83]. The hypothesis proposes that during preconditioning ischemia G protein activation following G protein coupling with adenosine receptors leads to PKC activation and subsequent translocation from the cytosol to the membrane where it phosphorylates substrate proteins to induce tolerance to subsequent ischemia [60]. However conflicting results in some species, particularly large animals where the concept does not apply consistently, would suggest that PKC activation is an epiphenomenon or secondary effect and not a primary mediator of the cardioprotective effects of preconditioning [84, 85]. Most of the evidence surrounding the PKC hypothesis is indirect and based on a pharmacological approach using PKC activators and inhibitors. Many of the inhibitors are not specific to PKC and are also isoform nonspecific. The above reviewers [84, 85] highlighted the limitations of pharmacological methods and also the fact that studies using isoform specific antibodies may not indicate activity of these specific PKC isoforms. Further, information on events downstream of PKC activation and the end effector of preconditioning is lacking at present.

In recent years, few studies have evaluated the evidence for involvement of PKC in preconditioning of the liver. This evidence is indirect and based on a pharmacological approach. Carini et al. [54] used an in vitro model of isolated rat hepatocytes and proposed that hypoxic preconditioning was mediated via PKC-mediated activation of vacuolar proton ATPase. In this study the increased tolerance of preconditioned hepatocytes to hypoxia was abolished by inhibition of PKC with chelerythrine or blocking vacuolar proton ATPase with bafilomycin A1 and mimicked by stimulators of PKC. 4β-phorbol-12-myristate-13-acetate (PMA) and 1,2-dioctanoyl-glycerol (1,2-DOG). The authors observed that the prevention of
intracellular acidosis and of cytosolic Na\(^+\) increase during hypoxia was associated with decreased hypoxic injury in preconditioned hepatocytes [54]. In another study, the same authors observed that preconditioning was abolished by adenosine A2a receptor antagonist and have proposed a signalling pathway involving adenosine A2a receptors, PKC and kinases downstream of PKC (p38 mitogen-activated protein kinase) to be involved in hypoxic preconditioning of isolated rat hepatocytes [55]. However downstream of this point, the mechanisms by which liver injury is decreased have not been explained. In the heart, it has been suggested that the kinase cascade activated during preconditioning leads to the opening of mitochondrial K\(_{ATP}\) channels [86] but there is no evidence that these are the end effectors. There is data to suggest that mitochondrial K\(_{ATP}\) channels may simply act as another signal transduction step [87]. The kinase cascade can also stimulate phosphorylation of HSPs [88], activation of the transcription factor NF-κB [89] and upregulation of inducible NO synthase [90] but the link with end effects of preconditioning has not been established. Ricciardi et al. [52, 53] have extended support for involvement of PKC and tyrosine kinase in liver IPC in larger animals. In one study, tolerance of ischemically preconditioned pig livers to cold ischemia was abolished by pretreatment with the PKC inhibitor chelerythrine [53]. In another study by the same authors pretreatment with tyrosine kinase inhibitor genistein abolished the preconditioning effect in cold-preserved pig livers [52]. While these data support a role for PKC in IPC, they still do not prove that PKC is responsible for preconditioning.

The Role of HSP

HSPs are intracellular stress proteins that have been shown to accumulate after hyperthermia and ischemia [91]. The concept of sublethal whole animal hyperthermia conferring tolerance to other stresses such as ischemia and lethal endotoxin exposure is referred to as hyperthermic preconditioning and has been associated with HSP accumulation [92, 93]. In the rat liver, tolerance to ischemic injury has been associated with production of various inducible HSPs: HSP72 [30, 94], HSP73 [30] and HSP70 and HO-1/HSP32 [95, 96]. Ishikawa et al. [61] have proposed that in heat shock-preconditioned rat livers HSPs maintain mitochondrial membrane integrity during the ischemic episode, to produce energy-rich phosphates during reperfusion and thus contribute to ischemic tolerance. In an in vivo study in rats by Kume et al. [29] the reduced posts ischemic hepatocellular injury and improved survival was associated with overexpression of HSP72 in ischemically preconditioned livers as well as in the livers preconditioned with heat shock. In this study HSP72 was detected within 6–72 h after heat exposure and the authors have proposed that HSP72 production is associated with a delayed protective effect of IPC. The link between HSP72 and delayed effect of IPC has not been explained. It is also not clear whether HSP production and accumulation is the reason for resistance to ischemia or merely a marker of tolerance [97].

While these studies demonstrate that HSPs are detected after preconditioning, the molecular mechanism of protection associated with HSP accumulation is not explained and these studies do not prove that HSPs are responsible for preconditioning.

The Role of NO

NO is a colorless, odorless, free radical gas which has been identified as an important signaling molecule in almost every tissue in the body. NO is produced from L-arginine by the enzyme NO synthase. In the liver, as in many other organs NO has many actions and cellular sources. Recent evidence supports the role of NO in regulating perfusion of the hepatic microcirculation [98]. The breakdown of microvascular perfusion with subsequent impairment of tissue oxygenation plays a central role in the pathophysiology of ischemia-reperfusion-induced injury of the liver [99]. Treatment of rats with nonspecific NO synthase inhibitors resulted in a failure of microvascular perfusion and development of patchy necrosis [100, 101]. Augmentation of NO synthesis with NO donors has been shown to attenuate hepatic ischemia-reperfusion injury and improve posttransplant survival [102]. NO may modulate microvascular perfusion through its vasodilatory effect [103] and through its anti-inflammatory actions including inhibitory effects on stellate cell activation [104], neutrophil adhesion [105] and platelet aggregation [101].

It has been proposed that NO plays a key role in both initiating and mediating IPC. While functional evidence in the heart indicates that NO modulates both acute and delayed preconditioning, downstream of this point in the biochemical pathway hypotheses are less well established [106, 107]. A recent study by Lochner et al. [108] has proposed that NO through generation of cGMP acts as a trigger of acute preconditioning in rat hearts. Parratt [109] has suggested that endocardial endothelium-derived mediators such as NO may mediate cardioprotective response of IPC by elevation of cGMP, which in turn could reduce energy demand by limiting myocardial cAMP levels by stimulation of cGMP-sensitive cAMP phosphodies-
terase enzyme. It appears that whereas the acute phase of preconditioning is protein synthesis independent, the late phase requires new protein synthesis. It has been proposed that eNOS-derived NO leads to activation of PKC and other kinases, which in turn through NF-κB and other transcription factors leads to an increase in transcription of iNOS [107]. The end effector of IPC in the supposed NO pathway is speculative and cGMP-dependent mechanisms and ATP-sensitive potassium channel have been proposed [107].

In the liver it has been suggested that depending on the rate of its production, NO may also play a mediating role in preconditioning [110]. NO has been implicated in IPC-associated decreased tissue damage in both warm ischemia [13] and cold ischemic storage [26] of the rat liver. However, the link between protective effects of IPC and NO is speculative. Peralta et al. [13] suggested that liver IPC in rats is mediated by the inhibitory action of NO on endothelin. In other studies in rats, the same authors have demonstrated that inhibition of adenosine and simultaneous administration of NO donor offered similar results to IPC [17] and have proposed that activation of adenosine A2 receptors with subsequent NO formation mediates IPC in the rat liver [20]. Yin et al. [26] have postulated that IPC increased resistance to cold ischemic liver injury in rats through stimulation of endogenous NO. In this study pharmacological NO stimulation mimicked and NO inhibition antagonized IPC-associated protection of liver grafts from preservation reperfusion injury in a rat liver transplantation model, but the mechanism has not been explained. Recently, our group has shown an increased hepatic intracellular oxygenation [42] and increased hepatic microcirculation [41] with IPC which was associated with increased NO levels. A recent report by Serafin et al. [47] has implicated NO in the preconditioning response for ischemia-reperfusion injury in fatty livers.

NO suppresses apoptosis in endothelial cells. In recent years it has been suggested that apoptosis is the dominant mechanism for cell turnover in the human liver. Apoptosis is a rapid process terminating in nuclear pyknosis and cell death. Recent evidence has shown that apoptosis of SEC and hepatocytes is a feature of ischemia-reperfusion injury in warm [111] and cold [112] ischemia of the liver. The signalling pathways leading to nuclear apoptosis in response to extracellular stimuli involve activation of cysteine proteases known as caspases and release of cytochrome c from the mitochondria [113]. Subsequent activation of downstream caspases such as caspase 3 ultimately executes nuclear apoptosis [114]. Antiapoptotic molecules such as Bcl-2 and caspase inhibitors have been shown to prevent release of mitochondrial cytochrome c [115]. In an experimental model of partial hepatic ischemia, IPC inhibited apoptosis of SEC and hepatocytes and was associated with inhibition of caspase 3 activity [27]. In the study IPC was not associated with higher Bcl-2 or Bcl-xl expression. The link between IPC and inhibition of caspase activity is speculative. NO has been shown to inhibit caspase activity in vitro [116]. Apoptosis in hepatocytes exposed to TNF-α and actinomycin D was prevented by NO. In this study NO produced by an NO donor or through iNOS gene expression inhibited caspase family proteases by S-nitrosylation and prevented cytochrome c release [113]. Other mechanisms for an anti-apoptotic effect of NO are increase in cGMP [116] and upregulation of Bcl-2 [117] and HSPs [118]. Thus, potentially liver IPC may be mediated through NO modulation of apoptosis cascade.

Role of IPC in Hepatic Surgery

Ischemia-reperfusion injury is a major cause of morbidity and mortality following liver surgery and transplantation. In the setting of liver resections, the effects of intermittent inflow occlusion, continuous inflow occlusion and total vascular exclusion during liver resections have been studied in clinical trials [119–121]. Whereas total vascular exclusion was effective in reducing blood loss, it led to unpredictable hemodynamic intolerance, increased morbidity and longer hospital stay [119]. This is not surprising since the state of total vascular exclusion is akin to the anhepatic phase of liver transplantation and hemodynamic consequences on reperfusion would be anticipated. In a prospective evaluation of intermittent inflow occlusion versus no inflow occlusion in patients undergoing liver resections, the former resulted in less blood loss and better preservation of liver function in the early postoperative period [120]. When intermittent versus continuous inflow occlusion were studied in patients undergoing liver resections [121], the group subjected to intermittent inflow occlusion was associated with decreased hepatocellular injury indicated by lower postoperative liver enzymes and serum bilirubin levels. However the intraoperative blood loss during liver transection was significantly higher in this group and this is most likely related to bleeding from the transected surface during successive reperfusion episodes [121]. Thus, the increased blood loss and likely increased duration of surgery due to successive reperfusion episodes may outweigh the benefit of intermittent occlusion on parenchymal tolerance to

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ischemia. Although some liver resections can be performed without vascular inflow occlusion, prolonged ischemia may be unavoidable to achieve radical tumor resection. An ideal protective strategy for human liver surgery would allow a bloodless parenchymal transection and an increased parenchymal tolerance to ischemia. In theory, IPC may obviate the need for intermittent releases of hepatic vascular occlusions and extend safe periods of ischemia by increasing hepatic tolerance to ischemia during hepatic surgery. The potential for clinical application of IPC for hemihepatectomies under inflow occlusion has been demonstrated by Clavien et al. [43]. In this study IPC protected against 30 min of inflow occlusion with patients showing a 2-fold decrease in serum transaminases compared to patients subjected to continuous ischemia only, but no significant differences in duration of surgery, need for intensive care or mortality. This study provides evidence that IPC occurs in the human liver.

In the setting of liver transplantation, ischemia time of the donor liver is a major determinant of graft outcome and patient survival after liver transplantation [122]. Liver transplantation requires mandatory organ ischemia. Warm ischemia to the graft may occur at organ harvest in an unstable donor and cold ischemia occurs during preservation of the liver for transplantation. During implantation of the graft in the recipient, the liver is subjected to further warm ischemia until the vascular anastomoses are completed. Finally reperfusion injury is inevitable following revascularization. Prolonged ischemia results in primary nonfunction or dysfunction of the transplanted liver graft and is associated with biliary and vascular complications [122, 123] often resulting in retransplantation. This adversely affects patient outcome and survival. Therefore IPC is an attractive strategy to assist liver preservation and protect the liver from ischemia-reperfusion injury during transplantation by increasing ischemic tissue tolerance of the liver. As yet there are no reported studies demonstrating clinical benefits of IPC in patients undergoing liver transplantation. Most animal studies have shown that IPC offers a degree of protection against cold ischemia in experimental liver transplantation. This data in animal models is encouraging and clinical studies are required to clarify the potential application of IPC in human liver transplantation.

Conclusions

The past decade has provided interesting new data establishing the existence of IPC in the liver. IPC is a powerful endogenous means to protect the liver from ischemia. To date one study has demonstrated human clinical benefits of liver IPC. Further clinical studies are required to prove unequivocally that IPC is possible in the human liver. However the central mechanism of IPC remains undefined. Current research has demonstrated that IPC is an endogenous adaptive phenomenon that can be reproduced easily in different models of warm and cold ischemia, and in animals as well as humans. However the causal relationship between the initiating event, biochemical pathways and end effector molecules remains mechanistically undefined and controversial. As the field advances with mechanistically descriptive studies, these controversies in interrelationships in the preconditioning cascade are likely to be resolved and will lead to pharmacological strategies for protecting the liver from ischemic injury.


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