Beneficial Effects of Ischemic Preconditioning in Patients Undergoing Hepatectomy

The Role of Neutrophils

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Hypotheses: Temporary vascular clampage (Pringle maneuver) during liver surgery can cause ischemia-reperfusion injury. In this process, activation of polymorphonuclear leukocytes (PMNLs) might play a major role. Thus, we investigated the effects of hepatic ischemic preconditioning on PMNL functions.

Design: Prospective randomized study. Patients who underwent partial liver resection were randomly assigned to 3 groups: group 1 without Pringle maneuver; group 2 with Pringle maneuver, and group 3 with ischemic preconditioning using 10 minutes of ischemia and 10 minutes of reperfusion prior to Pringle maneuver for resection.

Setting: University hospital, Munich, Germany.

Patients: Seventy-five patients underwent hepatic surgery mostly owing to metastasis.

Main Outcome Measures: Perioperative factors for PMNL activation, inflammation, and postoperative hepatocellular integrity.

Results: Ischemia-reperfusion of the human liver (mean±SD time to perform the Pringle maneuver, 35.5±2.6 minutes) caused (1) a decrease in the number of circulating PMNLs, (2) their intrahepatic sequestration, (3) their systemic activation, and (4) a significant correlation between the degree of their postischemic activation and the postoperative rise in liver enzyme serum levels. In parallel, cytokines with proinflammatory and chemotactic properties were released reaching the highest values when stimulation of PMNLs was most pronounced. When ischemic preconditioning preceded the Pringle maneuver, activation of PMNLs and cytokine plasma levels was reduced as evidenced by the attenuation of superoxide anion production, \( \beta \)-integrin up-regulation, and interleukin 8 serum concentrations, followed by a significant reduction in serum alanine aminotransferase levels on the first and second postoperative days.

Conclusions: These results demonstrate in humans that ischemic preconditioning reduces activation of PMNLs elicited by the Pringle maneuver. The down-regulation of potentially cytotoxic functions of PMNLs might be one of yet unknown important pathways that altogether mediate protection by ischemic preconditioning.


Resection is the most common curative treatment for liver diseases of malignant origin (eg, metastatic colorectal adenocarcinoma or hepatocellular carcinoma) and is also necessary for treatment of tumors of benign origin (eg, large hepatocellular adenomas or cysts from hydatid disease or Echinococcosis). For reducing the potentially lethal risk of massive bleeding and blood transfusions with adverse immunomodulatory adverse effects, major hepatic resection is frequently performed under clampage of the hepatic pedicle according to Pringle, Bismuth and Majno, and Arnoletti and Brodsky. As a consequence of vascular occlusion, in situ warm ischemia followed by reperfusion may result in severe organ dysfunction and organ failure, as evidenced by elevated serum liver enzyme levels. The spectrum of mechanisms leading to ischemia-reperfusion (I-R) injury is large and involves metabolic and mitochondrial changes, for example, adenosine triphosphate depletion, Kupffer cell activation, release of reactive oxygen species, cytokine secretion, and microcirculatory disturbances. In this scenario, granulocytes have been strongly suggested to play a key role in the orchestration and amplification of I-R injury ending up in apoptosis, necrosis, and organ failure. In vitro and in vivo experiments could demonstrate that the degree of postischemic tissue damage is not only dependent on energy depletion, but also on granulocyte activation and damage, but also on granulocyte activation and damage.
tween granulocyte activation caused by cold/warm liver I-R and postoperative liver tissue damage could also be demonstrated in patients who underwent orthotopic liver transplantation.17

Experimental therapies aiming at the prevention of I-R injury include the use of neutralizing antibodies (eg, anti–tumor necrosis factor, anti–intercellular adhesion molecule-1 antibodies, or anti-CD11b/CD18) or antioxidative-acting drugs (superoxide dismutase [SOD]; glutathione).18 Besides pharmacological attempts at tissue protection, beneficial effects were reported in recent years for ischemic preconditioning mostly in animals but recently in humans as well.19 Ischemic preconditioning refers to the phenomenon that most organs, for example, heart,20 brain,21 skeletal muscle,22 and liver tissue,23-27 can be adapted to the otherwise deleterious effects of ischemic stress by previous exposure to a brief period of I-R. In this clinical trial, we attempted to determine the role of granulocytes in warm hepatic I-R injury in humans and questioned whether the beneficial effects of ischemic preconditioning might result from the modulation of polymorphonuclear leukocyte (PMNL) activation. The primary outcome measures were factors of PMNL activation, inflammation, and postoperative hepatocellular integrity.

### METHODS

#### PROTOCOL

After approval of the study protocol by the local ethics committee and informed written consent, 100 adult patients were tested for eligibility after routine tumor staging (Table 1). Following enrollment and evaluation by the participating surgeons and anesthetists, patients were assigned by lot to 1 of the 3 study groups. Patients were excluded from randomization if they did not meet the inclusion criteria, that is, if the American Society of Anesthesiologists' Physical Status (ASA-PS) classification20 was 4 or 5 or if any additional extrahepatic tumor was diagnosed unexpectedly prior to liver surgery. Patients were also excluded intraoperatively (n=24) if drugs with anti-inflammatory effects were administered (eg, pentoxifylline or corticosteroids) or liver cirrhosis was identified. Patients also had to be excluded for surgical reasons (inoperability, additional surgical procedures like splenectomy, pancreas resection, or massive hemorrhage). The number of cases eligible for analysis was further reduced by postoperatively diagnosed cirrhotic or fatty liver diseases both of which were considered exclusion criteria. Moreover, accidental partial or total loss of blood samples sometimes occurred as a result of inappropriate handling or storage temperature. Thus, from 99 patients randomly assigned to 3 study groups, 75 patients who underwent elective major hepatectomy in our hospital from March 1, 1999, through August 31, 2000, were analyzed for the primary outcome measures. The 3 groups were as follows: group 1, the non-Pringle maneuver group (NPR, n=25) in whom liver resection was performed without clamping of the vessels in the hepatoduodenal ligament; group 2, Pringle maneuver group (PR, n=25) in whom during parenchymal transection the arterial and venous inflow was stopped by a clamp placed on the arteria hepatica and vena portae (PR); and group 3, the ischemic preconditioning group (IPC, n=25) in which the patients were treated the same as those of the PR group with the exception that clamping for resection was preceded by a period of 10 minutes of portal clamping followed by 10 minutes of reperfusion. Patients were anesthetized following a standardized protocol that was started by placement of an epidural catheter for intermittent injection of 0.5% bupivacaine hydrochloride. General anesthesia was induced by the intravenous injection of thiopental sodium (3-4.5 mg/kg-1 per body weight [BW]), cis-atracurium (0.05 mg/kg-1 per BW), and fentanyl (3-4 μg/kg-1 per BW). Patients were normoventilated with an air-oxygen mixture of 30% and anesthetized with use of desflurane (5-6 vol%). Arterial and central venous lines were used to monitor cardiovascular stability. After laparotomy, the liver was prepared and the hilus was exposed in all 3 of our study groups. In the NPR group, resection was started without clamping of the hepatic blood influx. Because portal lymph node dissection was performed in every patient with malignant liver disease, the blood vessels and bile duct were always isolated as was also done in the few benign cases. During the PR only blood vessels in the portal triad were clamped (hepatic artery and portal vein) to avoid any additional trauma to the bile duct. In the group who underwent IPC, the PR was preceded by 10 minutes of ischemia and 10 minutes of reperfusion. Clamping of the portal triad was performed without affecting the bile duct. Liver surgery was started immediately after inducing the PR that was kept effective until transection was finished. Parenchymal transection was performed by use of a water-jet cutter (Saphir Medical, Lyon, France). Hemostasis was secured by clipping small vessels. Bile ducts and major vascular structures were ligated with 4-0 monofilament sutures. Coagulation of minor bleeding from the cut liver surface was done with use of the argon laser beam (Erbe, Tübingen, Germany), and the transection area was covered with collagen fleece (TACH Comb; Baxter, Untersicheßheim, Germany).

The surface area of transection was quantified with a ruler and the volume was determined by the quantity of displaced fluid from a prefilled trough. Perioperative management and surgery were all performed by the same 3 experienced visceral surgeons and anesthetists.

<table>
<thead>
<tr>
<th>Variable</th>
<th>NPR</th>
<th>PR</th>
<th>IPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SEM, y</td>
<td>61.9 ± 2.1</td>
<td>56.3 ± 2.2</td>
<td>55.7 ± 2.9</td>
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<tr>
<td>Sex, F/M</td>
<td>9/16</td>
<td>8/17</td>
<td>12/13</td>
</tr>
<tr>
<td>Diagnosis (n = 25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorectal liver metastasis</td>
<td>11</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Hepatocellular cancer</td>
<td>7</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Cholangiocellular cancer</td>
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<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Liver metastasis of other origin</td>
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<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Echinococcus</td>
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<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Hemangiomia</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>PR, mean ± SEM, min</td>
<td>NA</td>
<td>35.6 ± 2.6</td>
<td>35.5 ± 2.7</td>
</tr>
<tr>
<td>Resection time, mean ± SEM, min</td>
<td>39.0 ± 4.5</td>
<td>33.2 ± 2.3</td>
<td>32.2 ± 2.0</td>
</tr>
<tr>
<td>Volume of resected liver, mean ± SEM, mL</td>
<td>351 ± 104</td>
<td>356 ± 61</td>
<td>392 ± 70</td>
</tr>
<tr>
<td>Resections (n = 25 for each group)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Hemipatectomy</td>
<td>5</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Segment resection</td>
<td>20</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>Pericystectomy and segment resections</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: IPC, ischemic preconditioning group; NA, not applicable; NPR, non-Pringle maneuver group; PR, Pringle maneuver group.

*For a more detailed explanation of IPC, NPR, and PR, see the “Protocol” subsection of the “Methods” section. Patients were randomly assigned to 1 of 3 study groups each containing 25 patients. Liver metastases of other primary origin, for example, the ovaries, pancreas, and throat, were sampled. None of the data were statistically significant using analysis of variance and χ2 tests.
Central venous blood samples were obtained before the start of liver transection (hereafter, preresection). In all 3 groups blood specimens were sampled intraoperatively at 3, 15, and 30 minutes as well as postoperatively at 2, 24, and 48 hours following resection of the liver. In addition, small pieces of liver tissue were cut from resting liver tissue preresection and 30 minutes after the end of the resection procedure. Tissue samples were kept frozen in liquid nitrogen.

**BLOOD ANALYSES**

Polymorphonuclear leukocytes were electronically counted in EDTA-anticoagulant blood (Coulter Counter STKS; Coulter Electronics, Lupton, England). Production of superoxide anions (O2\textsuperscript{−}) was determined in whole blood by the superoxide dismutase inhibitable reduction of cytochrome c as described in detail elsewhere.\textsuperscript{30} In brief, following stimulation of diluted whole blood with the chemotactic tripeptide N-formylmethionyl-leucyl-phenylalanine (fMLP) (fMLP concentration, 10\textsuperscript{-7}M; Sigma Chemicals, Deisenhofen, Germany), changes in the absorbance of cytochrome c were determined by photometric measurement (350 nm fitted with a 630-nm interference filter, Dynatec MRX 7000; Dynatec Laboratories Inc, Alexandria, Va). From the difference between the absorbances determined in samples in the absence and presence of superoxide dismutase, the fMLP-stimulated production of O2\textsuperscript{−} was calculated and expressed as nano moles of O2\textsuperscript{−} per 1×10\textsuperscript{6} PMNLs per 15 minutes. Cell surface expression of adhesion molecules (β\textsubscript{2}-integrins, CD18) on PMNLs was determined in ice-cold heparinized (10 IU/mL, VETREN; Byk Gulden, Konstanz, Germany) blood samples by fluorescein isothiocyanate–labeled monoclonal antibody IB\textsubscript{4}, which specifically binds to β\textsubscript{2}-integrins using a flow cytometer\textsuperscript{31} (Becton Dickenson, San Jose, Calif). Data are expressed in relative fluorescence units (rel fl U). Quantitative analyses of interleukin (IL) 6 and IL-8 were determined in liver biopsy specimens sampled before and 24 and 48 hours after resection.

**LIVER TISSUE**

To estimate I-R–induced accumulation of PMNLs in liver tissue, myeloperoxidase (MPO) was used as a marker enzyme that was quantified in liver biopsy specimens sampled before and 30 minutes after resection. Liver tissue samples were kept frozen in liquid nitrogen until determination of MPO concentrations. In brief, small pieces of liver tissue were homogenized in liquid nitrogen until determination of MPO concentration was quantified in liver biopsy specimens sampled before and 24 and 48 hours after resection. Normal distribution was assessed by Kolmogorov-Smirnov test. Comparison of changes within a group was performed by paired t test for normally distributed data and by Wilcoxon rank sum test otherwise. Comparison of data between the 3 treatment groups (NPR, PR, and IPC) was carried out by analysis of variance (ANOVA) and post hoc unpaired t test or by Kruskal-Wallis test and post hoc Mann-Whitney test, respectively. Because some factors were normally distributed and others were not. P<.05 was statistically significant. To analyze the relationship between activation of the PMNLs and hepatocellular damage, the correlation coefficient according to Spearman rank correlation (p) was calculated.

**RESULTS**

**PATIENTS**

Figure 1 shows the flow of allocation and the analysis of the study participants according to CONSORT.\textsuperscript{32} With the exception of 1 patient, the remaining 99 patients were initially allocated by lot with 28 patients to the NPR group, 33 patients to the PR group, and 38 patients to the IPC group. To not further increase the risk of the surgical procedure and to not endanger the success of resection, initial allocation had to be changed in a few patients intraoperatively for technical reasons: 2 patients in the NPR group (one with Echinococcus [or cysts from hydatid] and the other with cholangiocellular carcinoma) were cross-allocated to the PR group because surgery would have been infeasible without vascular inflow occlusion. Three patients from the PR group (2 with hepatocellular carcinoma and 1 with metastases of colon carcinoma) and 2 patients from the IPC group (1 with cholangiocellular carcinoma and 1 with Echinococcus) had to be cross-allocated to the NPR group because of the difficult prepa-
ration of the hilus. The final number of patients eligible for analysis was further decreased for reasons described in the “Protocol” subsection of the “Methods” section, ending up with 25 patients per group. No significant differences in the demographic data, duration of time for PR, or resected liver volume were found in any group between men and women or among the 3 groups, respectively (Table 1).

**PMNL COUNTS**

Following partial liver resection via NPR, no changes in the concentration of circulating PMNLs were observed within 30 minutes after completion of resection (Figure 2). When liver ischemia occurred (PR or IPC groups), PMNL counts significantly decreased at 3, 15, and 30 minutes after reperfusion (PR, *P*<.05 at 3 and 15 minutes compared with the presection group; IPC, *P*<.05 at 3, 15, and 30 minutes compared with the presection group). Although concentrations of PMNLs tended to be lower in patients subjected to IPC compared with patients who underwent PR only, the differences between both groups were not statistically significant. Irrespective of the treatment, all patients showed a significant postoperative granulocytosis 2 and 24 hours after resection or reperfusion.

Table 2. Myeloperoxidase Concentrations*  

<table>
<thead>
<tr>
<th>Time</th>
<th>NPR†</th>
<th>PR†</th>
<th>IPC†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preresection</td>
<td>112.7 ± 17.7</td>
<td>152.4 ± 17.1</td>
<td>162.6 ± 25.2</td>
</tr>
<tr>
<td>30 min after resection</td>
<td>136.0 ± 19.3</td>
<td>248.1 ± 69.8‡</td>
<td>310.4 ± 72.3‡</td>
</tr>
</tbody>
</table>

Abbreviations: IPC, ischemic preconditioning group; NPR, non-Pringle maneuver group; PR, Pringle maneuver group.

*Data are given as the mean ± SEM. The concentrations of myeloperoxidase were determined in liver tissue biopsy samples that were removed intraoperatively before and 30 minutes after resection and were calculated as follows: nanograms of myeloperoxidase per milligrams of dry-weight liver tissue.

†For a more detailed explanation of IPC, NPR, and IPC, see the “Protocol” subsection of the “Methods” section.

‡*P* < .05 compared with the presection of the PR and IPC groups, paired *t* test.

**MPO CONCENTRATION IN LIVER TISSUE**

Prior to partial liver resection the MPO concentration in liver tissue was not different among the 3 study groups (reference values as published by the manufacturer: 121 ng/mg of liver tissue) (Table 2). Thirty minutes after completion of the resection MPO concentrations were unchanged in the NPR group, but these concentrations almost doubled following PR (PR or IPC group). Interestingly, the rise in the MPO content of the postischemic liver was not decreased but slightly increased by ischemic preconditioning. However, the difference in liver MPO content between the PR and the IPC groups did not reach the level of statistical significance.

**O₂⁻ PRODUCTION BY PHAGOCYTES**

In all of the groups, the rate of O₂⁻ produced on stimulation of circulating blood phagocytes activated with fMLP (10⁻⁶M) (Figure 3) was in the same physiological range (approximately 28 nmol/10⁶ PMNL per 15 minutes) prior to liver resection. Soon after the PR was finished (3 minutes), O₂⁻ production increased significantly and showed the highest rates (PR group, 38 nmol/10⁶ PMNL per 15 minutes) compared with patients in the NPR group (32.5 nmol/10⁶ PMNL per 15 minutes) and patients pretreated with IPC (26 nmol/10⁶ PMNL per 15 minutes; *P* < .05, PR or NPR groups compared with IPC groups at 3 minutes). Twenty-four hours postoperatively, O₂⁻ production rates returned to baseline values (preresection).

**β₂-INTEGRINS ON PMNLs**

The expression of β₂-integrins (CD18) on resting PMNLs showed no intergroup differences prior to resection (mean value, 40 rel fl U. Figure 4). In the NPR group no significant perioperative changes were observed. After I-R (PR, IPC), expression of β₂-integrins increased significantly (*P* < .05 at 3, 15, and 30 minutes compared with the presection group). In the IPC group, however, β₂-integrin expression did not increase further compared with...
patients in the PR group (IPC compared with PR at 2 hours, \( P<.05 \), Figure 4).

**IL-6 AND IL-8 PLASMA CONCENTRATIONS**

There were no differences between study groups prior to resection with respect to plasma concentrations of IL-6 and IL-8 (IL-6, approximately 40-65 pg/mL, Figure 5A; and IL-8, approximately 20-28 pg/mL, Figure 5B). Plasma concentrations of IL-6 increased in all of the groups 30 minutes after partial liver resection, with this rise being slightly more pronounced at 2 hours in the PR group. Plasma concentrations of IL-8 followed the same kinetics; however, in the PR group IL-8 levels already had increased after 30 minutes and reached maximum values 2 hours after reperfusion/end of resection, these being significantly higher compared with the NPR group \((P=.04; 2 \text{ hours}, \text{PR compared with NPR})\). When IPC preceded the PR (IPC group), the rise of IL-8 concentrations was attenuated and concentrations were close to those observed in patients in the NPR group. On the first postoperative day (24 hours), both cytokine concentrations decreased and IL-8 levels returned to their baseline levels measured before liver surgery.

**ASSESSMENT OF HEPATIC INJURY**

Serum ALT levels increased in all groups significantly 24 and 48 hours after resection \((P<.05 \text{ compared with preoperative; Table 3})\). This increase was most pronounced in the PR group. However, the liver tissue damage due to PR was completely prevented by ischemic preconditioning (IPC compared with PR; \( P=.06, 24 \text{ hours}; P=.02, 48 \text{ hours} \)). No gender-specific differences between male and female patients were observed (data not shown).

**BIVARIATE CORRELATION ANALYSIS**

The expression of \( \beta_2 \)-integrins on the PMNL surface determined 2 hours after partial liver resection was tested for a possible relationship to serum levels of ALT determined on the first (24 hours) and second (48 hours) postoperative days. In patients of the control group (NPR) no correlations could be detected at any time. When global liver I-R occurred owing to PR (PR group), expression of \( \beta_2 \)-integrins on PMNLs significantly correlated with serum ALT levels indicating that the higher the levels of CD18 expressed on PMNLs, the higher was the postoperative rise in liver enzyme levels of ALT values (at 24 hours).

**Table 3. Hepatocellular Integrity**

<table>
<thead>
<tr>
<th>Time</th>
<th>NPR†</th>
<th>PR†</th>
<th>IPC†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative</td>
<td>21.47 ± 3.8</td>
<td>32.7 ± 9.8</td>
<td>23.9 ± 3.4</td>
</tr>
<tr>
<td>Postoperative</td>
<td>24 h</td>
<td>254.3 ± 62.4‡</td>
<td>519.4 ± 139.0§</td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td>195.8 ± 54.6§</td>
<td>497.0 ± 145.1§</td>
</tr>
</tbody>
</table>

Abbreviations: IPC, ischemic preconditioning group; NPR, non-Pringle maneuver group; PR, Pringle maneuver group.

*Data are given as the preoperative and postoperative (at 24 and 48 hours) mean ± SEM of serum levels of alanine aminotransferase measured in units per liter.
†\( P<.05 \) compared with the NPR group.
‡\( P<.05 \) compared with the preoperative values, Friedman and post hoc Wilcoxon test.
§\( P<.05 \) compared with the PR group, Kruskal-Wallis test and post hoc Mann-Whitney test.

\( * \) For a more detailed explanation of IPC, NPR, and PR, see the “Protocol” subsection of the “Methods” section.

\( \dagger \) For a more detailed explanation of IPC, NPR, and PR, see the “Protocol” subsection of the “Methods” section.

\( \ddagger \) For a more detailed explanation of IPC, NPR, and PR, see the “Protocol” subsection of the “Methods” section.

\( \§ \) For a more detailed explanation of IPC, NPR, and PR, see the “Protocol” subsection of the “Methods” section.

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hours, \( r = 0.43, \ P = .02 \); at 48 hours, \( r = 0.42, \ P = .02 \)). Such a statistically significant correlation, however, could not be shown in the IPC group (ALT at 24 hours, \( r = 0.31, \ P = .07 \)) until 48 hours after liver surgery (ALT at 48 hours, \( r = 0.46, \ P = .10 \)).

**COMMENT**

**I-R OF THE LIVER AND THE ROLE OF NEUTROPHILS**

Studies on the pathogenesis of I-R injury have shown that the degree of postischemic liver tissue damage is dependent on cellular energy depletion, hepaticocyte and endothelial cell swelling with impairment of microvascular perfusion, oxygen radical formation, Kupffer cell activation, leukocyte activation, entrapment, and inflammation. Among leukocytes, PMNLs may critically amplify this sequence of pathophysiological events after tethering to the vascular endothelium, adhesion, diapedesis, and migration to the site of injury, guided by a gradient of cytokine and chemokines.

The results of the present study further support the view that PMNLs are involved in I-R injury of the human liver as suggested by (1) the postischemic decrease in the numbers of circulating PMNLs (Figure 2); (2) their concomitant intrahepatic sequestration as evidenced by the rise in MPO concentrations in liver tissue (Table 2); (3) their systemic activation as demonstrated by the rise in \( \mathrm{O}_2^- \) production (Figure 3) and \( \beta_2 \)-integrin up-regulation (Figure 4); and (4) the correlation between the degree of their postischemic activation and the postoperative rise in serum liver enzyme values. In parallel to these signs of PMNL activation, cytokines with proinflammatory and chemotactic properties were released reaching the highest values when stimulation of the PMNLs was most pronounced (Figure 5). In contrast to a large body of evidence provided by animal studies, there is a lack of data on the role of neutrophils in warm hepatic I-R injury in man. To our knowledge, the study by Clavien et al is the only one to show an increase in hepatic neutrophil sequestration after hepatic vascular exclusion and reperfusion in patients with liver cirrhosis. In their study, the degree of leukocyte adhesion after reperfusion was correlated with several postoperative markers of liver injury. Even more support for postischemic liver damage by neutrophils comes from studies of patients undergoing liver transplantation. Although length and quality of ischemia (cold I-R) differs from warm ischemia, hepatic sequestration and activation of neutrophils are well-documented pathophysiological events during the reperfusion phase of orthotopic liver transplantation. Moreover, a direct relationship between sequestration of neutrophils and liver tissue damage after transplantation was suggested by the finding that \( \beta_2 \)-integrin expression in liver tissue measured 2 hours after reperfusion was associated with primary organ nonfunction. Thiel et al showed that determination of the expression of \( \beta_2 \)-integrins was also of predictive value for early graft dysfunction. In their study, hepatocellular damage was greater in patients with a postischemic increase in the expression of \( \beta_2 \)-integrins on PMNLs, indicating that PMNL activation was associated with hepatic I-R injury. Activation of circulating PMNLs was almost maximal 2½ hours after reperfusion, providing the rationale for choosing a similar time point (2 hours) in the present study. Thus, these clinical data are well in line with results of animal experiments in which a direct causative role of neutrophils in liver I-R injury could be shown. Using monoclonal antibodies either to deplete neutrophils or to block adhesive functions of \( \beta_2 \)-integrins, Jaeschke et al could decrease hepatic sequestration of PMNLs that significantly reduced the degree of liver tissue injury following warm I-R.

**EFFECTS OF IPC ON PMNL ACTIVATION AND POSTISCHEMIC LIVER DAMAGE**

Given the strong evidence that PMNLs contribute to liver tissue damage following warm and cold I-R, we asked whether ischemic preconditioning can prevent postischemic neutrophil activation. Interestingly, MPO liver content and, hence, hepatic PMNL accumulation was moderately higher in the IPC group than in the PR group. Although early accumulation of PMNLs in liver tissue was slightly higher in the IPC group (Table 2), ischemic preconditioning decreased the early rise in extracellular production of \( \mathrm{O}_2^- \) (Figure 3) and significantly inhibited the up-regulation of \( \beta_2 \)-integrins (Figure 4). The failure to detect an effect of ischemic preconditioning on early hepatic PMNL accumulation has to be discussed in the context of the following 3 different steps identified in experimental granulocyte-mediated postischemic liver injury: (1) sequestration of cells in sinusoidal capillaries, (2) capillary transmigration, and (3) adherence to hepatocytes. The first step seems to be independent of increased expression of \( \beta_2 \)-integrins and is most likely caused by mechanical factors following stoppage of hepatic blood influx. Because the total time of no blood flow was 10 minutes longer in the IPC group than in the PR group, higher neutrophil sequestration could have been expected to occur. By contrast, the second and third steps are mediated via the up-regulation of \( \beta_2 \)-integrins, a process that is triggered by cytokines and chemokines induced by I-R. Whereas early mechanical sequestration does not necessarily cause tissue injury (ie, benign granulocytosis), the ensuing capillary transmigration and \( \beta_2 \)-integrin–dependent contact formation with hepatocytes and subsequent release of toxic compounds (enzymes and oxygen radicals) are harmful PMNL-mediated late events. Thus, neutrophil recruitment is not affected by IPC while the ability of neutrophils to generate oxidants in response to stimulation and to change toward a more proinflammatory phenotype (increase in \( \beta_2 \)-integrins [CD18] expression) was decreased. These findings may therefore also point to the possibility of a dissociation in neutrophil activation induced with ischemic preconditioning because IPC increased mechanical sequestration but inhibited cytotoxic functions of these cells. However, postischemic neutrophil-mediated oxidative stress within the liver tissue is a complex process dependent on a large spectrum of factors.
of other cellular (eg, activated Kupffer cells, hepaticocytes, vascular endothelial cells) and noncellular events (eg, ferrylhemoglobin, xanthine dehydrogenase-oxidase). Thus, the question arises as to whether measuring the potentially tissue-toxic functions of PMNLs in the systemic circulation does really reflect the cytotoxic effects of PMNLs sequestered in the liver. To this end it would have been necessary to subject patients to transcutaneous liver biopsy. To circumvent ethical concerns of such procedures, we determined what the circulating cytokine (IL-6, IL-8) was to gain better insight into the degree of traumatic liver tissue damage and I-R-induced inflammation. Interestingly, plasma concentrations of IL-6 increased in all patient groups studied without any significant differences, suggesting that surgical trauma alone is the major factor accounting for the release of IL-6 to which I-R does not add any significant differences (Figure 5A). By contrast, IL-8 plasma concentrations were almost twice as high in patients subjected to the PR compared with patients who underwent NPR (Figure 5B, 2 hours). No such statistically significant difference was observed in patients pretreated by IPC when compared with liver NPR surgery. Thus, IPC attenuated the I-R-induced release of the proinflammatory and chemotactically active cytokine IL-8 to the level found in patients not subjected to the PR. Interleukin 8 is synthesized and released by a large variety of cell types as well (granulocytes, monocytes, endothelial cells, or epithelial cells) in response to various stimuli (eg, I-R, lipopolysaccharides, tumor necrosis factor α) and binds to the IL-8 chemokine receptors on the neutrophil surfaces, thereby causing the numerical up-regulation of CD18 on the cell surface of PMNLs. In addition, IL-8 has been shown to further induce the release of bactericidal and toxic metabolites and as a chemokine to attract PMNLs to sites of tissue injury. Thus, to our knowledge, the data of this clinical study show for the first time that hepatic IPC attenuates the activation of PMNLs and the release of the proinflammatory cytokine IL-8, effects that may well be involved in the protection of liver tissue from warm I-R injury. However, the improvement obtained with IPC on the modulation of PMNLs, IL-8 release, and postoperative liver enzyme levels was not always superior to the group subjected to PR only. On the other hand, one could not have expected a reduction of inflammation and tissue damage beyond the level observed in the NPR group because until now IPC has been considered to be a valuable procedure to protect one from I-R injury but not from surgical liver trauma per se.

**RELATIVE IMPORTANCE OF ATTENUATING CYTOTOXIC NEUTROPHIL FUNCTIONS IN LIVER PROTECTION BY IPC**

Of course, there is a large spectrum of other mechanisms that are discussed to explain the protection afforded by IPC including improvement of the cellular energy metabolism and attenuation of disturbances at the microcirculatory level. Especially as the latter are well known to be dramatically amplified by activated PMNLs and by an inflammatory response in general, the descript-

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**REFERENCES**


