Adenosine kinase inhibitor GP515 attenuates hepatic leukocyte adhesion after hemorrhagic hypotension

Clemens Bauer, Maarten G. Bouma, Isa Herrmann, Frans A. J. M. van den Wildenberg, Gary S. Firestein, Ingmar Marzi, and Wim A. Buurman. Adenosine kinase inhibitor GP515 attenuates hepatic leukocyte adhesion after hemorrhagic hypotension. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G1297–G1303, 1997.—Adhesion of leukocytes to the vascular endothelium hallmarks a key event in neutrophil-mediated organ injury after ischemia-reperfusion. The autacoid adenosine has been shown to inhibit activated neutrophil function and to interfere with leukocyte-endothelial adherence. Its therapeutic use in ischemia-reperfusion, however, has been limited by severe cardiovascular side effects. We therefore investigated the effects of the adenosine kinase inhibitor GP515 in vivo on hepatic leukocyte-endothelial interactions in a rat model of hemorrhagic hypotension and resuscitation, using intravital microscopy. Rats were pretreated with either GP515 (0.25 mg/kg) or saline in a randomized and blinded manner and subjected to pressure-controlled hemorrhagic hypotension at a mean arterial pressure of 40 mmHg for 60 min followed by 5 h of resuscitation. Five hours after resuscitation in saline-treated animals, firm leukocyte-sinusoidal adhesion was strongly enhanced in the periportal and midzonal sublobular regions, and sinusoidal diameters were also markedly reduced. Compared with saline treatment, GP515 significantly attenuated shock and resuscitation-induced leukocyte adhesion in both sublobular regions. Moreover, although GP515 did not significantly affect macrohemodynamical and hematological parameters, it enlarged narrowed sinusoidal diameters and tended to improve sinusoidal blood flow. We propose that the adenosine-regulating agent GP515 has a therapeutic potential to attenuate ischemia-reperfusion-induced inflammation by capitalizing on the beneficial anti-inflammatory effects of endogenous adenosine.

hemorrhagic shock; liver; leukocyte-endothelial interactions; resuscitation; intravital microscopy; rat

ISCHEMIA-REPERFUSION INJURY is an important pathological event that may result from restoration of blood circulation in a variety of serious clinical conditions, such as acute arterial obstruction, major vascular surgery, and hemorrhagic shock. Hypovolemic shock, as occurs after trauma or complex surgical procedures, followed by resuscitation, essentially represents a “whole body” ischemia-reperfusion insult and is often clinically associated with the development of a systemic inflammatory response syndrome and multiple-organ dysfunction syndrome (2, 21). Central to the pathogenesis of the inflammatory response to ischemia-reperfusion are the activation of neutrophils and their subsequent adhesion to the vascular endothelium (40). Strategies aimed at attenuating the inflammatory cascades elicited during ischemia-reperfusion include the use of antioxidants, platelet-activating factor antagonists, anti-cytokine monoclonal antibodies (MAbs), and MAbs to vascular and leukocyte adhesion molecules. During recent years, however, the anti-inflammatory potential of the endogenous metabolite adenosine has become an important focus of attention of studies seeking alternative therapeutic strategies to prevent or inhibit the detrimental inflammatory response after ischemia-reperfusion. In this context, adenosine has been termed a “retaliatory metabolite” (29). The reported anti-inflammatory effects of adenosine include inhibition of cytokine release (7, 8, 17, 31, 34), expression of endothelial adhesion molecules (8), leukocyte endothelial adhesion (18), and neutrophil function (6, 10, 13). Although numerous in vitro studies have demonstrated the antiadhesive effects of adenosine, few reports exist on the effects of adenosine on adhesion during postischemic conditions in vivo (23, 30). However, the therapeutic use of adenosine and its analogs has been limited by severe side effects, such as hypotension and bradycardia, as well as a short half-life. The strategy underlying the use of adenosine-regulating agents is to enhance endogenous adenosine concentrations at local sites of inflammation, thus enhancing the beneficial anti-inflammatory effects of adenosine while minimizing systemic toxicity. Under physiological conditions, adenosine is generated by dephosphorylation of AMP. This step is reverted by the enzyme adenosine kinase, which phosphorylates adenosine to AMP. Therefore one possibility for interference with the adenosine metabolism is to increase adenosine concentration to inhibit the rephosphorylation of adenosine by blocking adenosine kinase. GP515 is a novel adenosine kinase inhibitor, whose anti-inflammatory actions have indeed been related to enhancement of endogenous concentrations of adenosine (6, 12, 18, 33). This compound has been reported to provide protection from mortality in two rodent models of septic shock without displaying systemic side effects (17). Currently, however, its potential therapeutic use in ischemia-reperfusion has not been investigated. As outlined above, enhanced leukocyte-endothelial adhesion is a pivotal early step in the development of postischemic neutrophil-mediated injury of various target organs, such as the liver and lungs (24, 37, 38). We therefore examined the effects of the adenosine-regulating agent GP515 on leukocyte-endothelial interactions in the rat liver after hemorrhagic hypotension and resuscitation.
as a model of hemorrhagic shock, using intravital microscopy as described by Marzi et al. (26).

**MATERIALS AND METHODS**

Animals. The experiments were performed using female Sprague-Dawley rats (200–250 g body wt), obtained from Charles River, Sulzfeld, Germany. The study design was approved by the local veterinarian ethics committee. Rats had free access to standard rat food and water until the day of the experiment. The animals were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg). Body temperature was maintained at 37 ± 0.5°C using a temperature-controlled warming plate (Conrad Electronic, Hirschau, Germany).

Surgical preparation. All surgical preparations were performed under aseptic conditions. After induction of anesthesia and dermal disinfection, the rats were tracheotomized and intubated to maintain free airways and to allow spontaneous breathing. The animals were then instrumented for invasive monitoring of hemodynamical parameters as follows. The left carotid artery was cannulated with a thermistor-tipped 1.5-F catheter, which was advanced into the aortic arc for measurement of cardiac output (CO) by the transpulmonary thermodilution method (Cardiotherm 500, Columbus Instruments, Columbus, OH). The right internal jugular vein was cannulated with polyethylene tubing (0.4 × 0.8 mm, Portex, Hythe, UK) for infusion of GP515 and resuscitation fluids. The left femoral artery was cannulated with polyethylene tubing (0.4 × 0.8 mm) for continuous invasive monitoring of the mean arterial blood pressure (MAP) and heart rate (HR) using a pressure transducer (Hellige SMK 154–9, Freiburg, Germany). This line was used for shock induction and to take blood samples for laboratory analyses (blood gas analysis by ABL, Radiometer, Copenhagen, Denmark; hematological parameters by Sysmex 2000, Digitana, Frankfurt, Germany). Blood samples (300 µl each) were drawn at baseline, before shock induction, at the end of shock, and after 1 and 5 h of resuscitation. After shock, blood losses due to sampling were isovolumetrically substituted with shed blood.

Experimental groups. Animals were randomly assigned to two shock groups and two nonshock control groups, receiving the adenosine kinase inhibitor GP515 (kindly provided by Gensis, San Diego, CA) or an equal amount of vehicle (saline) in a blinded manner. Thus four experimental groups were formed: a shock/saline group (S-S), a shock/GP515 group (S-GP), a control/saline group (C-S), and a control/GP515 group (C-GP). GP515 was administered at a dose of 0.25 mg/kg diluted in 2 ml saline and infused intravenously during a 30-min period followed by an additional 30 min of steady state before shock induction. In preliminary dose-finding studies, 0.25 mg/kg infused over a 30-min period was found to be the critical dose to produce only a slight, nonsignificant initial hypotensive effect, with MAP stable thereafter. The experimental group assignment is summarized in Table 1. Two additional shock groups were investigated to examine the effects of GP515 on shock-induced inflammation and liver injury at 2 days after shock. Animals of the 2-day shock groups (n = 5 each) were treated either with GP515 or saline. After 2 days, the serum activity of liver transaminases and organ edema were measured and compared with a time-matched sham-control group.

**Hemorrhagic hypotension and resuscitation.** After pretreatment with either GP515 or vehicle, hemorrhagic hypotension was induced within 5 min by blood withdrawal from the femoral arterial line until MAP was 40 mmHg. Shed blood was citrated to inhibit coagulation. The shock model used was pressure controlled; i.e., if MAP was < 45 mmHg, additional blood was drawn. Hypotension was maintained at 40 mmHg for 60 min, and subsequently 60% of shed blood was retransfused. The animals were additionally resuscitated with lactated Ringer solution with twice the shed blood volume in the 1st h, once the shed blood volume in the 2nd and 3rd h, and with 10 ml·kg⁻¹·h⁻¹ in the 4th and 5th h. This resuscitation regimen was previously shown to sufficiently restore systemic circulation after hemorrhagic hypotension (3). Control animals were instrumented in an identical manner but were not subjected to hypotension and subsequent resuscitation.

**Intravital microscopy.** Five hours after the start of resuscitation from shock or at the corresponding time point in control animals, the hepatic microcirculation was investigated by intravital microscopy. The abdomen was opened by a midline laparotomy, and the left liver lobe was mobilized after dissection of the hepatic ligaments. The animal was positioned on its left side on a specially designed Plexiglas stage, which allowed gentle exteriorization of the left liver lobe, with its lower plane surface uppermost. The liver surface was covered with plastic foil and continuously superfused with saline at 37°C to prevent exsiccation. The hepatic microcirculation was then investigated using a Nikon MM-11 epifluorescence microscope (Tokyo, Japan) with a 100-W mercury lamp, a 545 nm excitation filter, a ×10/0.30 water immersion objective, a 0.7–2.25 zoom objective, a ×12 ocular, and a final magnification of ×330. The experiments were recorded with a low-light charge-coupled device camera (FK 6990, Pieper, Schwerte, Germany), which was connected through a serial time-date generator (VTG 33, FOR-A Company, Tokyo, Japan) with a SVHS video-recording system (Panasonic FS-1, Tokyo, Japan). Acridine orange (1 mg/ml in saline, Sigma, St. Louis, MO) as fluorescence marker of leukocytes was injected intravenously as bolus of 0.1 mg per recorded liver lobule. The microcirculation and leukocyte-endothelial interactions in five liver lobules were observed and recorded during 30 s for off-line evaluation. Next, five central venules were brought into the center of the image, and the pericentral region was recorded for comparable determination of sinusoidal diameters at distances of 90 µm from the central vein.

On completion of the experiments, the livers were harvested, and wet weight was determined. Next, the organs were dried at 60°C for 24 h and weighed again to determine wet-to-dry ratio as a marker of organ edema.

**Evaluation.** The recorded sequences of intravital liver microscopy were evaluated off-line by an independent observer blinded to experimental group assignment.

Leukocyte adhesion was determined as the number of leukocytes per square millimeter of liver surface for each of the three different sublobular fields of the liver acinus as described by Rappaport (32). According to recent previous studies (3, 26), two distinct types of leukocyte adhesion were differentiated: reversible, temporary adhesion (adhesion time: <20 s; mean adhesion time: 1–3 s), and mostly irreversible, firm adhesion (adhesion time: >20 s).

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**Table 1. Experimental groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Shock</th>
<th>Treatment</th>
<th>Reperfusion Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-S (control/saline)</td>
<td>8</td>
<td>No</td>
<td>Saline</td>
<td>5 h</td>
</tr>
<tr>
<td>C-GP (control/GP515)</td>
<td>8</td>
<td>No</td>
<td>GP515</td>
<td>5 h</td>
</tr>
<tr>
<td>S-S (shock/saline)</td>
<td>8</td>
<td>Yes</td>
<td>Saline</td>
<td>5 h</td>
</tr>
<tr>
<td>S-GP (shock/GP515)</td>
<td>8</td>
<td>Yes</td>
<td>GP515</td>
<td>5 h</td>
</tr>
<tr>
<td>2D-C (2-day control)</td>
<td>5</td>
<td>No</td>
<td>Saline</td>
<td>2 days</td>
</tr>
<tr>
<td>2D-S (2-day shock)</td>
<td>5</td>
<td>Yes</td>
<td>Saline</td>
<td>2 days</td>
</tr>
<tr>
<td>2D-GP (2-day shock/GP515)</td>
<td>5</td>
<td>Yes</td>
<td>GP515</td>
<td>2 days</td>
</tr>
</tbody>
</table>

n = no. of animals.
The hepatic microcirculation was quantitated by assessment of sinusoidal diameters and estimated sinusoidal blood flow, as determined by leukocyte velocity measurements using a computer-supported morphometrical image analysis system (Lobulus, Medvis, Homburg, Germany). Flow was calculated under the assumption of circular diameters of sinusoids using the following equation: \( F = v_L \cdot \pi \cdot \left( D_s / 2 \right)^2 \), where \( F \) is flow, \( v_L \) is leukocyte velocity, and \( D_s \) is sinusoidal diameter.

Statistical analysis. Data are presented as mean ± SE. Differences between groups were tested by analysis of variance and post hoc Student-Newman-Keuls test. Differences between various time points within one group were tested by paired \( t \)-test. \( P < 0.05 \) was considered statistically significant.

RESULTS

Hemodynamical parameters. At baseline, MAP, HR, and CO were identical in all experimental groups (Fig. 1). Infusion of 0.25 mg/kg GP515 over a 30-min period resulted in a moderate, nonsignificant reduction of MAP from 125.4 ± 5.6 to 102.9 ± 7.8 mmHg in the control group (C-GP) and from 128.9 ± 4.6 to 103.3 ± 6.2 mmHg in the shock group (S-GP). Also, after infusion of GP515, MAP in both GP515-treated groups did not differ significantly from both saline-treated control groups at the corresponding time point (t = 30 min). Similarly, intravenous administration of GP515 did not affect HR and CO before shock. Compared with baseline, a slight, but nonsignificant increase in CO was observed during the 1st h of the experiment, which was, however, identical in all experimental groups. Hemorrhagic hypotension was induced and maintained constantly at 40 mmHg for 60 min, resulting in a reduction of HR and CO that was the same in the saline- and GP515-treated groups. Resuscitation completely restored shock-induced hemodynamic changes in both shock groups, with MAP, HR, and CO being identical compared with both control groups. Moreover, no significant differences in these hemodynamical parameters were observed between saline- and GP515-treated animals.

Laboratory analysis. Before induction of shock, pH, blood gases, hemoglobin concentration (Hb), hematocrit (Hct), and leukocyte count were comparable in all experimental groups (Table 2). Compared with the end of steady state (t = 60 min), shock resulted in a significant elevation of arterial PO2 and a reduction of arterial PC02 as well as reduced Hb and Hct. These changes were not altered by pretreatment with GP515. At the end of shock, arterial PO2 and PC02, Hb, and Hct were significantly altered in shocked compared with nonshocked animals. During reperfusion, no significant differences with regard to these parameters were observed among any of the experimental groups. Neither in control animals nor in shock animals did GP515 affect the laboratory parameters determined.

Liver wet-to-dry ratio and transaminases. Liver wet-to-dry weight ratios revealed no differences among all experimental groups at 5 h after shock (data not shown). However, the significant increase in liver wet-to-dry ratio at 2 days after shock was prevented by GP515. In addition, at this time point, the shock-induced increase of serum activity of the transaminase aspartate aminotransferase (AST) was significantly reduced in the GP515 group (Fig. 2).

Leukocyte adhesion. Five hours after resuscitation from shock or at the corresponding time point in control
Table 2. pH, blood gases, hemoglobin concentration, hematocrit, and leukocyte count in experimental groups before shock

<table>
<thead>
<tr>
<th>Time, min</th>
<th>C-S</th>
<th>C-GP</th>
<th>S-S</th>
<th>S-GP</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.23 ± 0.02</td>
<td>7.34 ± 0.05</td>
<td>7.33 ± 0.04</td>
<td>7.33 ± 0.03</td>
</tr>
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<td>120</td>
<td>7.37 ± 0.03</td>
<td>7.36 ± 0.04</td>
<td>7.32 ± 0.04</td>
<td>7.35 ± 0.03</td>
</tr>
<tr>
<td>180</td>
<td>7.40 ± 0.02</td>
<td>7.38 ± 0.03</td>
<td>7.38 ± 0.02</td>
<td>7.39 ± 0.03</td>
</tr>
<tr>
<td>420</td>
<td>7.33 ± 0.02</td>
<td>7.33 ± 0.01</td>
<td>7.36 ± 0.02</td>
<td>7.37 ± 0.02</td>
</tr>
<tr>
<td>Arterial PO2, mmHg</td>
<td>70.5 ± 6.5</td>
<td>77.2 ± 13.4</td>
<td>65.7 ± 9.8</td>
<td>73.3 ± 6.4</td>
</tr>
<tr>
<td>120</td>
<td>80.2 ± 11.6</td>
<td>74.8 ± 10.9</td>
<td>107.9 ± 14.2†</td>
<td>105.2 ± 16.6†</td>
</tr>
<tr>
<td>180</td>
<td>84.1 ± 6.3</td>
<td>83.0 ± 18.7</td>
<td>68.5 ± 6.5</td>
<td>77.4 ± 6.7</td>
</tr>
<tr>
<td>420</td>
<td>108.1 ± 8.2</td>
<td>101.5 ± 11.6</td>
<td>99.7 ± 10.5</td>
<td>110.1 ± 12.4</td>
</tr>
<tr>
<td>Arterial PCO2, mmHg</td>
<td>32.5 ± 4.3</td>
<td>32.0 ± 5.1</td>
<td>38.2 ± 9.3</td>
<td>35.2 ± 6.1</td>
</tr>
<tr>
<td>120</td>
<td>31.0 ± 4.3</td>
<td>31.1 ± 5.8</td>
<td>21.4 ± 5.2†</td>
<td>23.2 ± 6.5†</td>
</tr>
<tr>
<td>180</td>
<td>28.8 ± 4.4</td>
<td>31.1 ± 2.9</td>
<td>32.3 ± 4.9</td>
<td>30.1 ± 3.2</td>
</tr>
<tr>
<td>420</td>
<td>37.8 ± 6.9</td>
<td>34.3 ± 5.9</td>
<td>39.2 ± 1.6</td>
<td>33.5 ± 9.4</td>
</tr>
<tr>
<td>Hb, mM</td>
<td>6.0 ± 0.7</td>
<td>6.7 ± 0.6</td>
<td>6.4 ± 0.6</td>
<td>6.6 ± 0.4</td>
</tr>
<tr>
<td>120</td>
<td>6.3 ± 0.5</td>
<td>6.5 ± 0.6</td>
<td>3.9 ± 0.6†</td>
<td>4.1 ± 0.4†</td>
</tr>
<tr>
<td>180</td>
<td>6.0 ± 0.4</td>
<td>6.1 ± 0.6</td>
<td>5.0 ± 0.2</td>
<td>5.1 ± 0.4</td>
</tr>
<tr>
<td>420</td>
<td>6.5 ± 0.6</td>
<td>6.4 ± 1.1</td>
<td>5.2 ± 0.8</td>
<td>5.3 ± 0.4</td>
</tr>
<tr>
<td>Hct, %</td>
<td>29.7 ± 2.6</td>
<td>30.5 ± 1.9</td>
<td>29.6 ± 2.1</td>
<td>30.5 ± 1.9</td>
</tr>
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<td>120</td>
<td>29.5 ± 2.0</td>
<td>29.4 ± 1.9</td>
<td>19.6 ± 2.6†</td>
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<td>180</td>
<td>27.2 ± 1.7</td>
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<td>23.9 ± 1.3</td>
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<td>420</td>
<td>29.2 ± 2.6</td>
<td>28.3 ± 4.1</td>
<td>24.8 ± 3.4</td>
<td>24.8 ± 1.7</td>
</tr>
<tr>
<td>WBC, ×10^9/l</td>
<td>5.2 ± 2.3</td>
<td>5.5 ± 1.4</td>
<td>4.5 ± 1.1</td>
<td>4.7 ± 1.9</td>
</tr>
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<td>120</td>
<td>6.8 ± 3.1</td>
<td>5.7 ± 1.2</td>
<td>3.9 ± 1.1</td>
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<tr>
<td>180</td>
<td>6.2 ± 2.3</td>
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<td>6.5 ± 1.8</td>
<td>5.0 ± 1.8</td>
<td>5.7 ± 1.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Hb, hemoglobin concentration; Hct, hematocrit; WBC, leukocyte count. *P < 0.05 vs. C-S; †P < 0.05 vs. 60-min value within same experimental group.

Animals, temporary leukocyte adhesion was identical in treated as well as untreated control and shock groups, ranging from 8.6 ± 0.7 to 9.7 ± 0.5% of the total number of sinusoidal leukocytes, and displayed no differences between the various sublobular regions (data not shown).

The number of leukocytes firmly adhering to the endothelial wall was comparable in both control groups (Fig. 3), with the highest adhesion numbers in the periportal region (C-S: 394 ± 16 leukocytes/mm², and C-GP: 352 ± 27 leukocytes/mm²). In the nontreated shock group (S-S), firm leukocyte adhesion was significantly enhanced in the periportal (1,032 ± 95 leukocytes/mm²) and midzonal (387 ± 35 leukocytes/mm²) regions of the liver acinus compared with both control groups. Also, in the pericentral field, the number of adhering leukocytes was 311 ± 77/mm² in the S-S group, compared with 126 ± 54/mm² in the C-S group (P < 0.05) and 172 ± 62/mm² in the C-GP group (P > 0.05). Pretreatment with GP515 markedly attenuated leukocyte adhesion after shock, with a significant reduction in the periportal as well as the midzonal field to 562 ± 22 and 286 ± 37 leukocytes/mm², respectively. Moreover, GP515 reduced shock-induced firm leukocyte adhesion in these two fields to a level comparable...
with saline-treated controls. GP515 also reduced shock-induced adhesion in the pericentral region to 224 ± 89 leukocytes/mm², but without reaching statistical significance compared with saline-treated shock animals.

Hepatic microcirculation. Mean sinusoidal diameter in the saline-treated control group was 11.6 ± 0.3 µm, and was nonsignificantly increased by ~7% in the GP515-treated control group (Fig. 4A). After shock and resuscitation, sinusoids were markedly narrowed to a mean diameter of 7.8 ± 0.2 µm. In comparison, pretreatment with GP515 partly prevented this sinusoidal narrowing, resulting in a mean sinusoidal diameter of 9.8 ± 0.1 µm, a significant increase of ~26%. In parallel, GP515 tended to enhance reduced sinusoidal blood flow after shock (Fig. 4B), almost reaching statistical significance (37.5 ± 1.1 and 50.8 ± 3.5 µl/s in S-S vs. S-GP, respectively; P = 0.06). In control animals, however, GP515 did not affect sinusoidal blood flow.

DISCUSSION

In the present study we have determined the effects of pretreatment with the adenosine kinase inhibitor GP515 on firm leukocyte-endothelial adhesion in the liver after 1 h of hemorrhagic shock followed by 5 h of reperfusion. Previous studies using this animal model have revealed two different patterns of leukocyte adhesion in the liver after hemorrhagic shock. First, temporary (short-term) adhesion is maximally elevated in the 1st h of resuscitation and declines thereafter (26). This type of reversible leukocyte-endothelial interaction may be the correlate of postcapillary leukocyte rolling and represents an early step in neutrophil recruitment to inflammatory sites. Second, firm (long-term) adhesion, also referred to as leukocyte sticking, increases gradually during the reperfusion period and is significantly elevated 5 h after resuscitation from shock (3). Adherence of neutrophils to the vascular endothelium is an important step in neutrophil-mediated injury of endothelial cells, a key pathological event in ischemia-reperfusion (40). Although initial rolling is considered a prerequisite for firm adherence (22), it is the strong adhesive neutrophil-endothelial interaction that is thought to create a sequestered microenvironment in which activated neutrophils release proteolytic enzymes and reactive oxygen metabolites that cause the endothelial damage, eventually resulting in increased microvascular permeability and organ edema (39, 40). Therefore, to study firm adhesion of leukocytes to the liver sinusoids, we selected the 5-h reperfusion time frame. Indeed, our present results confirm the extent of increase of leukocyte adhesion as well as these earlier observed adhesion patterns, with firm adhesion highly elevated in both shock groups compared with both control groups and temporary adhesion being identical in all experimental groups. Also, the sublobular adhesion pattern with increasing numbers of firmly adhering leukocytes in the pericentral, midzonal, and periporal regions, respectively, was consistent with previous results in this model (26). Firm leukocyte adhesion to sinusoidal endothelium as observed in this study was previously shown not to be due to mechanical trapping of leukocytes in narrowed sinusoid but was suggested to be a receptor-specific mechanism (4). The results of the study cited clearly indicate the involvement of adhesion receptors such as intercellular adhesion molecule-1 (ICAM-1) and the corresponding CD11b/CD18 complex in the process of firm leukocyte adhesion (4).

The most prominent finding of this study is that GP515 strongly attenuated shock-induced firm adhesion of leukocytes to the sinusoidal endothelium. As reported by Vedder et al. (37, 38) and Mileski et al. (28), inhibition of CD18-dependent firm neutrophil adherence effectively reduces neutrophil-mediated injury to the lungs, liver, and gastrointestinal tract after hemorrhagic shock in rodents as well as in subhuman primates. Similarly, in isolated hepatic ischemia-reperfusion models, it has been established that massive neutrophil infiltration contributes to posts ischemic liver injury (24, 36). Although in the aforementioned hemorrhagic shock models gross organ injury was evident after long-term resuscitation, in the present study no gross liver edema, as determined by liver wet-to-dry ratios, was present after 5 h of resuscitation. However, at 2 days after shock, a significant increase in liver wet-to-dry ratio was observed, possibly due to tissue injury by activated and transmigrated leukocytes.
GP515 had protective effects at this time point indicated by reduced liver wet-to-dry ratio and significantly reduced serum activity of AST.

The observed antiadhesive effect of the adenosine-regulating agent GP515 is consistent with reported effects of adenosine and adenosine A2 receptor agonists on neutrophil-endothelial interactions in vitro (9, 11, 16, 18) and in vivo (1, 23, 30, 33). Although we did not measure in vivo adenosine levels in this study, GP515 has been demonstrated to enhance local endogenous adenosine concentrations in vitro (18) and ex vivo (6), as well as in vivo (12). Although activation of neutrophil A2 receptors has been implicated in the antiadhesive effect of adenosine, it has not currently been established which specific types of leukocyte adhesion molecules are affected by adenosine. Firestein et al. (18) have observed that adenosine interferes with L-selectin-mediated adhesion and does not affect CD18-dependent adhesion in a static adhesion assay. Others have demonstrated that in vitro integrin-mediated adhesion can also be modulated by adenosine (14, 41). Interestingly, adenosine has been shown to inhibit stimulated upregulation of the CD11/CD18 integrin on human neutrophils in vitro (41). Because GP515 attenuated the firm adhesive leukocyte-endothelial interactions, our results would be in support of altered integrin-mediated adhesion, although we cannot rule out an early effect on selectin-mediated rolling. The present results are paralleled by previous similar findings in this same model obtained with the natural glycoprotein neutrophil inhibitory factor, which inhibits CD11b/CD18-mediated adhesion (4).

Adenosine A2 receptors are thought to couple unifor- mally to adenylate cyclase, with activation resulting in enhanced generation of intracellular adenosine 3’5’-cyclic monophosphate (cAMP) (35). Elevated neutro- phil intracellular cAMP has indeed been related to inhibition of CD11b/CD18 surface expression in vitro (14), thus supporting a role for A2 receptors in down-modulating postischemic integrin-mediated adhesion. In line with this, the cAMP-raising agent pentoxifylline attenuated hemorrhagic shock-induced leukocyte-sinusoidal adhesion in this animal model in an identical fashion as described here for GP515 (27).

Although neutrophils have clearly been identified as targets for the anti-inflammatory actions of adenosine, endothelial cells have not been ruled out as potential targets for adenosine. On the contrary, adenosine has been shown to inhibit expression of E-selectin and vascular cell adhesion molecule-1, by activated human endothelial cells in vitro (8). Also, the adenosine analog 3-deazaadenosine inhibits ICAM-1 biosynthesis in tumor necrosis factor-α-stimulated cultured human endothelium (25). It therefore remains to be determined whether interference of adenosine with endothelial expression of adhesion molecules is involved in the inhibition of neutrophil-endothelial interactions in vivo.

A second interesting observation in this study is that GP515 displayed a tendency to improve local microcirculation in the liver after resuscitation from shock. Although sinusoidal diameters in nontreated shock animals were significantly reduced compared with sham-operated animals, GP515 significantly enhanced sinusoidal diameters after shock. GP515 also slightly enlarged sinusoidal diameters in control animals but had a relatively stronger effect on sinusoidal diameter after shock. In parallel, although sinusoidal blood flow was significantly reduced after shock, GP515 tended to improve sinusoidal flow after shock, almost reaching statistical significance (P = 0.06), but was without effect on blood flow in control animals. Although adeno- sine has been shown to exert negative chronotropic and dromotropic effects (20) and to cause hypotension (5), GP515, compared with saline treatment, did not affect the cardiovascular parameters MAP, HR, and CO in this study. Moreover, systemic hematological parameters were not altered by GP515. Together, these data sug- gest that GP515 acts to enhance endogenous adenosine at its local site of formation, thereby limiting systemic side effects and at the same time capitalizing on well-recognized beneficial microcirculatory effects of adenosine during ischemia-reperfusion (15, 19).

In summary, using an experimental model that allowed direct visualization of hepatic leukocyte-endothelial interactions and microcirculation, we have demonstrated that systemic administration of the adenosine-regulating agent GP515 has significant antiadhesive and mild beneficial microcirculatory effects in the early inflammatory response to hemorrhagic hypoten- sion and resuscitation, which are paralleled by reduced liver injury at the long term. Our results point to a possible therapeutic potential of GP515 and possibly other adenosine-regulating agents (39–41) in the treat- ment of ischemia-reperfusion injury.

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