Superior Mesenteric Artery Occlusion Models Shock-Induced Gut Ischemia-Reperfusion

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Background. Superior mesenteric artery occlusion (SMAO) is a simple and reproducible model of shock-induced gut ischemia/reperfusion, but some argue that it is not clinically relevant. The purpose of the current study was to compare SMAO to a standard model of controlled hemorrhage (CH) and uncontrolled hemorrhage (UH).

Methods. Rats had femoral lines and a jejunal mucosal laser Doppler placed followed by SMAO (60 min of ischemia, no resuscitation), controlled hemorrhage (40 mm Hg for 60 min, 2:1 resuscitation shed blood and lactated Ringers), or uncontrolled hemorrhage (liver injury, 3:1 resuscitation with lactated Ringers). Base deficit, lactate, and jejunal mucosal flow (as a percentage of baseline) were recorded during ischemia and for 120 min after reperfusion. Jejunal tissue was harvested for morphological evaluation. Comparison among groups was by analysis of variance (ANOVA), and significance was set at \( P < 0.05 \).

Results. Mucosal blood flow was similar among groups at the onset of reperfusion (CH, 16.9 \pm 5.0\% versus UH, 10.9 \pm 3.1\% versus SMAO, 13.9 \pm 6.2\%) and during the initial period of reperfusion. By 120 min, however, flow in CH (75.4 \pm 2.5\%) was significantly higher than in either UH (36.4 \pm 13.1\%) or SMAO (31.7 \pm 8.4\%). Histological injury was less with CH, while base deficit was significantly higher in CH at the onset of reperfusion (\(-24 \pm 2\) versus UH, \(-10 \pm 3\) and SMAO, \(-6 \pm 3\) mM/L) but comparable by the end (CH, \(-17 \pm 4\) versus UH, \(-16 \pm 3\) and SMAO, \(-17 \pm 2\) mM/L).

Conclusion. SMAO is a clinically relevant model of shock-induced gut ischemia/reperfusion.

Key Words: rodent model; shock; ischemia/reperfusion; superior mesenteric artery occlusion; uncontrolled hemorrhage; controlled hemorrhage.

INTRODUCTION

Gut ischemia/reperfusion (I/R) is thought to be a prime inciting event for post injury multiple organ failure [1, 2]. Clinically, shock has consistently been shown to be a strong predictor of multiple organ failure [3]. In the laboratory, it has been demonstrated that shock causes disproportionate splanchnic vasoconstriction that persists despite adequate systemic resuscitation [4, 5]. This has been verified in the ICU, where persistent gastric mucosal hypoperfusion (documented by tonometry) in patients undergoing shock resuscitation predicts multiple organ failure and death [6, 7]. Finally, it has been convincingly shown that shock-induced gut I/R elaborates mediators that cause remote organ injury [8–10].

The optimal laboratory model to study shock, however, has not been well agreed upon and depends upon the research question being asked. The model of controlled hemorrhage (CH) dates back to the early 1900s, when Carl Wiggers, a physiologist, was studying the phenomenon of irreversible shock [11]. CH was then adopted and adapted (i.e., the modified “Wiggers Prep”) by surgeons, the most notable being Shires et al. and Moyer et al., to study optimal methods of shock resuscitation [12, 13]. This pioneering work culminated in
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our current standard care of isotonic crystalloid resuscitation (primarily lactated Ringers) and blood at a ratio of 3:1. However, in 1980, Rocha E. Silva and associates published a landmark study in which they demonstrated that small-volume resuscitation with hypertonic saline in CH had remarkably better survival compared to an equal volume of isotonic saline [14]. Subsequent investigators identified that hypertonic saline caused increased bleeding, presumably due to arteriolar vasodilatation, and they developed models of uncontrolled hemorrhage (UH), arguing that CH was not clinically relevant [15, 16]. Unfortunately, these models (tail resection [16], hole punch in aorta [17], splenic laceration [18], and hepatic laceration [19]) are difficult to use due to the variable blood loss, blood pressure, and mortality response to similar anatomic injuries.

Our laboratory is interested in shock-induced gut I/R and its effect on gut inflammation and function. To investigate this, we have used a simple and reproducible model of superior mesenteric artery occlusion (SMAO). However, some reviewers criticize this model as not being clinically relevant. The purpose of the current study, therefore, was to compare systemic perfusion, mucosal perfusion, and mucosal injury in three established models of shock: CH, UH, and SMAO.

METHODS

Sprague Dawley rats weighing 250–350 grams were used after a period of acclimatization. Rats were fasted overnight but allowed free access to water. Anesthesia was induced and maintained with 2% isoflurane, and body temperature was maintained at 37°C by use of a warming blanket. At the conclusion of the experiment, cardiac puncture and exsanguination were used to achieve euthanasia. All procedures performed were under approved protocols by the Animal Welfare Committee of the University of Texas-Houston School of Medicine.

A polyethylene catheter (PE-50) was introduced into the femoral artery and a 14-gauge angiocatheter into the vein for blood pressure and hemodynamic measurements, blood sampling, and intravenous fluid administration. Mean arterial pressure (MAP) was recorded at 1-min intervals throughout the study period using a continuous data collection system (Micro-Med, Louisville, KY) connected to the arterial line. Blood (250 μL) was drawn into a heparinized syringe for arterial blood gas analysis (i-STAT, Abbott Laboratories, East Windsor, NJ).

An upper midline laparotomy was performed and the jejunum identified 5 cm distal to the ligament of Treitz. A Teflon-coated laser optic flow probe (Peri flux PF409, flexible probe with 0.25-mm fiber separation) was inserted into a small enterotomy and positioned along the antimesenteric border of the proximal jejunum [20]. Mucosal blood flow was continuously recorded with a laser Doppler flow monitor (Peri Flux 4001 Master; Perimed, Jaarnfalla, Sweden). Measurements were taken as the average flow (in arbitrary perfusion units) [21] over a 5-min period following an initial 30-min period of stabilization and recorded as a percentage of baseline flow. The use of laser Doppler-measured tissue perfusion is a reliable technique that has been validated in the gastrointestinal mucosa [22].

Shock was then introduced by one of three methods (n = 4-6/group): controlled hemorrhage, uncontrolled hemorrhage, or superior mesenteric artery occlusion, and animals were followed through out 120 min of reperfusion. At the time of sacrifice, full thickness segment of jejunum just proximal to enterotomy for the laser Doppler was harvested for microscopic examination. Sections were stained with hematoxyline and eosin and then examined under a light microscope in a blinded manner (HTH). Mucosal injury was scored on a scale from 0 to 5 as described by Chiu et al. [23]. The grading system was as follows: grade 0, normal mucosal villi; grade 1, subepithelial Gruenenhagen’s space, capillary congestion; grade 2, extension of subepithelial space with moderate lifting of epithelial layer from lamina propria; grade 3, massive epithelial lifting down sides of villi, few tips denuded; grade 4, denuded villi with lamina propria and dilated capillaries exposed; and grade 5, digestion and disintegration of lamina propria, hemorrhage and ulceration.

Controlled Hemorrhage

Hemorrhagic shock was induced by withdrawing blood from the femoral artery into a heparinized syringe over 10 min to achieve and then maintain a mean arterial pressure (MAP) of 40 mm Hg [24, 25]. Following 60 min of shock, the animals were resuscitated to a MAP of 80 mm Hg by administration of the remaining shed blood plus two times the shed blood volume as lactated Ringers solution. The onset of reperfusion was marked by the reinfusion of blood. Blood samples were obtained at baseline, after 60 min of hemorrhage, and at the end of 120 min of resuscitation.

Uncontrolled Hemorrhage

The capsule of the median lobe of the liver was scored 1.5 cm from the suprahepatic vena cava, and the portion of the liver distal to the mark was sharply excised. Because of established variability inherent to this model, rats were excluded if the weight of the excised lobe of the liver divided by the baseline total body weight was either less than 0.8% or greater than 1.2% [19]. Five minutes after the onset of hemorrhage, Floseal (4 mL, Fusion Medical Technologies, Mountain View, CA) was applied to the cut surface of the liver to aid in hemostasis. Resuscitation to the baseline MAP with three times the shed blood volume as lactated Ringers solution was begun 5 min after the onset of hemorrhage [17]. Blood gas samples were obtained at baseline, 5, and 120 min after the onset of hemorrhage. Five minutes marked the onset of reperfusion, as resuscitation was instituted at this time. Due to the severity of the insult, longer times of hemorrhage were not tolerated, and a hemostatic agent was required to ensure survival through 120 min of reperfusion.

Superior Mesenteric Artery Occlusion

The superior mesenteric artery was isolated at its origin and clamped for 60 min [26] followed by reperfusion for 120 min. Blood gas samples were obtained at baseline, at the end of 60 min of ischemia, and after 120 min of reperfusion.

Statistical Analysis

Comparison among groups was performed using one-way analysis of variance (ANOVA) followed by Tukey’s test. Significance was set at P < 0.05; data are reported as mean ± SEM.

RESULTS

Systemic Perfusion

Mean Arterial Pressure

MAP was similar for all groups prior to the onset of ischemia (CH, 92 ± 5 mm Hg; UH, 91 ± 3 mm Hg;
SMAO, 90 ± 6 mm Hg) but diverged by the end of ischemia/onset of reperfusion, with SMAO (91 ± 7 mm Hg) being significantly higher than CH (39 ± 3 mm Hg) or UH (61 ± 10 mm Hg). MAP data throughout reperfusion is shown in Fig. 1. Within the first 5 min of reperfusion, MAPs converged and remained similar through 20 min of reperfusion (at 20 min: CH, 92 ± 6 mm Hg; UH, 99 ± 4 mm Hg; SMAO, 87 ± 9 mm Hg), but then began to diverge for the remaining period of reperfusion. By the end of reperfusion (120 min), MAP was significantly lower in CH (56 ± 9 mm Hg) than UH (101 ± 6 mm Hg) or SMAO (73 ± 16 mm Hg).

Arterial Blood Gas Determinations

Arterial blood gas results are shown in Table 1 for each model of shock at baseline, the onset of reperfusion, and the end of reperfusion. In each group, base deficit was increased by the onset of reperfusion and remained elevated throughout reperfusion. However, CH had a significantly higher base deficit at the onset of reperfusion compared to both UH or SMAO. By the end of reperfusion, all groups were comparable.

Local Gut Perfusion

Jejunal mucosal blood flow was comparable among groups at the onset of reperfusion (CH, 16.9 ± 5.0%; UH, 10.9 ± 3.1%; SMAO, 13.9 ± 6.2%) and during the initial 30 min of reperfusion (CH, 50.8 ± 8.0%; UH, 43.4 ± 6.2%; SMAO, 41.8 ± 6.3%). CH thereaf- ter began to diverge from UH and SMAO and was significantly higher than SMAO by 90 min (CH, 72.6 ± 12.5%; SMAO, 29.7 ± 10.8%) and higher than both SMAO and UH by 120 min (CH, 75.4 ± 2.5%; UH, 36.4 ± 13.1%; SMAO, 31.7 ± 8.4%) (Fig. 2).

<table>
<thead>
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<th>Parameter</th>
<th>CH</th>
<th>UH</th>
<th>SMAO</th>
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<tbody>
<tr>
<td>Baseline</td>
<td>pH</td>
<td>7.39 ± 0.06</td>
<td>7.36 ± 0.02</td>
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<td></td>
<td>P_{CO_2} (mm Hg)</td>
<td>38 ± 5</td>
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<td></td>
<td>P_{O_2} (mm Hg)</td>
<td>543 ± 28</td>
<td>497 ± 13</td>
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<tr>
<td></td>
<td>HCO_3 (mM/L)</td>
<td>23 ± 2</td>
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<td></td>
<td>Base excess (mM/L)</td>
<td>2.3 ± 1.1</td>
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<tr>
<td>Onset of reperfusion</td>
<td>pH</td>
<td>7.39 ± 0.06</td>
<td>7.27 ± 0.08</td>
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<tr>
<td></td>
<td>P_{CO_2} (mm Hg)</td>
<td>38 ± 5</td>
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<tr>
<td></td>
<td>P_{O_2} (mm Hg)</td>
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<td>512 ± 48</td>
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<tr>
<td></td>
<td>HCO_3 (mM/L)</td>
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<td>14 ± 7</td>
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<td>Base excess (mM/L)</td>
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<tr>
<td>End of reperfusion</td>
<td>pH</td>
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<td>7.31 ± 0.03</td>
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<tr>
<td></td>
<td>P_{CO_2} (mm Hg)</td>
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<td>22 ± 4</td>
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<td>HCO_3 (mM/L)</td>
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<tr>
<td></td>
<td>Base excess (mM/L)</td>
<td>−16.8 ± 3.9</td>
<td>−15.5 ± 2.6</td>
</tr>
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</table>

^a P < 0.05 versus UH.

^P < 0.05 versus SMAO.
Mucosal Injury

Morphological injury was significantly less in CH compared to UH and roughly correlated with mucosal perfusion at the end of reperfusion (Table 2).

DISCUSSION

Our laboratory interest is to study the effects of shock on gut inflammation, injury, and dysfunction. The goal of this study was to compare perfusion using three commonly used models of shock to determine which model(s) could mimic the clinical scenario of shock-induced gut I/R. All three models of shock resulted in impairment of both systemic and local gut perfusion. Base deficit increased by the end of ischemia and remained elevated throughout reperfusion. Similarly, gut perfusion remained impaired throughout reperfusion in all models and roughly correlated with the degree of mucosal histological injury. However, CH resulted in a more significant early systemic insult but less of a local gut insult (enhanced perfusion and less mucosal injury) than either UH or SMAO.

There are a number of inherent differences among models, including the degree of ischemic insult, the method of resuscitation, and the degree of hemodynamic impairment. First, the duration of ischemia varied among models: in UH, ischemia lasted for approximately 5 min (time when hemostatic agent applied), compared to both CH and SMAO, which used 60 min of ischemia. Because of the variable blood loss and physiological response using uncontrolled hemorrhage, only a brief period of hemorrhage is tolerated. Despite this marked difference in ischemic time, systemic and gut perfusion closely mimicked SMAO. The second difference involved the use and type of resuscitative fluid. CH allows the reinfusion of shed blood plus the use of lactated Ringers as needed to maintain MAP. Because of the difficulty with collecting and then reinfusing blood shed from the peritoneal cavity, only lactated Ringers was infused to maintain MAP with UH. On the other hand, SMAO used no type of fluid resuscitation. Third, the degree and extent of hemodynamic impairment among models differed. CH allows for a carefully maintained MAP during ischemia (40 mm Hg in the current study), whereas MAP is a function of blood loss and autoregulation in UH. With SMAO, there was a brief decrease in MAP with the onset of ischemia, but MAP was otherwise essentially unchanged from baseline. This is in contrast to a recent report by Khanna et al. that demonstrated a steep rise in MAP with onset of

**TABLE 2**

Jejunal Mucosal Injury

<table>
<thead>
<tr>
<th>Shock model</th>
<th>Chiu Score</th>
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<tr>
<td>SMAO</td>
<td>3.7 ± 0.7</td>
</tr>
<tr>
<td>Uncontrolled hemorrhage</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>Controlled hemorrhage</td>
<td>2.3 ± 0.5 *</td>
</tr>
</tbody>
</table>

* P = 0.01 versus uncontrolled hemorrhage.
ischemia that returned to baseline by the end of ischemia [27]. Similar to the current study, they also found a sustained decrease in MAP with reperfusion, which has been shown to be mediated by the release of platelet-activating factor from the postischemic intestine [28–30]. With UH, MAP was increased over baseline throughout most of reperfusion. This may be due to overly aggressive administration of fluid at a time when the animals are hemodynamically unstable coupled with rapid hemorrhage control. Interestingly, despite a high MAP, gut mucosal perfusion remained low throughout reperfusion. CH initially followed a similar pattern, with an initial overshoot of MAP that gradually returned to the target MAP (80 mm Hg). However, after the first 60 min of reperfusion, MAP was persistently decreased. We found that it was difficult to maintain MAP in this group as reperfusion continued. Additional parameters of systemic perfusion, such as oxygen delivery, could provide additional information on the differences in hemodynamic impairment among models. The current study only examined gross indices of perfusion, blood pressure and base deficit, as these parameters are those typically used in the acute management of shock.

The differences in perfusion demonstrated in the current study with CH may be due to the intensity and duration of ischemia and reperfusion chosen, rather than to inherent differences in the model. Other investigators have chosen longer periods of ischemia [31, 32], which may be desirable if the purpose of the experiment is to study gut injury and dysfunction (to increase the extent). However, the greater systemic perturbation exerted by increasing ischemia in this model may also exert a confounding effect on other organs that may then affect the gut. Even with a more significant ischemic insult, (CH 90 min with MAP 35–40 mm Hg), Tisherman et al. in a recent study also found gut injury to be minimal [33].

For the perfusion parameters examined, the two models that most closely resembled each other were SMAO and UH, suggesting that these models may be comparable for examining gut function (such as perfusion and injury) following shock-induced gut I/R. Further studies are needed to determine if additional parameters of gut function, such as transit and absorption, are similarly affected. Clearly, uncontrollable hemorrhage is a more clinically relevant model in that it mimics acute, uncontrollable blood loss followed by attempts at hemorrhage control and then fluid resuscitation. This model, however, can be quite variable and more difficult to reproduce [34, 35]. SMAO is simple, highly reproducible, and has been suggested as a useful model for studying intestinal I/R injury [25].

In conclusion, SMAO results in systemic and local gut perfusion comparable to hemorrhagic shock models, particularly uncontrollable hemorrhage. It may therefore be considered as a clinically relevant model of shock-induced gut ischemia/reperfusion.

REFERENCES


