Blood Flow Limitations of Solute Transport Across the Visceral Peritoneum

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Abstract. In a previous study, no limitations to urea transfer across the parietal peritoneum were demonstrated with decreases in local blood flow of 70%. It was hypothesized that the visceral peritoneum would have similar characteristics. To address this problem at the tissue level, diffusion chambers were affixed to the serosal side of the stomach, cecum, or liver of anesthetized rats (n = 6 each tissue), and solutions containing $^{14}$C urea were placed in the chamber. During each experiment, the local chamber blood flow was measured with laser Doppler flowmetry, and, simultaneously, the disappearance of the tracer versus time was determined under three conditions: control, after 60 to 70% blood flow reduction, and postmortem (flow = 0). The results showed no difference in the urea mass transfer coefficient (MTC; mean ± SEM; cm/min × 10$^4$) between control and blood flow reduction for the stomach (4.0 ± 0.4 versus 3.6 ± 0.3) or for the cecum (4.6 ± 0.3 versus 4.0 ± 0.3). However, the MTC was significantly decreased by local blood flow reduction in the liver (5.4 ± 0.2 versus 2.6 ± 0.2). Postmortem data demonstrated significant reductions in the MTC with blood flow equal to zero. It is concluded that a 60 to 70% blood flow reduction from control values does not limit solute transperitoneal transfer in the hollow viscera but causes significant changes in the mass transfer across the liver surface. Because the liver makes up only a small portion of the effective exchange area, overall transperitoneal solute transfer should not be greatly affected by significant decreases in blood flow. (J Am Soc Nephrol 8: 1946–1950, 1997)

In response to a recent hypothesis that blood flow to the tissues surrounding the peritoneal cavity may limit transport (1), we developed a technique to assess the effect of blood flow on the mass transfer of small solutes across the parietal peritoneum (2). That study by Ronco et al. demonstrated that decreases of blood perfusion to 30% of control values do not limit the transperitoneal transport of urea or mannitol. Because a larger portion of the peritoneum covers the visceral tissues (chiefly the gastrointestinal tract and the liver) and because the micro-circulations of these tissues are different from that of the abdominal wall, the hypothesis of a blood flow limitation during peritoneal dialysis remains. In this article, we have applied the technique that was presented in our previous work (2) to three representative visceral tissues: liver, stomach, and cecum of the rat. The results demonstrate that the stomach and cecum have transport characteristics similar to the abdominal wall: no blood flow limitation if blood flow is ≥30 to 40% of control. Blood flow through the liver, on the other hand, demonstrated significant limitation of the transport of urea. The possible impact of low blood flow on peritoneal solute transport is discussed.

Materials and Methods
Because all methods were thoroughly documented in the previous article (2), the following has been abbreviated.

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In all experiments, 200- to 300-g female Sprague Dawley rats (Charles River Labs, Wilmington, MA) were used. All animals were housed in standard rat cages at the University of Rochester vivarium and had ample access to water and standard rat chow. All procedures were reviewed and approved by the University of Rochester Committee on Animal Resources.

Surgery
Animals were anesthetized with an initial intramuscular loading dose of sodium pentobarbital (60 mg/kg) and maintained with subsequent intravenous injections. Upon loss of the blink reflex, the animal was placed in the supine position, a tracheostomy was performed, and catheters were placed into both femoral arteries and one femoral vein for blood sampling, pressure monitoring, and infusion of fluids and drugs, respectively. A midline laparotomy was performed to gain access to the target tissues. The blood supply to each target tissue was carefully isolated in preparation for the reduced flow portion of the experiment, and 4-0 silk sutures were placed around the appropriate vessels. For the stomach, the left gastric artery was isolated from the other branches of the celiac trunk. The occlusion of this supply vessel resulted in reduction of blood flow to both the pyloric and cardiac regions of the stomach, thereby reducing the effect of the location of the chamber on the stomach as a variable in local blood flow. For the cecum, a suture was placed around the ileocolic artery near its anastomosis with the superior mesenteric artery. This allowed uniform blood flow reduction to the entire surface of the cecum. For the liver, clamping of the portal vein or hepatic arteries was found to be ineffective in the reduction of local blood flow. Therefore, a loop of 4-0 silk suture was placed around the proximal region of the left lobe. After occlusion, the entire area of the lobe distal to the tie would experience blood flow reduction. The lobe of the liver that had been tied off by a loop of suture was the only tissue noted to visibly deteriorate in appearance by the end of the 75-min period of reduced
blood flow. Unfortunately, it was not possible to control the degree of blood flow reduction to investigate whether more moderate decreases in perfusion would affect the rate of transport without visible deterioration of the tissue. No attempt was made to ascertain the degree of damage in this tissue. It is presumed that the process of cellular necrosis had begun due to the decrease in local blood flow.

The tissue to be tested was cleared of any interfering visceral tissue, and the body of the animal was repositioned to provide the largest accessible area for chamber attachment. Care was taken to avoid any damage to the mesothelium or stretching of the tissue. After gently padding the tissue dry with gauze pads, the diffusion chamber was affixed to the desired tissue with a thin layer of cyanoacrylate glue and held in place for 3 to 5 min until secure. The surrounding tissue surfaces were covered with gauze pads saturated with warmed Krebs-Ringer-bicarbonate solution.

The temperature of the animal was continuously monitored with a rectal probe and maintained within the range of 37 ± 1.5°C, using a Servo-controlled warming blanket (Harvard Apparatus, South Natick, MA) and overhead heating lamps. The exposed tissue was covered with gauze pads saturated with isotonic saline solution and warmed to 39°C to maintain physiological conditions. The tissue under the chamber was assumed to be stable and to follow the core body temperature. However, we cannot rule out some temperature differential in the tissue during the postmortem period when there was no tissue perfusion. According to the Nernst-Einstein equation (3), the diffusion coefficient is proportional to the absolute temperature, T, and therefore a temperature differential of 12°C (37 to 25°C) would translate into a maximal possible change of 4% in the rate of transport (310/298 = 1.04).

Materials

14C urea was purchased from Moravek Biochemicals (Brea, CA) and certified to be 99% pure. The activity was determined by liquid scintillation counting (Beckman LS60001C, Fullerton, CA). The chamber tracer solution was comprised of approximately 1 μCi of 14C urea in 1 ml of Krebs-Ringer-bicarbonate solution. The solution osmolality was adjusted to 290 ± 5 mosmol/kg with the addition of NaCl and filtered through a 0.45-μm membrane (Nalgene, Rochester, NY). The solution was stored at 4°C. Before use, the pH was adjusted to 7.4 and the solution was warmed to 39°C.

The diffusion chambers were constructed from 15-ml polystyrene centrifuge tubes (Dow Corning, Corning, NY). Extensive testing on the effects of the chamber and glue on the tissue indicated an absence of any alterations to the tissue isolated by the chamber or to local tissue blood perfusion (2).

Measurements

Blood flow was monitored with the Perimed Periflux PF3 laser Doppler flowmeter (Perimed, Stockholm, Sweden). This device returns a signal that does not provide an absolute measurement of local blood flow or distinguish between small and large vessels. However, extensive testing has demonstrated that the perfusion readings within a single tissue preparation are linearly proportional to local blood flow (4). In manipulating blood flow, we assume that the perfusion through the microvessels actively engaged in the peritoneal–blood transport process was directly proportional to the total blood flow in the tissue.

Experimental Protocol

This study of blood flow limitations on small solute transport through peritoneal tissues consisted of three phases for each of the tissues studied: a period of control blood flow, a period of reduced blood flow (typically ≤50% of control), and a postmortem period in which blood flow is zero. During each phase, measurements of blood perfusion, chamber volume, and tracer concentration in the plasma and chamber solution were made.

Perfusion was measured by placing the laser Doppler probe perpendicular to the surface of the tissue, directly over the mesothelium isolated by the diffusion chamber (see Figure 1 of reference 2). Blood perfusion determinations in the tissue underlying the chamber were made every 15 min.

To initiate the control phase, the chamber fluid containing approximately 1 μCi of 14C urea was carefully injected into the chamber. Chamber fluid samples were taken in 15-min intervals, beginning with an initial sample at time zero. At each interval, the chamber fluid was mixed by pulling the solution into a syringe and needle and then injecting it back into the chamber. Then, a 5-μl sample was taken using Microcaps (Drummond, Bromwall, PA). The chamber fluid was removed and weighed at the end of each 75-min phase to account for volume loss. Additionally, a plasma sample was taken at this time to determine the plasma concentration of tracer.

Despite the mixing at 15-min intervals, there is the possibility that stagnant boundary layers existed at the bottom of the chamber. More vigorous mixing would likely have resulted in the removal of mesothelial cells by shear forces. From our previous work with the transfer of mannitol from diffusion chambers into peritoneal tissue, we have estimated that the maximal thickness of such a stagnant layer would amount to 12% of the total resistance to transport (5). The relative magnitude of this added resistance would not interfere with the detection of a limitation due to blood flow.

At the end of the control phase of each experiment, the chamber fluid was removed for volume measurement, and the silk sutures, previously placed around the blood supply for the tissue, were used to occlude flow through the vessel and to reduce the local blood supply. After a period of 15 min to allow for initial transients in perfusion measurements to decay, the experiment was repeated as described in the control blood flow phase. After the 75-min reduced blood flow phase, a condition of zero blood flow condition was established by giving the animal an overdose of anesthetic and observing the BP to be zero for at least 15 min before repeating the mass transfer measurements.

During control and blood flow reduction periods, the systolic BP remained ≥100 mmHg. The reduction of blood flow to each of the studied tissues did not produce any adverse effects on the animal as a whole.

Calculations

The mass transfer coefficient (MTC) was calculated for each phase of the experiment by fitting the following first-order differential equation (program: Scientist, Micro-Math, Salt Lake City, UT) to the data gathered for tracer mass within the chamber:

\[
\frac{dM}{dt} = \frac{\text{Mass transferred}}{\text{Duration of experiment}} = \frac{MTC\times A_{\text{chamber}}}{C_{\text{chamber}} - C_{\text{plasma}}}
\]

where \(A_{\text{chamber}}\) is the surface area isolated by the chamber, and \(C\) is the tracer concentration. The tracer mass was determined by the product of the measured quantities: \(C_{\text{chamber}}\) times the chamber fluid volume (corrected for sampling). \(C_{\text{plasma}}\) was always less than 1% of \(C_{\text{chamber}}\) due to the small area of transport in relation to the size of the animal and was set equal to zero in the analysis. The initial 15 min of each experimental phase was considered a period of unsteady-state transport and not used in the evaluation of MTC.
Statistical Analyses

A two-way ANOVA was performed on the MTC for the effects of tissue type and of blood flow. In addition, paired t tests of the MTC were performed between the control and reduced flow, control and zero flow, and reduced flow and zero flow phases. t tests assuming unequal variance were also performed to compare the perfusion readings for the control and reduced blood flow phases of each tissue and determined that the mean perfusion readings for each phase were significantly different from the others. A difference was considered significant if P ≤ 0.05. All statistical analyses were performed with either Microsoft Excel (t tests) or Number Cruncher Statistical Systems (Kaysville, UT) (ANOVA).

Results

Figures 1 through 3 display the chamber urea mass data for the three tissues studied. For the cecum (Figure 1) and the stomach (Figure 2), there are insignificant differences between the slopes (rate of mass transfer) of the urea mass curves versus time for the first two periods. In contrast, the liver (Figure 3) displayed a significant decrease in the slope of the mass curves during the period in which blood perfusion is reduced to 27% of control. All tissues demonstrated a marked decrease in the rate of urea mass loss during the postmortem period.

Table 1 lists the mean MTC (mean ± SEM), calculated for each period studied and for each tissue. Data from our previous work on the abdominal wall (2) are included for comparison. The MTC clearly demonstrate that the rate of transport of urea is not limited by blood flow (≥40% of control values) in the abdominal wall and in the two hollow viscera. However, transport across the surface of the liver appears to be significantly decreased with a 73% reduction in blood flow. For the data listed in Table 1, the two-way ANOVA demonstrated a clear dependence of the MTC on blood flow but not on the specific tissue. t tests demonstrated significant differences between control periods and postmortem periods and between periods of reduced blood flow and postmortem. The only significant decrease in the rate of transfer occurred in the postmortem period (asterisk).
difference between the control and reduced blood flow periods was in the liver.

**Discussion**

Theoretical models of peritoneal cavity–blood transport currently depend on the assumption that, under normal physiologic conditions, blood flow does not limit the transfer of solutes (6). The assumption has been based on the clearance of gas from the peritoneal cavity, which is equated to the functional or effective blood flow available for transport and which exceeds rates of solute transfer. The effective blood flow available for transport will be only a fraction of the total blood flow through the tissues surrounding the peritoneal cavity, because most of the exchange capillaries are too far from the cavity to be active in the exchange process (6,7) or they are contained in tissues not in contact with the solution in the cavity. Clearance of various gases from the peritoneal cavity of animals (7–10) and humans (11) has provided estimates of peritoneal blood flow of 2 to 7% of the cardiac output during dialysis. In a typical human, the minimal effective peritoneal blood flow would be approximately 100 ml/min (2). This is at least two to three times the estimated maximum peritoneal urea clearance of 30 to 40 ml/min (12). This excess capacity to deliver solute to the peritoneal exchange vessels is supported by dog studies, in which profound hemorrhagic shock causes peritoneal urea clearances to decrease only 10 to 25% (13).

Our studies address the question of blood flow limitations at the level of individual tissues, which has recently been posed by Ronco and colleagues (1). The pathways of small solute transport are the same whether solutes leave the blood exchange vessels in the tissue surrounding the peritoneal cavity and diffuse through the interstitium to enter the cavity or the solutes take the reverse path (7). We have also previously demonstrated that the transport rate of mannitol and the MTC are the same for either direction of transport (5). Therefore, we carried out these experiments in one direction only (from cavity to the blood) and assume that the results in the opposite direction would be the same. For blood flow reductions of ≤60 to 70%, our results indicate that there is no decrease in the rate of urea transport across the peritoneum of the abdominal wall and of the hollow viscera. Because the structure of the abdominal wall is similar to skeletal muscle, we can assume similar characteristics for transfer of solutes across the muscles of the retroperitoneum and of the diaphragm. In the rat, the parietal peritoneum and the peritoneum covering the hollow viscera make up 86% of the total dissected peritoneal area, with the remaining surface made up of liver (5). Therefore, it is unlikely that blood flow to tissues underlying nearly 90% of the anatomic peritoneum limits solute transport across the peritoneum.

The liver, in contrast to other visceral structures, demonstrated a significant change in the rate of urea transport between the peritoneum and the cavity with manipulation of blood flow. This result would be expected because of the structure of the hepatic sinusoids and previous data that have shown that transcapillary transport of not only small solutes (urea or glucose), but also serum albumin, is limited by blood flow (14). However, we also cannot rule out the possibility that ischemia and apparent cellular degeneration, which we observed in the liver, had direct effects on the transport process. Unfortunately, we were not able to control the degree of blood flow reduction to test this possibility. We observed that with a decrease of blood perfusion of 73%, the MTC decreases to a value of approximately 50% of the control value. As we have pointed out previously (2), for a flow-limited system:

\[ \text{MTC} = (Dq)^{0.5} \]

where \( D \) is the effective interstitial diffusivity, and \( q \) is the blood perfusion through the tissue. If we assume that \( D \) re-

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**Table 1.** Mass transfer coefficients (MTC): effect of blood flow

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Blood Flow Condition</th>
<th>( n )</th>
<th>Mean Perfusion Unit Ratio</th>
<th>SEM</th>
<th>Mean MTC (cm/min)</th>
<th>SEM</th>
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<tbody>
<tr>
<td>Cecum</td>
<td>Control</td>
<td>6</td>
<td>1</td>
<td>0.04</td>
<td>0.0046</td>
<td>0.003</td>
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<tr>
<td></td>
<td>Reduced flow</td>
<td>6</td>
<td>0.39</td>
<td>0.04</td>
<td>0.0040</td>
<td>0.003</td>
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<tr>
<td></td>
<td>Postmortem</td>
<td>6</td>
<td>0</td>
<td>0.00</td>
<td>0.0007</td>
<td>0.004</td>
</tr>
<tr>
<td>Stomach</td>
<td>Control</td>
<td>6</td>
<td>1</td>
<td>0.04</td>
<td>0.0040</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Reduced flow</td>
<td>6</td>
<td>0.29</td>
<td>0.02</td>
<td>0.0036</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Postmortem</td>
<td>6</td>
<td>0</td>
<td>0.00</td>
<td>0.0016</td>
<td>0.002</td>
</tr>
<tr>
<td>Liver</td>
<td>Control(^c)</td>
<td>6</td>
<td>1</td>
<td>0.00</td>
<td>0.0054</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Reduced flow(^c)</td>
<td>6</td>
<td>0.27</td>
<td>0.02</td>
<td>0.0026</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Postmortem</td>
<td>6</td>
<td>0</td>
<td>0.00</td>
<td>0.0011</td>
<td>0.002</td>
</tr>
<tr>
<td>Abdominal wall(^d)</td>
<td>Control</td>
<td>6</td>
<td>1</td>
<td>0.00</td>
<td>0.0037</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Reduced flow</td>
<td>6</td>
<td>0.28</td>
<td>0.03</td>
<td>0.0043</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Postmortem</td>
<td>3</td>
<td>0</td>
<td>0.00</td>
<td>0.0014</td>
<td>0.005</td>
</tr>
</tbody>
</table>

\(^a\) All postmortem periods demonstrated significant decreases in the rate of mass transfer when compared with either other period.

\(^b\) Perfusion Unit Ratio = \( \frac{\text{PU}_{\text{each period}} - \text{PU}_{\text{postmortem}}}{\text{PU}_{\text{control}} - \text{PU}_{\text{postmortem}}} \) (mean ± SEM for \( n \) animals).

\(^c\) Significant difference between control and reduced blood flow periods (paired \( t \) test; \( P < 0.001 \)).

\(^d\) Data from reference 2 added into table for comparison.
mains the same after the 73% reduction in flow, we would anticipate that $MTC$ for the reduced blood flow period would have a mean value of $(0.27)^{0.5} = 0.5 \times MTC_{control}$. This is in good agreement with the ratio of the $MTC_{reduced~flow}$ to the $MTC_{control}$, which is equal to 0.48 (from Table 1: $0.0026/0.0054 = 0.48$).

Will this flow limitation of transfer across peritoneum covering the liver affect overall transperitoneal transport during periods of low blood flow? Despite the fact that the dissected area of the liver makes up as much as 12 to 14% of total area of the peritoneum, the actual area exposed to fluid in the cavity is likely a much smaller quantity and makes up only a small fraction of the total "exposed" peritoneum (5). Zakaria and colleagues (15) have demonstrated that removing the liver from the transport process by sealing or by surgical excision does not affect the overall peritoneal mass transfer coefficient of small solutes. So, the transport limitation that is observed in the liver, whether caused by blood flow limitation, ischemic damage to the liver parenchyma, or some combination of these two, will likely not affect the overall small solute transfer during peritoneal dialysis.

Acknowledgments

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References