Blood Flow Limitation In Vivo of Small Solute Transfer during Peritoneal Dialysis in Rats

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Abstract. The aim of this study was to determine whether or to what extent transperitoneal flux of small solutes is reduced at low blood flows during peritoneal dialysis (PD) in rats. Peritoneal blood flow reductions were achieved by bleeding anesthetized (300 g) rats by 25% of their blood volume. After bleeding, a 2 h PD dwell was started using standard PD fluid. The permeability-surface area product (PS) for $^{51}$Cr-EDTA and glucose were assessed, as well as the transperitoneal clearance (Cl) of albumin. Control animals were not bled. After bleeding, peritoneal blood flow declined from 145 ± 17 perfusion units (PU) to 59 ± 12 PU ($P = 0.001$). Concomitant with this reduction, PS for $^{51}$Cr-EDTA fell from 0.284 ± 0.01 ml/min to 0.216 ± 0.01 ml/min ($P = 0.006$) and PS for glucose from 0.338 ± 0.02 ml/min to 0.294 ± 0.01 ml/min ($P = 0.046$). Mean arterial BP (MAP) dropped from 133 ± 4 mmHg to 61 ± 5 mmHg ($P = 0.008$). Cl of albumin fell largely in proportion to the estimated capillary hydrostatic pressure drop, i.e., from 6.1 ± 0.7 μl/min to 2.3 ± 0.3 μl/min ($P = 0.001$).

The results demonstrate that the transperitoneal clearances of small solutes are blood flow limited during PD, when peritoneal perfusion is markedly reduced. The level of flow limitation was, however, much lower than expected and observed in other tissues. Albumin transport, which is not blood flow limited, was reduced largely in proportion to the calculated capillary hydrostatic pressure decrease.

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Materials and Methods

General Surgery

Experiments were performed on 23 male Wistar rats (Møllegaard, Copenhagen, Denmark) having an average body weight of 334 ± 9 g.
The rats had free access to food and water until the day of experiment. Anesthesia was induced by an intraperitoneal injection of sodium pentobarbital (50 mg/kg of body weight) and was maintained through repeated intraarterial injections of the same drug. The rat was placed in a supine position on a heating pad, and the fur over the abdomen and the back was closely shaved. The tail artery was cannulated for continuous monitoring of BP on a polygraph (Grass Instruments Co., Quincy, MA) and for administration of anesthesia and tracer (125I-albumin). A tracheotomy was performed to facilitate breathing. The left carotid artery was cannulated for blood sampling, and the left jugular vein was cannulated for infusions of saline or 51Cr-EDTA.

**Blood Flow Reductions**

Blood flow reductions were achieved in nine rats by withdrawing approximately 25% of the total blood volume from the carotid artery. The procedure was done in two steps. First, two thirds of the intended volume was withdrawn, and the rats were then allowed to stabilize for a few minutes, after which the remainder was withdrawn. Blood flow was measured using a laser-Doppler flowmeter (Periflux 5000; Perimed, Stockholm, Sweden). A small hole was made in the skin laterally in the abdominal wall to gain access to the external surface of the abdominal muscle, which has a thickness of <1 mm in this area. The blood flow was measured as the local microcirculatory flow in this part of the parietal peritoneum, and we assumed that the blood flow reduction was largely proportional to that in the whole tissue. The probe (PF407–1; Perimed) was carefully placed in the hole and was fixed to avoid movements. This probe is able to register red blood cell velocity in the whole thickness (∼1 mm) of the tissue.

**Peritoneal Dialysis Protocol**

PD dwells were performed in control rats and in bled rats using 16 ml of 1.36% Dianeal (Baxter Health Care, Castlebar, Ireland). A catheter (Venflon Ø 1.7 mm; BOC Ohmeda, Helsingborg, Sweden) was inserted into the lower left quadrant of the abdominal wall for dialysis fluid infusion and sampling. A bolus dose of 51Cr-EDTA (0.09 MBq in 0.2 ml of saline; Amersham Life Science Ltd., UK) was given through the jugular vein catheter at the start of the dialysis dwell and then as a constant infusion of 3 ml/h (0.22 MBq/ml). The tracer appearance in dialysis fluid was measured and used to calculate the permeability-surface area product (PS or MTAC) of 51Cr-EDTA.

Albumin clearance (Cl) calculations were performed as described in previous publications, from the mass transfer of tracer (RISA) per unit time divided by the average plasma concentration of tracer, assessed from the area under the plasma tracer concentration curve (7–10). PS for 51Cr-EDTA and glucose were averaged from sequential measurements throughout the dwell, setting the sieving coefficient at 0.55 (11). Dialysate volume, as well as the albumin clearance from peritoneum to plasma (as a marker of direct lymphatic absorption), were assessed from separate experiments using RISA as a volume marker in control rats (n = 6) and bled rats (n = 3). This technique has been described previously (7,11). An average dialysate volume versus time curve was then used to calculate (Cl) and PS for 51Cr-EDTA as well as PS for glucose.

All data are expressed as means ± SEM. Statistics were obtained using the Mann-Whitney test or the Wilcoxon signed ranks test (for hematocrit change and MAP). All statistical calculations were made using SPSS for Macintosh release 10.0.7a (SPSS Inc. Chicago, IL).

**Results**

After bleeding, the peritoneal blood flow fell (P = 0.001) from 145 ± 17 arbitrary units (perfusion units, PU) during control to 59 ± 12 PU, i.e., by 59% (Figure 1) (n = 7). There was a significant drop in hematocrit after bleeding (P = 0.008) from 48 ± 0.4% to 37 ± 0.5% (n = 9). Concomitant with the blood flow reduction, there was a parallel drop in PS for 51Cr-EDTA (Figure 2) from 0.284 ± 0.01 ml/min (n = 8) to 0.216 ± 0.01 ml/min (n = 9; P = 0.006), and for glucose (Figure 3) from 0.338 ± 0.02 ml/min during control (n = 8) to 0.294 ± 0.01 ml/min (n = 9; P = 0.046). PS for 51Cr-EDTA was thus reduced by ∼24%, and PS for glucose by ∼13%.

Mean arterial BP (MAP) was reduced by 53% (P = 0.008) from 133 ± 4 mmHg to 61 ± 5 mmHg after bleeding (n = 9), resulting in a drop in capillary pressure, which can be estimated to at least equal or actually exceed the drop in MAP (Figure 4). The clearance of albumin fell largely in proportion to the estimated capillary hydrostatic pressure drop (P = 0.001), from 6.1 ± 0.7 μl/min (n = 8) to 2.2 ± 0.3 μl/min (n = 6), i.e., by ∼60% (Figure 5). This is what could be expected for convective-driven transport. Furthermore, the clearance of albumin from dialysate to blood (as a marker of direct lymphatic absorption) increased in bled animals (P = 0.036), from 6.1 ± 1.2 μl/min (n = 6) to 12.8 ± 0.8 μl/min (n = 3).

![Figure 1. Microcirculatory blood flow measured by laser-Doppler flowmetry. After bleeding rats by 25% of their blood volume, blood flow was decreased by 59% (P = 0.001). Blood flow was measured in arbitrary units (PU) in the lateral side of the peritoneal wall after making a small hole in the skin. Black bar represents blood flow during control; gray bar represents blood flow after bleeding (n = 7).](image-url)
Discussion

The present results clearly demonstrate the presence of blood flow limitation of transperitoneal small solute transfer when blood flow was reduced during PD in rats. The level of flow limitation was, however, much less than expected, judging from what has been found in other organs. Conceivably, this is due to the transport modification of the total blood-peritoneal transport offered by the peritoneal interstitium, which is coupled in series with the capillary wall. Blood flow limitation of transcapillary small solute diffusion in whole organs is a well-studied phenomenon (1). At very low blood flows, i.e., for PS/Q ratios < 1 (Q symbolizes flow), backdiffusion of tracer from the tissue during a single capillary transit will mainly account for the phenomenon. At higher blood flows (PS/Q > 2), heterogeneity in microvascular design, i.e., the fact that individual capillaries vary with respect to area and permeability, as well as in perfusion, will be the dominant causative factor. Even at maximal flows, there will always be some capillary blood flow limitation for a small solute due to the heterogeneity factor (1). Most interestingly, however, is the fact that increases in blood flow per se seems to be able to augment the capillary small solute transfer capacity (PS), even in individual capillaries (5).

The capillary wall is highly permeable to small diffusible solutes. Small solute transport across the peritoneal capillary wall is, like in most organs, highly blood flow limited; furthermore, the transport may decrease even further in low flow conditions.

Figure 2. Permeability-surface area coefficient (PS) for $^{51}$Cr-EDTA in control (black bar, $n = 8$) and bled rats (gray bar, $n = 9$). PS for $^{51}$Cr-EDTA was decreased by 24% ($P = 0.006$) after bleeding.

Figure 3. Permeability-surface area coefficient (PS) for glucose in control (black bar, $n = 8$) and bled rats (gray bar, $n = 9$). PS for glucose was decreased by 13% ($P = 0.046$) after bleeding.

Figure 4. Mean arterial blood pressure (MAP) during control condition (black bar) and after bleeding rats (gray bar). MAP was decreased by 53% after bleeding the rats by 25% of their total blood volume ($P = 0.008; n = 9$).

Figure 5. Clearance of albumin from plasma to peritoneal cavity for control rats (black bar, $n = 8$) and bled rats (gray bar, $n = 6$). The albumin clearance was reduced by 60%, in due proportion to the reduction in arterial pressure ($P = 0.001$).
conditions due to a reduced exchange surface area by derecruitment of capillaries. It is not possible to differentiate between derecruitment of capillaries and reductions in capillary blood flow (caused by the bleeding) with respect to the effects on small solute PS. Both factors will reduce small solute PS. Derecruitment of capillaries reduces the surface area available for small solute diffusion, while decreasing the capillary blood flow reduces the concentration gradient of solutes acting over the capillary wall due to rapid transcapillary equilibration of highly diffusive molecules. In the article by Haraldsson and Rippe (3), a maximally vasodilated tissue was investigated, and the capillary surface area could hence be kept constant when tissue perfusion was markedly reduced. Thus, the effects of changing the flow per se could be investigated independently of surface area changes, demonstrating the flow heterogeneity effect. In an article by Paaske (12), on the other hand, the combined effects of flow heterogeneity and derecruitment of capillaries were assessed. Here the effect of reducing flow was much more pronounced than in the study of Haraldsson and Rippe (3).

In the present model, we assessed the transport across two barriers coupled in series: the peritoneal interstitium and the peritoneal capillary walls. The interstitium conceivably offers a significant resistance to small solute transport due to volume exclusion, tortuosity, and solute-matrix interactions (sieving), resulting in diffusion rates two (to three) orders of magnitude lower than through the corresponding thickness of water (13). This is of less importance for large solutes, because the main transport barrier will be in the capillary wall, but the interstitium may be an important transport barrier for small solutes.

In a simplified manner, the total peritoneal transport resistance \(1/PS_{\text{tot}}\) may be regarded as the sum of the resistance of the capillary wall \(1/PS_{\text{cap}}\) and that of the interstitium \(1/PS_{\text{int}}\) (9):

\[
\frac{1}{PS_{\text{tot}}} = \frac{1}{PS_{\text{cap}}} + \frac{1}{PS_{\text{int}}}
\]

This formula implies that the total PS will be mainly governed by the barrier that offers the highest resistance. In an attempt to investigate the effect of peritoneal blood flow on peritoneal transport, Waniewski et al. (14) derived a distributed mathematical model similar to that of Flessner of diffusive peritoneal transport. To account for the transiently elevated PS of small solutes frequently observed early during PD dwells, the authors increased the peritoneal blood flow in the model by a factor of six. The impact of such an elevated blood flow was, due to the serial arrangement of capillaries and interstitium, an increase in creatinine flux of just about 60%.

It is important to point out that even if the mass transfer coefficients for small solutes are decreased by blood flow reductions, the volume exchange during a PD dwell may still be adequate, or even higher than normal, under blood flow reduced conditions. This is because the glucose osmotic gradient will then dissipate at a slower rate and give a prolonged osmotic gradient over the dwell time (15). A further consequence of the improved ultrafiltration is that the overall plasma clearance of small solutes is much less affected than their PS by blood flow limitation.

The current work is obviously in agreement with most of the literature data on transcapillary small solute diffusion and blood flow in various organs (see chapter by Renkin [1]), e.g., the works by Haraldsson and Rippe (3), Kajimura and Michel (5), as well as Ronco et al. (4). Furthermore, in a study by Erbe et al. (16), in which dogs were subjected to hemorrhagic shock by a similar approach as the present study, the peritoneal clearance of urea decreased by 26% after bleeding. This is almost identical to the reduction in PS for \(^{51}\)Cr-EDTA found in the present study. These results are, however, at variance with the work by Kim et al. (6). The cause of this discrepancy may be manifold. Our results were obtained in a peritoneum that was exposed to PD, while Kim et al. measured small solute disappearance from a small area of the peritoneum after gluing a chamber to the inner side of the abdominal wall. Hence, a midline laparotomy was necessary in their experiments to access the peritoneal membrane. Opening and exposure of the peritoneal cavity always involves a risk of dehydrating or perturbing the tissue. Dehydration of the peritoneal tissue and cells may give rise to changes in the tissue and may change the transport characteristics across the peritoneal membrane. This would have its largest impact in the no flow condition, because there is no blood supply replacing some of the evaporated interstitial fluid and shrunken cells. It is conceivable that such tissue changes may have partly curtailed the blood flow-related transport characteristics. Furthermore, the model described by Kim et al. may be rather insensitive to changes in blood flow, due to the fact that the tissue partly acts as a sink vis-a-vis small solutes. The tracer disappearance technique was used in another article by Demissachew et al. (17), with similar results as the one by Kim et al.

Peripheral vasoconstriction due to increased sympathetic activity during bleeding will induce blood flow reductions in muscle, particularly in the splanchnic area. We cannot differentiate between the blood flow reduction in the parietal muscle, where the blood flow was assessed in our model, and that in the visceral organs. It is reasonable, however, to assume that the reduction in gastrointestinal blood flow was at least as large as that assessed in the parietal peritoneum. On the other hand, as has been shown previously by Zakaria et al. (11), the major site for small-solute mass transfer (60 to 70%) in stationary rats is the parietal peritoneum, based on evisceration experiments. Despite a 60% reduction of the peritoneal surface area after removing most of the visceral organs, PS for small solutes was reduced by a mere 10 to 30% (18–20). Thus, the parietal peritoneal microvascular blood flow is, after all, the most relevant parameter determining the peritoneal small solute exchange under the present conditions.

The blood flow reductions of this study would probably have been even higher if we had measured the perfusion in the parietal wall after shock without ongoing PD. This is because the PD fluid seems to act as a vasodilator (21); furthermore, it may also be a potential source for fluid replacement. The effect of the anesthesia used is a moderate suppression of the sympathetic vasoconstrictor response. In an unanesthetized
animal, we would probably have seen a stronger peritoneal flow reduction, but the effect observed in our anesthetized animals was still surprisingly large.

There was a seemingly parallel drop in MAP and in albumin transcapillary transport. Albumin has a capillary extraction fraction (E) of less than 1% (22) and can, on theoretical grounds, not be blood flow-limited (1). The transport is almost completely dependent on the transport hindrance offered by the capillary wall. If there is a hydraulic pressure-dependent transport, then transport will be dependent on the level of capillary pressure. The relative capillary pressure drop in our experiments could be calculated to at least equal or exceed the percentage of the MAP drop. The present study may thus provide further evidence for albumin being convected through water-filled channels, i.e., through large pores. Part of the reduction in clearance may also be ascribed to derecruitment of capillaries, even though after bleeding (and sympathetic activation), the capillary filtration coefficient (LpS) is usually not reduced, but rather increased (23,24).

A quite unexpected finding in the present study was the fact that the clearance of albumin from dialysate to plasma (“direct lymphatic absorption”) increased significantly during bleeding. In a previous study, awake sheep were bled by 25% of their blood volume, and this resulted in a doubling of the intrinsic pumping of isolated mesenteric lymphatics (25). It is speculated that this might contribute to recruit fluid and protein to the vascular compartment during hemorrhagic shock.

The major result from the present study was that there was a significant blood flow limitation of small solute transfer during PD in intact rats when blood flow was decreased. The level of blood flow limitation was, however, much lower than that observed in other tissues under similar conditions. This may be due to the transport modification offered by the interstitium in the peritoneum, which curtailed the large blood flow limitation theoretically expected at the capillary level. Furthermore, the transperitoneal clearance of albumin decreased largely in proportion to the estimated capillary hydrostatic pressure reduction.

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