Plasma Appearance Rate of Intraperitoneal Macromolecular Tracer Underestimates Peritoneal Lymph Flow

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The magnitude of peritoneal lymph flow is an issue of great controversy in peritoneal dialysis (PD) research. Because no single lymphatic duct drains the entire peritoneal cavity, peritoneal lymph flow is indirectly measured as lymphatic removal of intraperitoneal macromolecular tracer. In rats, the peritoneal clearance (K) of such a tracer is 5 times the approximately 8 μL/min determined from the tracer appearance rate in blood (Cl). The fractional contribution of tissues bordering the peritoneal cavity to the overall Cl was determined to be diaphragm, 55%; viscera, 30%; and abdominal wall, 15%. The present study determines whether direct measurement of visceral peritoneal lymph flow matches the 30% (approximately 2.5 μL/min) contribution of the visceral peritoneal lymph flow as measured indirectly by the Cl method.

The mesenteric lymph duct that exclusively drains lymph from the gut, liver, and mesentery was cannulated in 15 rats, and lymph flow from the duct was collected at hourly intervals up to 6 hours under near-normal physiologic conditions and under conditions of simulated PD. Changes in mesenteric lymph flow that resulted from a challenge with 3 mL intravenous saline were captured using real-time video.

*We observed no significant differences between the hourly lymph volumes collected over 6 hours in naïve animals (*n = *5,* p > *0.05). Under conditions of simulated PD with dialysis fluid in the peritoneal cavity, the mesenteric duct lymph flow averaged 8.67 ± 1.41 μL/min (*n = *10). That flow is similar to reported data on total peritoneal Cl in rats; and 4 times the 2.5 μL/min visceral peritoneal contribution to the total peritoneal Cl. The intravenous saline challenge significantly increased mesenteric lymph duct output to 30.9 ± 1.6 μL/min (*n = *5,* p < *0.01) and reduced the lymph-to-plasma concentration ratio (L/P) by 43%. The reflection coefficient for total proteins (σ_{prot}) across the intestinal capillaries as calculated from the filtration rate–dependent L/P ratio when the transcapillary fluid escape rate and the mesenteric lymph flow were both high was more than 0.87.*

We concluded that (A) under near-normal physiologic conditions, the mesenteric lymph duct flow is steady, but quite low; (B) under conditions of simulated PD, the mesenteric lymph duct flow increases significantly from the physiologic norm; (C) mesenteric lymph duct flow is sensitive to the peritoneal fill volume; (D) during simulated PD, the fractional visceral peritoneal lymph flow measured indirectly from plasma appearance of intraperitoneal tracer underestimates the directly measured mesenteric duct lymph flow; and (E) the increased transcapillary fluid escape rate is rapidly buffered by augmentation of mesenteric lymph duct output.

Key words
Peritoneal lymph flow, capillary filtration, mesenteric duct, interstitium

Introduction

Direct cannulation of the relevant lymphatics draining the peritoneal cavity is the most sensitive and specific method to provide accurate measurements of peritoneal lymph flow. This method is not relevant in humans, and it has proven very difficult in small experimental animals, given that no single lymphatic duct drains the entire cavity. In large animals, such as sheep, the thoracic duct has typically been cannulated to provide estimates of peritoneal lymph flow

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after corrections for the greater fraction of the peritoneal lymph flow that is accounted for by the right lymphatic duct (1–4). However, peritoneal uptake of ^{125}I–fibrinogen and ^{51}Cr–erythrocyte in rats indicated that only about 30% of these tracers pass through the thoracic duct (5).

Collectively, the foregoing data suggest that the peritoneal lymphatic system is quite complex and that a minor fraction of the peritoneal lymph flow passes through the thoracic duct, which drains nearly the whole body's lymph (6,7). More relevant is the understanding that 50% – 70% of the peritoneal lymph flow passes through the right lymphatic duct that predominantly drains the diaphragm and the parietal tissues bordering the peritoneal cavity (8). Because of this complexity, direct measurement of the total peritoneal lymph flow is not currently possible. However, it can be estimated clinically and experimentally from the kinetics of an intraperitoneally administered macromolecular tracer such as radioactive iodinated serum albumin (RISA) or dextran 70. When these macromolecular tracers are used, their appearance rate in the blood averages only 20% of their disappearance rate from the peritoneal cavity (9–12). Because of the discrepancy in the kinetics of the intraperitoneal macromolecular tracer, controversy exists today whether to consider the disappearance rate or the blood appearance rate as an appropriate estimate of the magnitude of peritoneal lymph flow (13,14).

In a previous study, we compared the clearance out of the peritoneal cavity and the simultaneous plasma appearance rate of an intraperitoneally administered RISA in intact rats and in rats after exclusion of various parts of the peritoneal membrane surfaces (12). Data from these studies indicated that the disappearance clearance is 5 times the tracer appearance rate in blood (12). In addition, the fractional contribution of tissues bordering the peritoneal cavity to the tracer appearance rate in blood was determined to be diaphragm, 55%; viscera, 30%; and abdominal wall, 15%. Other investigators subsequently reached similar results (15).

Recently, we developed a technique to cannulate the mesenteric lymph duct that drains lymph exclusively from the liver, mesentery, and gut. In the present study, we used that technique to determine if direct measurement of visceral peritoneal lymph flow from the mesenteric lymph duct would match the 30% (approximately 2.5 μL/min) contribution of the visceral peritoneal lymph flow as measured indirectly by the appearance rate method (12).

Materials and methods

Animal preparation

Male Sprague–Dawley rats (weight: 200 – 215 g; Harlan, IN, U.S.A.) were used for this study. Animals were maintained in a facility approved by the American Association for the Accreditation of Laboratory Animal Care. Animals were acclimated for 2 weeks before the experimental use and received a standard rat chow (15 g daily) and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Care and Use Committee and Biohazard Safety Committee at the VA Medical Center, Louisville, KY. Anesthesia was induced with intraperitoneal pentobarbital (50 mg/kg), and supplemented hourly by subcutaneous injections equal to 25% of the initial dose to maintain a surgical plane of anesthesia throughout the experimental protocol. Before surgical preparation, each animal received 2 mL of normal saline subcutaneously to compensate for body fluid loss during surgical preparation and equilibration periods. Body temperature was maintained throughout the experiment at 37 ± 0.5°C using a rectal probe and a servo-controlled heating pad. Surgery was performed after loss of blink and withdrawal reflexes. Tracheostomy was performed to reduce airway resistance and the animals were allowed to breathe spontaneously. Catheters (PE-50) were inserted into the right femoral vein and the right carotid artery to allow for continuous monitoring and recording of blood pressure on a pressure measurement system (Digi-Med, Louisville, KY, U.S.A.).

Mesenteric duct cannulation and lymph collection

A midline laparotomy was performed on the abdominal wall to access the peritoneal cavity. The gut was pushed posteriomedially to visualize the base of the mesentery. The mesenteric lymph duct was located superior and parallel to the superior mesenteric artery. Under a dissection microscope, the mesenteric lymph duct was aseptically cannulated with Silastic tubing that exited the right flank to drain lymph by gravity into a collection tube (Figure 1). Lymph travels quickly along the Silastic cannula to reach the collection tube within approximately 2 min. At this point, the Silastic tube was secured with superglue.

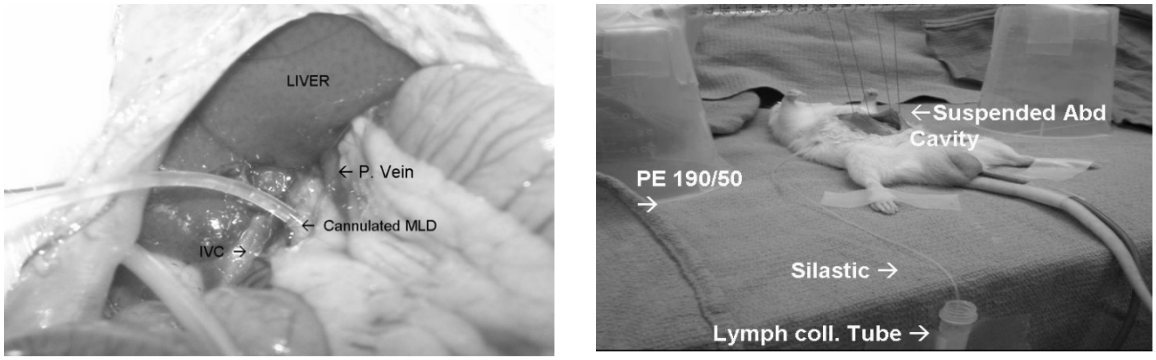

FIGURE 1 Technique of mesenteric lymph duct cannulation in rats. P. vein = portal vein; MLD = mesenteric lymph duct; IVC = inferior vena cava; Abd = abdominal; coll. = collection.

Experimental protocol

In the first experimental protocol, mesenteric lymph output from five naïve rats without intraperitoneal solution was collected hourly for 6 hours, and the mesenteric duct flow was calculated assuming that 1 g lymph was 1 mL in volume. In the second experimental protocol, peritoneal dialysis (PD) was simulated by intraperitoneal instillation of a PD solution ($n = 10$). To start the experiment, 20 mL of pre-warmed clinical PD solution (Delflex: Fresenius U.S.A., Ogden, UT, U.S.A.; containing 5.67 g/L sodium chloride, 3.92 g/L sodium lactate, 0.257 g/L calcium chloride, and 0.152 g/L magnesium chloride at a pH of 5 – 6 and an osmolality of 398 mOsm/L) was instilled into the peritoneal cavity. Lymph collection was continued at hourly intervals for another 4 hours. In 5 experiments, a new lymph collection vial was placed after 4 hours, and 3 mL of normal saline was injected intravenously over approximately 2 minutes. The change in mesenteric lymph flow as a result of this saline challenge was captured using real-time video. Collected lymph samples at the hourly intervals and after the saline challenge were analyzed for total proteins. Estimates of the reflection coefficient for total proteins (σ_{rot}) across the visceral peritoneal capillaries were provided from the filtration rate–dependent lymph-to-plasma (L/P) protein ratio when the transcapillary fluid escape rate and the mesenteric lymph flow were both high.

Statistics and data analysis

Data are presented as mean ± standard error of the mean unless otherwise stated. Differences in means between time points were statistically analyzed using the Welch unpaired *t*-test. Statistics were considered significant at $p < 0.05$.

Results

All animals remained hemodynamically stable during the experimental protocol (Figure 2, upper and middle panels). As shown in the bottom panel of Figure 2, the mesenteric lymph-duct flow under nearly normal physiologic conditions in naïve animals without intraperitoneal solution was steady, but quite low. We observed no significant differences between the hourly lymph flow rates collected from the mesenteric lymph duct over 6 hours. Although the mesenteric lymph duct flow during the first hour differed numerically from the subsequent flows at the 5 hourly intervals, differences between the means were not statistically significant ($n = 5$, $t = 1.49$, $p > 0.05$).

The data in Figure 3 depict the pooled mesenteric duct lymph flows in animals that underwent simulated PD with 20 mL of Delflex solution ($n = 10$) and in animals that underwent intravenous saline challenge with 3 mL saline. As seen in the figure, the mesenteric duct lymph flow under simulated PD averaged 8.67 ± 1.41 μL/min ($n = 10$), which is a significant increase from the near normal physiologic lymph flow in naïve animals ($p < 0.05$). The intravenous saline challenge in PD-simulated rats significantly increased mesenteric lymph duct output to 30.9 ± 1.6μL/min ($n = 5$, $p < 0.01$), and decreased the L/P concentration ratio by 43%.

The σ_{prot} across the visceral peritoneal capillaries as calculated from the filtration rate–dependent L/P ratio when the transcapillary fluid escape rate and the

mesenteric lymph flow were both high was greater than 0.87.

Discussion

The salient findings of the present study are that (A) the mesenteric lymph duct output under conditions of open abdomen and no fluid in the peritoneal cavity yields very low lymph flow from the liver, gut, and mesentery; (B) under conditions of simulated PD, mesenteric lymph duct output increases markedly to surpass the total peritoneal lymph flow as measured indirectly from the plasma appearance rate of intraperitoneal macromolecular tracer; (C) rapid hydration of the interstitia of tissues bordering the peritoneal cavity is associated with a marked increase in the mesenteric lymph duct output; and (D) the reflection coefficient of total proteins across the visceral capillaries is close to unity.

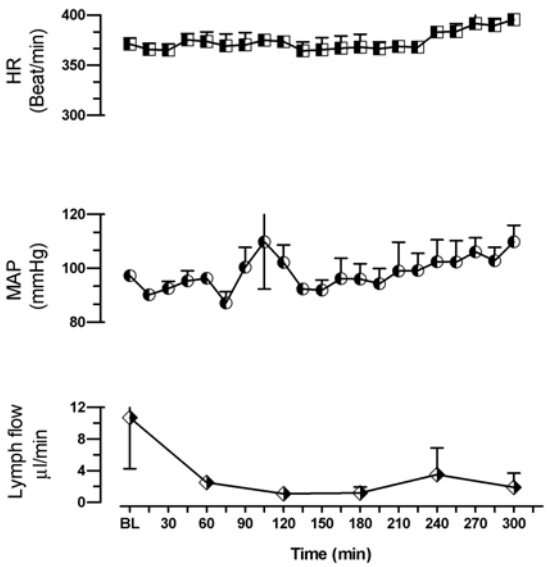

FIGURE 2 Hemodynamics and mesenteric lymph duct flow in naïve rats (n = 5). HR = heart rate; MAP = mean arterial pressure.

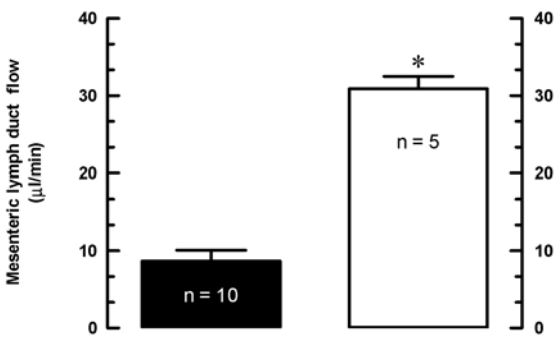

FIGURE 3 Average mesenteric lymph flow data (solid bar) from simulated peritoneal dialysis rats (n = 10) and mesenteric lymph flow after saline challenge (open bar, n = 5, $p < 0.01$) by Welch unpaired t-test.

As indicated in the present study, the mesenteric lymph duct output was low under relatively normal physiologic conditions, but it increased markedly with simulated PD. These data suggest that the mesenteric lymph duct output is sensitive to the presence of fluid in the peritoneal cavity (16). In a previous study, a fill volume equivalent to 10% of a sheep's body weight caused a marked increase in lymph flow from the cannulated caudal mediastinal node efferent lymphatics (17). The perturbations of intraperitoneal or interstitial hydrostatic pressures that result from opening the abdomen and from fill volume are unlikely to explain the incremental mesenteric lymph duct output in the PD-simulated animals, because the pressure perturbations under conditions of simulated PD in the present study are modest (18,19). During PD in rats, the fractional contribution of the visceral peritoneal lymph flow as determined from the plasma appearance rate of an intraperitoneally administered macromolecular tracer is approximately of the order 2.5 µL/min. That flow was measured indirectly after *exclusion* of peritoneal surfaces either by sealing or by combined sealing and evisceration (12).

As shown in the present study, the directly measured mesenteric lymph duct output was 4 times that of the fractional contribution of the visceral peritoneal lymph flow as determined by the *exclusion* method (12). This discrepancy does not preclude the validity of measurements of peritoneal lymph flow by indirect methods. Instead, it acknowledges fundamental differences between the two techniques in the dynamics of the interstitium, which is the source of lymph. In the exclusion experiments, the interstitium was either not present because of evisceration, or its fluid and protein (including exogenous tracer) kinetics were altered by sealing. The direct cannulation technique does not

provide absolute measurements of peritoneal lymph flow, because no single lymphatic duct exclusively drains the entire peritoneal cavity. Therefore, estimates of peritoneal lymph flow can currently be determined only indirectly.

A controversy exists as to whether the disappearance rate of an intraperitoneal macromolecular tracer (14,20–23) or its appearance rate in blood (5,10,12,24–27), represents peritoneal lymph flow. The rate of disappearance is at least 4 times that of its appearance in blood. This discrepancy is attributed to the typical non steady-state conditions prevailing during acute experiments. Under these conditions, the disappearance of the intraperitoneal macromolecular tracer occurs partially and relatively faster by convection directly into the diaphragmatic lymphatics, and partially by slow hydrostatic-driven convection over several days into the peritoneal tissue interstitium, particularly that of the abdominal wall (28). Thus, after several days, the peritoneal macromolecular tracer influx to the interstitium and its outflux through the scarce parietal interstitial lymphatics will reach a steady state (29,30). Based on this reasoning, it is likely that, under steady-state conditions, either the peritoneal disappearance or the blood appearance provides a reasonable estimate of the magnitude of peritoneal lymph flow. This reasoning can also be valid when the inherent assumptions between the two clearance methods and likely apparent uncoupling between fluid and protein kinetics within the interstitium are appreciated. This validity is important, because, as shown by our present study, rapid hydration of the interstitium triggers simultaneous augmentation of lymph output to offset interstitial expansion.

Conclusions

Under nearly normal physiologic conditions, flow through the mesenteric lymph duct is steady, but quite low. Under conditions of simulated PD, that flow increases significantly from the physiologic norm. Mesenteric lymph duct flow is sensitive to the peritoneal fill volume. During simulated PD, the fractional visceral peritoneal lymph flow measured indirectly from the plasma appearance of intraperitoneal tracer underestimates the directly measured mesenteric duct lymph flow. The increased transcapillary fluid escape rate is rapidly buffered by augmentation of mesenteric lymph duct output.

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References

1 Abernethy NJ, Chin W, Hay JB, Rodela H, Oreopoulos D, Johnston MG. Lymphatic drainage of the peritoneal cavity in sheep. Am J Physiol 1991;260:F353–8.
2 Tran L, Rodela H, Hay JB, Oreopoulos D, Johnston MG. Quantitation of lymphatic drainage of the peritoneal cavity in sheep: comparison of direct cannulation techniques with indirect methods to estimate lymph flow. Perit Dial Int 1993;13:270–9.
3 Tran L, Rodela H, Abernethy NJ, *et al.* Lymphatic drainage of hypertonic solution from peritoneal cavity of anesthetized and conscious sheep. J Appl Physiol 1993;74:859–67.
4 Yuan Z, Rodela H, Hay JB, Oreopoulos D, Johnston MG. Lymph flow and lymphatic drainage of inflammatory cells from the peritoneal cavity in a casein-peritonitis model in sheep. Lymphology 1994;27:114–28.
5 Flessner MF, Parker RJ, Sieber SM. Peritoneal lymphatic uptake of fibrinogen and erythrocytes in the rat. Am J Physiol 1983;244:H89–96.
6 Barrowman JA. Physiology of the gastro-intestinal lymphatic system. Cambridge, U.K.: Cambridge University Press; 1978.
7 Barrowman JA, Granger DN. Lymphatic drainage of the peritoneal cavity. In: Bengmark S, ed. Peritoneum and peritoneal access. London, U.K.: Wright; 1989: 85–93.
8 Yoffey JM, Courtice FC. Lymphatics, lymph, and the lymphomyeloid complex. London, U.K.: Academic Press; 1970.
9 Daugirdas JT, Ing TS, Gandhi VC, Hano JE, Chen WT, Yuan L. Kinetics of peritoneal fluid absorption in patients with chronic renal failure. J Lab Clin Med 1980;85:351–61.
10 Rippe B, Stelin G, Ahlmen J. Lymph flow from the peritoneal cavity in CAPD patients. In: Maher JF, Winchester JF, eds. Frontiers in peritoneal dialysis. New York: Field, Rich and Associates; 1986: 24–30.
11 Zakaria ER, Rippe B. Intraperitoneal fluid volume changes during peritoneal dialysis in the rat: indicator dilution vs. volumetric measurements. Blood Purif

1995;13:255–70.
12 Zakaria ER, Simonsen O, Rippe A, Rippe B. Transport of tracer albumin from peritoneum to plasma: role of diaphragmatic, visceral, and parietal lymphatics. Am J Physiol 1996;270:H1549–56.
13 Flessner MF. Effective lymphatic absorption rate is not a useful or accurate term to use in the physiology of peritoneal dialysis. Perit Dial Int 2004;24:313–16.
14 Krediet RT. The effective lymphatic absorption rate is an accurate and useful concept in the physiology of peritoneal dialysis. Perit Dial Int 2004;24:309–12.
15 Kumano K, Go K, He M, Sakai T. Role of diaphragmatic, visceral, and parietal pathways in peritoneal fluid absorption in rat peritoneal dialysis. Perit Dial Int 1996;16(Suppl 1):S80–3.
16 Zhu Q, Carlsson O, Rippe B. Clearance of tracer albumin from peritoneal cavity to plasma at low intraperitoneal volumes and hydrostatic pressures. Perit Dial Int 1998;18:497–504.
17 Drake RE, Gabel JC. Abdominal lymph flow response to intraperitoneal fluid in awake sheep. Lymphology 1991;24:77–81.
18 Zakaria ER, Lofthouse J, Flessner MF. *In vivo* hydraulic conductivity of muscle: effects of hydrostatic pressure. Am J Physiol 1997;273:H2774–82.
19 Zakaria ER, Lofthouse J, Flessner MF. *In vivo* effects of hydrostatic pressure on interstitium of abdominal wall muscle. Am J Physiol 1999;276(Pt 2):H517–29.
20 Khanna R, Mactier R. Role of lymphatics in peritoneal dialysis. Blood Purif 1992;10:163–72.
21 Krediet RT, Struijk DG, Koomen GC, Hoek FJ, Arisz L. The disappearance of macromolecules from the peritoneal cavity during continuous ambulatory peritoneal dialysis (CAPD) is not dependent on molecular size. Perit Dial Int 1990;10:147–52.
22 Mactier R, Khanna R, Twardowski ZJ, Nolph KD. Estimation of lymphatic absorption and intraperitoneal volume during hypertonic peritoneal dialysis. ASAIO Trans 1988;34:82–3.
23 Mactier RA, Khanna R, Twardowski Z, Nolph KD. Role of peritoneal cavity lymphatic absorption in peritoneal dialysis. Kidney Int 1987;32:165–74.
24 Flessner MF, Dedrick RL, Rippe B. Estimation of lymphatic absorption and intraperitoneal volume during hypertonic peritoneal dialysis. ASAIO Trans 1989;35:178–81.
25 Heimbürger O, Waniewski J, Werynski A, Park MS, Lindholm B. Lymphatic absorption in CAPD patients with loss of ultrafiltration capacity. Blood Purif 1995;13:327–39.
26 Rippe B, Zakaria ER. Lymphatic versus nonlymphatic fluid absorption from the peritoneal cavity as related to the peritoneal ultrafiltration capacity and sieving properties. Blood Purif 1992;10:189–202.
27 Zakaria ER, Rippe B. Peritoneal fluid and tracer albumin kinetics in the rat. Effects of increases in intraperitoneal hydrostatic pressure. Perit Dial Int 1995;15:118–28.
28 Spencer PC, Farrell PC. Continuous ambulatory peritoneal dialysis. Edinburgh: Churchill Livingstone; 1986.
29 Flessner MF, Dedrick RL, Reynolds JC. Bidirectional peritoneal transport of immunoglobulin in rats: compartmental kinetics. Am J Physiol 1992;262:F275–87.
30 Flessner MF, Dedrick RL, Reynolds JC. Bidirectional peritoneal transport of immunoglobulin in rats: tissue concentration profiles. Am J Physiol 1992;263:F15–23.
31 Zakaria ER, Mays CJ, Matheson PJ, Hurt RT, Garrison RN. Plasma appearance rate of intraperitoneal macromolecular tracer underestimates peritoneal lymph flow (Abstract). Perit Dial Int 2008;28(Suppl 1):S7.

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