ABDOMINAL LYMPH FLOW RESPONSE TO INTRAPERITONEAL FLUID IN AWAKE SHEEP

R.E. Drake, J.C. Gabel

Center for Microvascular and Lymphatic Studies and Department of Anesthesiology, The University of Texas Medical School, Houston, Texas, USA

ABSTRACT

Lymphatic vessels are important in draining excess fluid from the abdominal space and preventing ascites. In sheep, diaphragmatic lymph vessels draining the abdominal space run to the caudal mediastinal lymph node and efferent vessels from the node drain into veins in the neck. To estimate the lymph flow response to excess intraperitoneal fluid in sheep, we cannulated a caudal mediastinal node efferent lymphatic in 5 sheep. After the sheep recovered from the surgery, the lymph flow (Q_L) was 154±161 (SD) μl/min and the lymph protein concentration (C_L) was 3.7±9g/dl. Lymph flow decreased linearly with increases in lymphatic outflow pressure > 6cmH$_2$O. From this linear Q_L vs. outflow pressure relationship, we estimated the effective pressure driving lymph flow as the outflow pressure at which Q_L=0. At baseline, the driving pressure was 24.7±14.0 cmH$_2$O. After we infused Ringers solution (10% body weight) into the abdominal space, Q_L increased significantly to 7.0±4.1 times baseline and C_L decreased significantly to 0.7±0.6g/dl. Although the abdominal pressure increased significantly from 10.6±2.8 cmH$_2$O to 15.8±2.1 cmH$_2$O, we found no increase in lymphatic driving pressure.

Lymph vessels are important for maintaining normal fluid balance within the abdominal space (1-3). Abdominal fluid enters diaphragmatic lymph vessels through stoma on the abdominal surface of the diaphragm (1) and, in the sheep, many of the diaphragmatic vessels drain to the large caudal mediastinal lymph node in the chest cavity (4). Efferent lymphatics from the node then carry the fluid to veins within the neck. Although the abdominal lymph flow rate is normally low, it may increase substantially to remove excess fluid from the abdominal space. Thus, lymphatic vessels serve as an important safety mechanism against ascites.

Several factors may determine the rate of lymphatic flow. First, according to Zink and Greenway (5), abdominal lymph flow is directly proportional to intra-abdominal pressure. Thus, increases in abdominal fluid pressure might be one means by which abdominal lymph flow is increased. Second, lymph may be passively pumped due to compression of the lymphatic vessels by motion of the surrounding tissues. Passive pumping could explain the observation that there is little flow from diaphragmatic lymph vessels unless the diaphragm is moving (4,6). Third, some investigators believe that fluid is actively pumped through the lymphatics by rhythmic contraction of the lymphatic vessel smooth muscle (7,8). The abdominal fluid pressure and the pressure generated by active and passive pumping must force fluid through the resistance of the lymphatic vessels and nodes and against the pressure in the

neck veins.

In this study, we measured the lymphatic flow response to excess intraperitoneal fluid in awake sheep. We measured the lymph flow rate at several different outflow pressures and we used these data to estimate the effective pressure driving lymph flow. As expected, lymph flow increased when we infused fluid into the abdominal space, but we were surprised to find no increase in the effective pressure driving lymph flow.

METHODS

Five female sheep (28-38Kg) were anesthetized with halothane and ventilated with O_2. We placed catheters into a femoral artery and vein and we placed another catheter into the abdominal space via a small incision in the right abdominal wall. Next we injected 25mg of indocyanine-green dye into the abdominal catheter and massaged the abdomen to cause the dye to spread within the abdominal space and fill the lymphatic vessels. Then we opened the right chest at the 6th rib. We located a dye-filled caudal mediastinal node efferent lymphatic vessel and cannulated it with 0.025 inch inside diameter silastic tubing. The lymphatic cannula was tunneled through the chest wall and secured to the side of the sheep. We used standard surgical techniques to close the chest. Our technique to cannulate the lymphatic was essentially the same as the technique described at Staub et al (9), except we did not resect the tail of the node and we did not inject dye directly into the node.

We allowed all sheep to recover from the surgery for 1-2 days before the experiments. During the experiments, the sheep were awake and standing in their cages with free access to food and water.

Lymph flow vs. outflow pressure

We have previously described our technique in detail (10). We placed an extension of 1.2mm ID polyethylene tubing onto the lymphatic cannula. The outflow end of the extension was connected to a graduated pipette and the lymph flow was determined by timing the flow down the pipette. First, we measured the lymph flow rate (Q_L) with the pipette fixed ~20cm below the olecranon. Then we raised the pipette in 4-10cm steps and measured Q_L at each pipette height. At each height, we estimated the lymphatic vessel outflow pressure (Po) as the height plus the product of Q_L and the cannula resistance. Q_L was plotted vs. Po and a regression line was estimated. From the regression line, we estimated the effective pressure driving lymph flow (P_L) as the $Q_L = 0$ intercept.

The experiments

First we determined the baseline Q_L vs. Po relationship. Then we infused 2.8-3.8L (10% body wt.) warmed Ringers solution into the abdominal space. The infusion required approximately 40 min. We remeasured the Q_L vs. Po relationships 60, 140 and 300 min after the start of the infusions.

To determine the time course of the lymphatic response to the abdominal infusions, we measured the lymph flow rate each 10-20 min. For these measurements, we held the pipette level with the olecranon (zero height). We also used a refractometer to estimate the protein concentration of each lymph sample and the protein concentration of plasma samples. Plasma (blood) samples were taken from the femoral artery catheter each hour.

In 4 of the sheep, we determined the abdominal fluid pressure. We filled the abdominal catheter with Ringers solution and raised the open end of the catheter above the sheep. We recorded the abdominal pressure as the height of the Ringers solution within the catheter. The abdominal pressure was measured at baseline and 60 and 300 min after the start of the infusions.

We used the olecranon as the zero reference level for all pressures because we have used it as the reference level in our previous studies with awake sheep (10,11). The olecranon (elbow) is easy to

see on all sheep and, in a standing sheep, it is near the level of the atria.

At the end of the experiments, we euthanized the sheep with an overdose of thiopental sodium. We opened the right chest and removed the tip of the cannula from the lymphatic vessel. We allowed the tip of the cannula to rest on the mediastinal tissue near the lymphatic. Then we connected the external end of the cannula to a lymph-filled pipette and measured the flow rate as we held the pipette at several different heights above the sheep. The cannula resistance was calculated from the flow vs. height data as Δheight/Δflow. With this technique, we estimated the cannula resistance = 0.016 ± 0.003 cmH$_2$O•min/μl.

Statistics

Data are given as mean ± 1 SD in the text and mean ± SE in the figures. We used the method of least squares to determine the best fit lines to the lymph flow vs. pressure data. Two way analysis of variance was used to test for differences in data between sheep and between times. We accepted $p<0.05$ as indicating significant differences.

RESULTS

None of the sheep showed any sign of distress during the experiments. However, in each sheep, the abdomen became distended as we infused the Ringers solution. In one sheep, the Ringers solution began to leak through the abdominal wall around the abdominal catheter. We euthanized this sheep 140 min after the infusion.

The baseline lymph flow rate for all sheep=154±161μl/min. As shown in Fig. 1, the lymph flow rate increased and the lymph protein concentration decreased almost as soon as we began the infusions (both changes significant). The lymph flow remained elevated and the lymph protein remained low for the rest of the experiment.

The Q$_L$ vs. Po relationships at baseline and 140 min after the start of the infusions are shown in Fig. 2. The Q$_L$ vs.
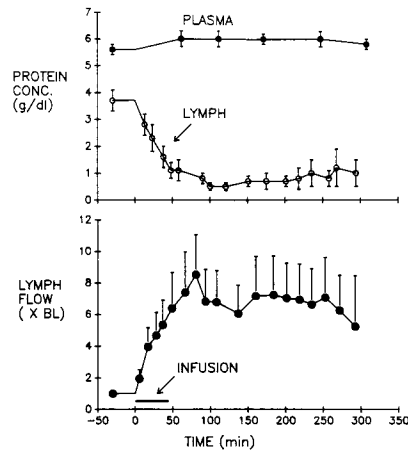

Fig. 1. Lymph flow rate (times baseline) and lymph and plasma protein concentrations.
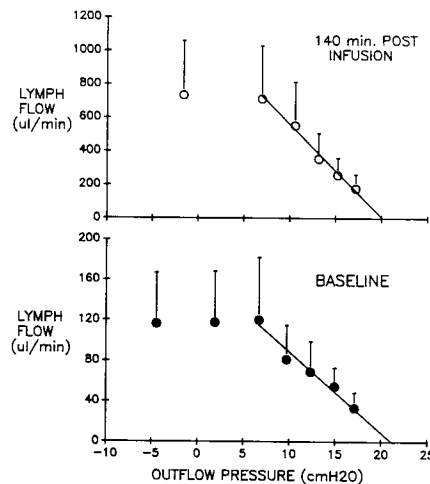

Fig. 2. Lymph flow rate (Q$_L$) vs. outflow pressure (Po) at baseline and 140 min after the start of the infusions.

Po relationships at 60 and 300 min were similar to the relationship at 140 min. Q$_L$ did not decrease significantly until we increased Po>6 cmH$_2$O. For Po>6 cmH$_2$O, Q$_L$ decreased linearly with increases in Po. As shown in Fig. 3, the abdominal pressure increased significantly but the lymphatic driving pressure did not increase after the intra-abdominal infusions.
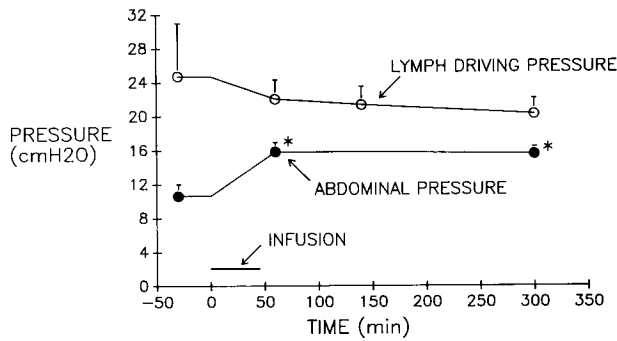
*Fig. 3. Effective lymphatic driving pressure (P_L) and abdominal pressure.
Indicates data significantly different from baseline.

DISCUSSION

Our results are important for several reasons. First, we believe this is the only report of the abdominal lymph flow response to excess intraperitoneal fluid in unanesthetized, spontaneously breathing animals. Anesthetic agents may depress active lymphatic pumping (7). Furthermore, several studies have shown that contraction of the diaphragm muscle is important in causing diaphragm lymph flow (6,11,12). Thus we felt it was important to perform our experiments in awake, spontaneously breathing sheep.

As shown in *Fig. 1*, the lymph flow increased almost immediately after we began the intra-abdominal infusions and the flow reached 6-8 times the preinfusion flow rate. Although the caudal mediastinal node efferent vessels we cannulated carry lymph from several sources, the substantial decrease in lymph protein concentration indicates that the post-infusion flow was primarily from the protein-free Ringers solution we placed into the abdominal space.

After the infusions, the lymph flow rate was 0.50±0.27ml/min. Thus the lymphatic vessels we cannulated removed approximately 150ml (0.5ml/min x 300 min) or ~5% of the infused volume in 5 hrs. However, this is an underestimate of the total volume removed through all the lymphatics which drained the abdominal space. Also, an even larger volume may

have been absorbed by the venous capillaries. Nevertheless, judging from the elevated intra-abdominal pressure at 5 hrs and large volumes of intraperitoneal fluid post mortem, it is clear that the removal of excess intraperitoneal fluid in sheep is a slow process.

Another important finding was that the increased lymph flow rate was not associated with an increase in the effective pressure driving lymph flow. We expected that the driving pressure would increase because the intra-abdominal pressure increased. Zink and Greenway (5) found that, in anesthetized cats, the abdominal lymph flow depends directly on the intra-abdominal pressure. However, the effective driving pressure should depend on the pressure generated by active and passive lymphatic pumping as well as on the intra-abdominal pressure. Part of the passive pumping is probably due to motion of the diaphragm. Furthermore, diaphragmatic motion may have been restricted by the large volume of intraperitoneal fluid in our sheep. Thus it is possible that a decrease in the pressure generated by lymphatic pumping offset the increase in intra-abdominal pressure so that there was no increase in the effective driving pressure.

The lack of an increase in driving pressure is important because lymphatic vessels drain into veins. In many diseases, the venous pressure is elevated above

normal. Thus, to remove excess fluid from the abdominal space, the lymph must be pushed against an elevated pressure (13). Because the pressure driving abdominal lymph flow was relatively low after we infused fluid into the abdominal space (20-22 cmH_2O), the abdominal lymph drainage could be significantly slowed or stopped by the elevated venous pressures commonly found in humans with ascites (13). This can be seen in *Fig. 2*. According to the data in that figure, abdominal lymph flow would be slowed by 50% at an outflow pressure of only 13 cmH_2O.

Although diaphragmatic lymph vessels may be important in removing excess intraperitoneal fluid, they may not be necessary to maintain normal fluid volume in the peritoneal cavity. For instance, Raybuck and his associates (14) found that obliteration of the diaphragmatic lymphatics in rats did not lead to ascites. On the other hand, rats with lymphatic obliteration and portal hypertension had much more intraperitoneal fluid than rats with portal hypertension alone.

In conclusion, after intra-abdominal infusions of Ringers solution (10% body weight) caudal mediastinal lymph flow increased to 7 times baseline and the increased flow was sustained for 5 hrs. Although abdominal pressure increased, we found no increase in the effective pressure driving lymph flow.

REFERENCES

1. Granger, DN, JA Barrowman: Gastrointestinal and liver edema. In: *Edema*, Staub, NC, AE Taylor (Eds.), New York, Raven (1984), 615-656.
2. Witte, CL, MH Witte, AE Dumont: Lymph imbalance in the genesis and perpetuation of the ascites syndrome in hepatic cirrhosis. Gastroent. 78 (1980), 1059-1068.
3. Witte, CL, MH Witte, AE Dumont: Pathophysiology of chronic edema, lymphedema, and fibrosis. In: *Edema*, Staub, NC, AE Taylor (Eds.), New York, Raven (1984), 521-542.
4. Drake, RE, JC Gabel, T Adair, et al: Contamination of caudal mediastinal node efferent lymph in sheep. Am. J. Physiol. 241 (1981), H354-H357.
5. Zink, J, CV Greenway: Control of ascites absorption in anesthetized cats: Effects of intraperitoneal pressure, protein, and furosemide diuresis. Gastroent. 73 (1977), 1119-1124.
6. Courtice, CF, B Morris: The effect of diaphragmatic movement on the absorption of protein and of red cells from the pleural cavity. Austral. J. Exp. Biol. 31 (1953), 227-238.
7. Elias, RM, MG Johnston, A Hayashi, et al: Decreased lymphatic pumping after intravenous endotoxin administration in sheep. Am. J. Physiol. 253 (1987), H1349-H1357.
8. McHale, NG, IC Roddie: The effect of transmural pressure on pumping activity in isolated bovine lymphatic vessels. J. Physiol. 261 (1976), 255-269.
9. Staub, NC, RD Bland, KL Brigham, et al: Preparation of chronic lung lymph fistulas in sheep. J. Surg. Res. 19 (1975), 315-320.
10. Drake, R, M Giesler, G Laine, et al: Effect of outflow pressure on lung lymph flow in unanesthetized sheep. J. Appl. Physiol. 58 (1985), 70-76.
11. Drake, RE, SJ Allen, JC Gabel, et al: Effect of endotoxin on diaphragm lymph contamination in unanesthetized sheep. J. Appl. Physiol. 62 (1987), 706-710.
12. Morris, B: The effect of diaphragmatic movement on the absorption of protein and of red cells from the peritoneal cavity. Austral. J. Exp. Biol. 31 (1953), 239-246.
13. Witte, MH, AE Dumont, RH Clauss, et al: Lymph circulation in congestive heart failure. Effect of external thoracic duct drainage. Circulation 39 (1969), 723-733.
14. Raybuck, HE, T Weatherford, L Allen: Lymphatics in genesis of ascites in the rat. Am. J. Physiol. 198 (1960), 1207-1210.

Robert E. Drake, Ph.D.
The University of Texas Medical School
Department of Anesthesiology
6431 Fannin, MSB 5.020
Houston, Texas 77030