

THE EFFECT OF ANESTHESIA AND SURGERY ON DIAPHRAGMATIC LYMPH VESSEL FLOW AFTER ENDOTOXIN IN SHEEP

J.R. Elk, T. Adair, R.E. Drake, J.C. Gabel

Center for Microvascular and Lymphatic Studies and Department of Anesthesiology (JRE, RED, JCG), The University of Texas Medical School, Houston, Texas and Department of Physiology (TA), University of Mississippi Medical School, Jackson, Mississippi, USA

ABSTRACT

Increases in diaphragmatic lymph vessel flow (Q_{di}) may be important in preventing ascites because diaphragmatic lymph vessels drain the peritoneal space. However, lymphatic vessel function may be depressed in anesthetized, open chested animals. To test this hypothesis, we cannulated diaphragmatic lymph vessels in five sheep which were anesthetized with 1-2% halothane. We performed a thoracotomy and cannulated a diaphragmatic lymph vessel in each sheep. Then we infused 0.75-1.0 $\mu\text{g}/\text{kg}$ of E. coli endotoxin intravenously and we measured Q_{di} and the lymph protein concentration for 2-4 hrs. The data were compared to previously reported data for five unanesthetized sheep (J. Appl. Physiol. 62:706-710, 1987). At baseline $Q_{di}=0.8\pm 0.7$ (SD) in the anesthetized sheep and $1.0\pm 0.8\text{ml}/\text{hr}$ in the unanesthetized sheep. After endotoxin, Q_{di} increased to $4.5\pm 3.1\text{ml}/\text{hr}$ in the unanesthetized sheep ($p<0.05$) but Q_{di} did not change in the anesthetized sheep. However, the lymph protein concentration increased similarly in each group, indicating that endotoxin caused the same degree of injury in each group. Our results indicate that diaphragmatic lymph vessel function is depressed in anesthetized, open chested sheep.

Diaphragmatic lymph vessels are one of the main drainage pathways for the peritoneal space and these vessels are important in preventing or limiting ascites in several diseases (1-3). In unanesthetized sheep, flow through the diaphragmatic vessels is greatly increased after endotoxin administration (4). This response may be important in preventing fluid accumulation within the peritoneal space. However, for several reasons, diaphragmatic lymph vessel function may be suppressed during surgery in anesthetized animals. First, diaphragmatic lymph vessels contract rhythmically and this probably aids lymph flow (5). Some volatile anesthetic agents depress the spontaneous contractions of lymphatic vessels (6). Consequently, diaphragmatic lymph vessel flow could be suppressed in anesthetized animals. Second, diaphragmatic lymph vessel flow is facilitated by diaphragmatic movement. For instance, Morris found that diaphragmatic paralysis caused a reduction in the rate at which diaphragmatic lymph vessels removed red cells and protein from the peritoneal cavity in rats (7). Furthermore, Florey found that increases in intra-abdominal pressure may facilitate diaphragmatic lymph flow (5). Activity of the diaphragmatic and abdominal muscles is often depressed in anesthetized animals or patients (8) and this could slow lymph flow. Third, lymphatic function could be

altered by some of the numerous metabolic and humoral changes which occur during surgery. Fourth, during intrathoracic surgery, the normal negative pleural pressure is absent. Also, the flow through lymphatics running within the pleural cavity may be influenced by the changes in pleural pressure which occur with respiratory movements (9). Because the diaphragmatic lymph vessels run through the chest cavity, diaphragmatic lymph vessel flow could be altered when the chest is opened.

In this study, we measured the flow from diaphragmatic lymph vessels in anesthetized, open chested sheep following an infusion of *E. coli* endotoxin. We found that the lymph flow response was much less than we previously found in unanesthetized sheep. These findings support our hypothesis that diaphragmatic lymph vessel function is suppressed in anesthetized, open chested animals.

MATERIALS AND METHODS

We anesthetized five sheep initially with thiopental sodium (10mg/kg IV bolus). Following endotracheal intubation, mechanical ventilation was instituted. Anesthesia was maintained with halothane 1-2% in air supplemented with O₂. A balloon tipped catheter was inserted into a jugular vein and directed into the pulmonary artery in each sheep. We used this catheter to measure the pulmonary arterial pressure (zero reference level = right atrium). Next we made a left posterolateral incision into the sixth intercostal space. We resected ribs 7-9 in order to expose the left side of the diaphragm, and the lower left lung lobe. Approximately 0.1ml of Evans blue dye was injected below the diaphragm as previously described (8). In each sheep we found several diaphragmatic lymph vessels which filled with dye and drained into the caudal mediastinal lymph node. We cannulated diaphragmatic lymph vessels in each sheep and measured the lymph flow rates (Q_{di}) by timing the flow of lymph through calibrated pipettes. The outflow ends of the lymph cannulas were

placed approximately 5cm below the sites of cannulation. We used a refractometer to estimate the protein concentrations in samples of lymph and plasma (4,8).

We recorded baseline data for approximately 1 hr, then infused 0.75-1.0g/kg *E. coli* endotoxin (0127:B8, Difco) into the femoral venous catheters over a 30 min period. The lymph flows, protein concentrations and pulmonary artery pressures were recorded for 2-4 hrs after the endotoxin infusions.

The pulmonary arterial pressure response to endotoxin in unanesthetized sheep has been described by many investigators (4,10,11). We measured the pulmonary arterial pressure in this study in order to confirm that endotoxin caused a similar response in anesthetized and unanesthetized animals.

We compared the diaphragmatic lymph vessel data in this study with the data of a previous study from our laboratory (4). In the previous study, five sheep were anesthetized and managed in a similar fashion to the sheep of the present study. However, the cannulae in the diaphragmatic lymph vessels were exteriorized between ribs 11 and 12. The sheep were then allowed to recover. The day after surgery, the lymph flows, protein concentrations and pulmonary artery pressures were measured for 2-4 hrs after the infusion of *E. coli* endotoxin (0.5-1.0 μ g/kg) into the awake sheep.

We used two-way analysis of variance to test for differences in the anesthetized vs unanesthetized sheep data and to test for changes in the data with time. $P < 0.05$ was accepted as indicating significant differences in the data. Data are presented as mean \pm 1 SD in the text and mean \pm 1 SE in the figure.

RESULTS

The pulmonary arterial pressure response to endotoxin in the anesthetized animals was typical of the response in unanesthetized animals (4,10,11). The pressure increased from its baseline of 17.1 ± 3.0 mmHg to 40.0 ± 5.1 mmHg 30 min after the start of the endotoxin, then

decreased to 24.4 ± 11.1 mmHg over the next hour.

At baseline Q_{di} was 0.8 ± 0.7 ml/hr and the lymph to plasma protein concentration ratio (L/P) was 0.76 ± 0.09 in the anesthetized sheep. Q_{di} and L/P were 1.0 ± 0.8 ml/hr and 0.74 ± 0.15 , respectively at baseline in the unanesthetized sheep of the previous study (4). Fig. 1 (top) shows the Q_{di} response to endotoxin in anesthetized (closed squares) and unanesthetized (open circles) sheep. There was no change in Q_{di} for the anesthetized sheep but lymph flow increased 6.4 times baseline in the unanesthetized sheep. The difference in the lymph flow responses in the two groups of sheep was significant.

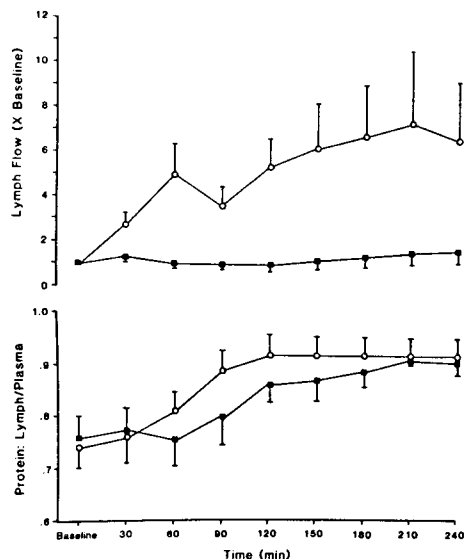


Fig. 1. Diaphragmatic lymph vessel flow and protein concentration for anesthetized (solid squares) and unanesthetized (open circles) sheep before (baseline) and after endotoxin infusion.

Fig. 1 (bottom) shows the L/P data after endotoxin for anesthetized (closed squares) and unanesthetized (open circles) sheep. Although L/P increased significantly in both groups of sheep, the response in the anesthetized sheep lagged behind the response in the unanesthetized sheep. We found no significant change in the plasma protein concentration during the experiments (baseline = 5.9 ± 1.5 g/dl, final = 5.8 ± 1.5 g/dl).

DISCUSSION

Comparison of lymph data in anesthetized and unanesthetized sheep

Peritoneal fluid is derived mostly from fluid which weeps from the surfaces of the abdominal organs or fluid which has filtered through the microvessels at the abdominal wall or peritoneum (1,2). The protein concentration of peritoneal fluid reflects the protein concentration of the microvascular filtrate (2). Toxic substances which injure the walls of microvessels often cause an increase in lymph flow and protein concentration because the injured microvessels allow fluid and protein to rapidly leak from the plasma (10-12). For instance, *E. coli* endotoxin causes an increase in lung microvascular permeability and an increase in lung lymph flow and protein transport (4,10). Thus, the increased L/P in our study may have resulted from endotoxin-induced injury to the peritoneal microcirculation. The similar increase in L/P for unanesthetized and anesthetized, acutely operated sheep suggests that the endotoxin caused similar injury in each group of sheep (Fig. 1). The increase in lymph flow in the unanesthetized sheep indicates that the lymphatics in those animals responded to the injury by removing excess fluid from the peritoneal space. On the other hand, the lack of a lymph flow increase in the anesthetized, open chested sheep suggests that the lymphatics were depressed in those sheep.

The delayed increase in L/P in the anesthetized sheep (relative to the unanesthetized sheep) may have been due to the slow lymph flow. Because Q_{di} was slower in the anesthetized vs unanesthetized sheep, it took longer for the lymph fluid to transverse the length of the lymph vessels and cannulas. We estimate that the diaphragmatic vessels were approximately 0.2 cm in diameter and 15 cm long (volume = 0.47 cm^3). The cannula volumes were approximately 0.09 cm^3 (20 cm of PE 60 tubing) so that the total volume from the entrance of the lymph vessels to the outflow end of the cannula was approxi-

mately 0.56cm^3 . After endotoxin administration, Q_{di} averaged 4.5ml/hr ($0.077\text{cm}^3/\text{min}$) in the unanesthetized sheep so we estimate it took 7 min ($0.56/0.077$) for lymph to travel through the vessels and cannulas. After endotoxin in the anesthetized sheep, Q_{di} was 0.8ml/hr ($0.013\text{cm}^3/\text{min}$) so the transient time should have been 43 min. The difference in these two times (36 min) is very close to the lag time between the L/P responses in the anesthetized vs unanesthetized sheep (Fig. 1).

Although we cannulated over 1-2 vessels per experiment, we estimate that each sheep had at least 10-15 diaphragmatic vessels similar to the ones we cannulated. If our unanesthetized sheep lymph flow data accurately reflect the flow in uncannulated vessels, then the total diaphragmatic lymph vessel flow increased by $35/50\text{ml/hr}$ after endotoxin. This increase in flow may serve as a safety mechanism to prevent the accumulation of at least $35\text{-}50\text{ml/hr}$ of fluid within the peritoneal space. Our results indicate that this diaphragmatic lymph vessel flow safety mechanism may be absent in anesthetized, open chested animals. Possible causes of absence of a diaphragmatic lymph vessel response to endotoxin include 1) suppression of the lymphatic pumping mechanism by the anesthetic (6), or a mediator released during surgery, 2) decreased diaphragmatic or abdominal muscle activity (5), and 3) the effect of the open chest on flow through lymphatic vessels within the chest (9).

ACKNOWLEDGEMENT

The authors thank Margaret Lee and Linda Larsen for their technical assistance, and Verna Jasso and Caroline Buggs-Warner for typing this manuscript.

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.), Raven, New York (1984), 521-542.
4. 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.
5. Florey, H: Reactions of, and absorption by, lymphatics with special reference to those of the diaphragm. *Br. J. Exp. Path.* 8 (1927), 479-491.
6. Elias, RM, MG Johnston, A Hayashi, et al: Decreased lymphatic pumping after intravenous endotoxin administration in sheep. *Am. J. Physiol.* 253 (1987), H1349-H1357.
7. 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.
8. Drake, RE, JC Gabel, T Adair, et al: Contamination of caudal mediastinal node efferent lymph in sheep. *Am. J. Physiol.* 241 (1981), H354-H357.
9. 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.
10. Brigham, KL, RE Bowers, J Haynes: Increased sheep lung vascular permeability caused by *Escherichia coli* endotoxin. *Circ. Res.* 45 (1979), 292-297.
11. Esbenshade, AM, JH Newman, PM Lams: Respiratory failure after endotoxin infusion in sheep: Lung mechanics and lung fluid balance. *J. Appl. Physiol.* 53 (1982), 967-976.
12. Garcia-Szabo, RR, DF Kern, R Bizios, et al: Comparison of alpha and gamma thrombin on lung fluid balance in anesthetized sheep. *J. Appl. Physiol.* 57 (1984), 1375-1383.

Robert E. Drake, Ph.D.
Professor, Department of Anesthesiology
The University of Texas Health
Sciences Center at Houston
6431 Fannin, 5.020 MSMB
Houston, TX 77030 USA