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SHEEP LUNG LYMPH SHUNTING

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ABSTRACT

The caudal mediastinal lymph node (CMN) has several efferent lymph vessels in most sheep. When investigators cannulate one of the CMN efferent vessels in order to collect lung lymph, it is possible that lymph may be shunted between the cannulated vessel and other vessels which drain from the CMN into the systemic veins. If shunting does occur then an increase in venous pressure could cause lymph to be shunted to the cannulated lymph vessel. This would increase the flow of lymph from the cannula and could falsely indicate that lung lymph flow had increased. To test this possibility we cannulated CMN efferent vessels in 7 sheep and then used a balloon to raise the pressure in the superior vena cava. Because uncannulated CMN efferent vessels ultimately drain into the superior vena cava, an increase in pressure may cause lymph to be shunted through the lymph cannula. We found, however, that lymph flow increased in only one of seven sheep and conclude that lymph shunting is uncommon when operative preparation includes meticulous ligation of collateral common efferent lymph vessels.

We have shown that the flow rate from cannulated lung lymph vessels in anesthetized dogs is extremely sensitive to the pressure at the outflow end of the vessels (1). Small increases in outflow pressure cause large decreases in lymph flow. We have used the lymph flow vs. outflow pressure relationships to estimate the resistance of the lymph vessels within the lung tissue.

We have also shown that the lung lymph from unanesthetized sheep is sensitive to outflow pressure (2). For that study, we used the preparation for collecting lung lymph after it passes through the large caudal mediastinal lymph node (CMN). With this preparation, the investigator cannulates an efferent vessel from the CMN, then ligates all other visible efferent vessels. However, it is usually difficult to locate all the CMN efferent vessels so that vessels draining the CMN in parallel with the cannulated vessel may not be ligated (3). In this case, increases in outflow pressure to the cannulated vessel could cause lymph flow to be shunted from the cannulated vessel to other collateral CMN efferent vessels (2,4). The "shunted" lymph would drain through the collateral lymph vessels, into the thoracic duct, and finally empty into the veins in the neck. If our lymph flow vs. outflow pressure relationships do represent in fact lymph shunting then some of our conclusions about intrapulmonary lymph vessel resistance in sheep would be invalid.

Lymph vessel shunting could also have affected the results of studies by other investigators who have used the sheep lung lymph preparation. Many investigators have tested the effects of various substances on lung fluid balance by measuring lung lymph flow before and after giving the sheep a dose of the substances. An increase in lymph flow is usually taken as evidence that the lung microvascular filtration rate has increased.

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However, if a test substance caused an increase in systemic venous pressure, then the flow of lymph in CMN efferent vessels entering the venous system (via the thoracic duct) might be shunted to the cannulated vessel. Thus shunting could lead investigators to the incorrect conclusion that lung lymph flow had increased. For example, E. coli endotoxin, a substance frequently studied with the sheep lymph preparation, does cause an increase in central venous pressure (5).

In order to test for lymph vessel shunting, we measured lung lymph flow from the CMN in sheep before and during a period of elevated central venous pressure. In most sheep we found no lymph vessel shunting.

MATERIALS AND METHODS

We prepared 7 sheep for the collection of CMN lymph as described by Staub et al (8). The sheep were anesthetized with thiopental, intubated and ventilated with 1-2% halothane in O_2. The right chest was opened at the 9th rib and the tail of the CMN was ligated and resected. We injected Evans blue dye into the node. Next we opened the right chest at the 6th rib, located a dye-containing CMN efferent vessel and cannulated it. We also ligated any other CMN efferent vessels we saw (usually 0-1). A 30cc Foley balloon catheter was placed into the right jugular vein and advanced into the superior vena cava. A second catheter was placed in the superior vena cava upstream of the balloon. During the experiments this catheter was connected to a pressure transducer so that we could monitor the pressure in the superior vena cava (the pressure at the outflow of uncannulated lymph vessels).

Four experiments were performed in acutely prepared, anesthetized sheep. In the other 3 sheep, we closed the chests and allowed the animals to recover for at least one day before we preformed experiments on the awake sheep. We used sterile surgical techniques on these 3 sheep.

The experiments

We placed the outflow end of the lymph cannulas approximately level with the right atrium and measured the lymph flow rate ($\dot{Q}_L$) by timing the flow of lymph into a pipette. Then we inflated the superior vena caval balloon to elevate the superior vena caval pressure to 19-32 cmH_2O. We maintained the elevated pressure for 1-5 min and measured $\dot{Q}_L$ during this time.

Statistics

All summary data is reported as mean ± SD. We used Student's t-test on the paired data and accepted $p < 0.05$ as indicating significant differences.

RESULTS

In 6 of the 7 experiments we found no increase in $\dot{Q}_L$ when we increased superior vena caval pressure. $\dot{Q}_L$ during elevated venous pressure (163 ± 77 μl/min) was not increased from the $\dot{Q}_L$ of 180 ± 85 μl/min we measured at baseline venous pressure. The baseline and increased venous pressures were 7.1 ± 5.6 and 26.4 ± 5.4 cmH_2O respectively for these 6 experiments.

In one experiment, however, there was clear evidence of lymph shunting. $\dot{Q}_L$ increased within seconds following a 10 cmH_2O increase in venous pressure and reached a steady state 4x its baseline level after approximately 4 min (*Fig. 1*). In this sheep, $\dot{Q}_L$ increased with each step elevation in venous pressure from 0 to 15 cmH_2O. Because $\dot{Q}_L$ was so sensitive to venous pressure in this sheep, an increase in venous pressure of 7.5 cmH_2O (such as we have measured in sheep after endotoxin administration (5)) would have caused a 3- to 5-fold increase in $\dot{Q}_L$.

DISCUSSION

It is possible that true lung lymph flow decreased when we increased superior vena caval pressure. The increased venous pressure could have caused pooling of blood in the peripheral veins and resulted in a reduced cardiac output. Theoretically this would have led to a reduction in lung microvascular filtration rate and, ultimately, a reduction in lung lymph flow. In this case, the rate of flow from the cannulated lymph vessel might have

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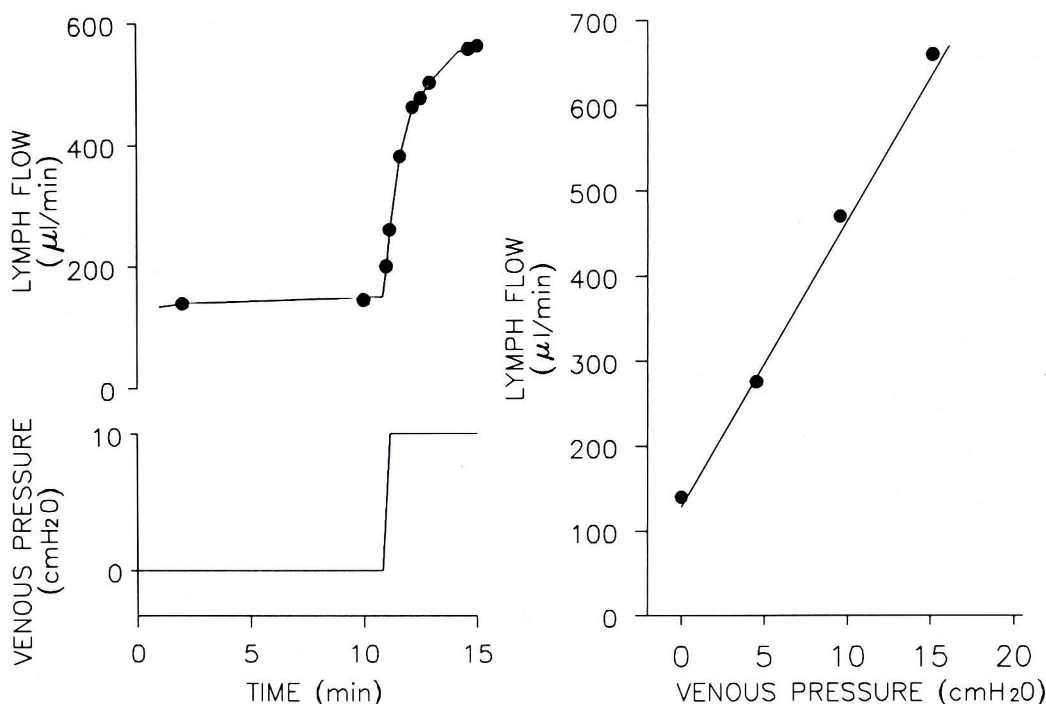

Fig. 1: (Left) Lymph flow rate from a cannulated CMN efferent vessel following an increase in pressure in the superior vena cava. The rapid rise in lymph flow is evidence of shunting of lymph from uncannulated lymph vessels to the cannulated vessel. (Right) Relationship between steady state lymph flow rate and pressure in the superior vena cava of the same sheep. The data of this figure were the exception because there was no lymph shunting in the other 6 sheep (see Results).

remained unchanged if the decrease in lung lymph flow matched the increase in lymph shunt flow. We attempted to avoid this problem in two ways. First, we raised the pressure only in the superior vena cava above the entrance of the vertebral and azygos veins. Thus there should have been much less pooling of blood than if we had elevated the pressure throughout the venous system. Second, we measured $\dot{Q}_L$ for only 1-5 min after the venous pressure elevation. If lung filtration rate did decrease after we elevated venous pressure, we expected that it would take several minutes for true lung lymph flow to decrease because there is a delay between a change in filtration rate and the corresponding change in lymph flow (6,7). Our results show that, when lymph shunting did occur, $\dot{Q}_L$ increased within seconds after the increase in venous pressure (*Fig. 1*).

Staub et al (8) also found no significant increase in $\dot{Q}_L$ when they elevated venous pressure in sheep. However, they elevated the pressure throughout the body and measured $\dot{Q}_L$ over a longer period of time. Thus their failure to find any increase in $\dot{Q}_L$ may have been due to a decrease in true lung lymph flow.

We found evidence for lymph vessel shunting in only one of seven sheep. This result indicates that our previous study of the $\dot{Q}_L$ vs outflow pressure relationship was not significantly influenced by shunting because we found that $\dot{Q}_L$ decreased with increases in outflow pressure in all sheep of the previous study.

The percent of sheep with lymph shunting probably varies from one laboratory to another depending on each investigator's success at ligating collateral CMN efferent

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vessels. In order to avoid shunting, we recommend that investigators adjust the height of the cannula so that the pressure at the outflow end of the cannulated lymph vessel equals the venous pressure. With equal pressure opposing the flow from cannulated and uncannulated vessels, there should be no shunting of lymph between the vessels (9).

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