Chronic lymph flow responses to hyperproteinemia

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Manning, R. Davis, J. R. Chronic lymph flow responses to hyperproteinemia. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R135–R140, 1998.—The long-term responses of lymph flow, lymph protein transport, and the permeability-surface area (PS) product to hyperproteinemia have been studied in conscious dogs. Plasma protein concentration (PPC) was increased by high intravenous infusion of a human plasma protein concentrate (PPC) was increased by daily intravenous infusion of previously collected autologous plasma for 9 days. Lymph flow was determined by collecting lymph from a lymphatic afferent to the popliteal node in the hind leg. Compared with the average value during the normal period, the following changes occurred during 10 days of high PPC: lymph flow decreased from 12.3 ± 1.1 to 3.8 ± 0.6 µl/min, lymph protein transport decreased from 241 ± 24 to 141 ± 21 µg/min, PS product decreased from 4.7 ± 0.5 to 3.0 ± 0.6 µl/min, PPC increased from 7.1 ± 0.1 to 8.8 ± 0.4 g/dl, lymph protein concentration increased from 1.9 ± 0.1 to 3.8 ± 0.1 g/dl, plasma colloid osmotic pressure increased from 18.6 ± 0.8 to 24.2 ± 2.1 mmHg, and lymph colloid osmotic pressure increased from 4.8 ± 0.2 to 10.4 ± 0.7 mmHg. In conclusion, long-term hyperproteinemia in dogs results in chronic decreases in lymph flow, lymph protein transport, and the PS product and chronic increases in lymph protein concentration and lymph colloid osmotic pressure. The marked decrease in lymph flow during hyperproteinemia decreased lymph protein transport and thus contributed to the increase in lymph protein concentration. In addition, the decreases in PS product and lymph protein transport suggest that transcapillary protein flux decreases during hyperproteinemia.

SEVERAL STUDIES HAVE INDICATED that the lymphatic system plays an important role in the control of extracellular fluid volume during hypertension (22, 23), after hemorrhage (12), and during hypoproteinemia (4, 10). Our previous studies showed that during moderate hypoproteinemia blood volume decreases very little (15), and extracellular fluid volume increases only mildly because of a decrease in interstitial protein concentration (1, 24). The decrease in interstitial protein concentration in this condition has been referred to as a "wash-down phenomenon" and is caused by an increase in lymph flow that returns interstitial protein to the circulation and thus depletes the interstitial protein. Therefore, increased lymph flow results in a decrease in interstitial protein concentration during hypoproteinemia and thus helps to prevent edema formation.

Although significant hyperproteinemia occurs in multiple myeloma, sarcoidosis, lymphogranuloma, liver diseases, parasitic conditions, and dehydration (3), no studies have determined the lymph flow responses to chronic increases in plasma protein concentration (PPC). A study in our laboratory has shown that prenodal lymph protein concentration increases markedly in dogs with chronic hyperproteinemia (14). However, lymph was collected acutely during anesthesia (14), which could have affected lymph protein concentration, and lymph flow was not determined. A previous acute study in cats showed that intravenous infusions of albumin cause marked increases in plasma colloidal osmotic pressure and large decreases in the lymph flow and the capillary filtration coefficient (CFC) of an intestinal segment (8). In this study, the net capillary filtration pressure was normal 2 h after the albumin infusion, but lymph flow and the CFC decreased 75% (8). However, whether lymph flow remains at subnormal levels during chronic increases in PPC is not known. The goal of this study was to test the hypothesis that lymph flow decreases chronically during hyperproteinemia, which will reduce lymph protein transport and thus allow lymph protein concentration to increase. Therefore, the responses of lymph flow, lymph protein transport, and the permeability-surface area (PS) product to chronic hyperproteinemia were studied in the long term in conscious dogs in which PPC was elevated by daily intravenous infusion of previously collected autologous plasma.

METHODS

Animal preparation and experimental protocol. Experiments were performed over a 23-day period on five conscious dogs with an average body weight of 31.2 ± 3.3 kg. The project had the approval of the local Institutional Animal Committee. All dogs were splenectomized and equipped with chronically arterial and venous catheters and a chronic catheter in a prenodal lymphatic afferent to the popliteal lymph node in the hind leg.

During the first surgical procedure, a splenectomy was performed through a midline abdominal incision and catheters were implanted in the aorta and inferior vena cava through the femoral artery and vein. Anesthesia was initiated with thiopental sodium (Pentothal, 25 mg/kg iv; Abbott Laboratories, North Chicago, IL) and maintained with a mixture of methoxyflurane (Penthrane, Abbott Laboratories) and oxygen. Appropriate gas concentrations were delivered to the dogs through an endotracheal tube connected to an Ohio Medical Products anesthesia machine (Kinetic-O-Meter). The catheters were tunneled subcutaneously and exited the back between the dogs' shoulders. A period of 10–14 days of recovery followed surgery, during which the dogs were trained to lie quietly in their cages. Water intake was ad libitum throughout the experiment. Sodium intake was maintained at ~75 meq/day during the control period (days 1-7) and the normal-PPC period (days 19-23) by feeding the dogs 894 g/day of K/D prescription diet dog food (Hills Pet Food) to which 45 meq of sodium chloride (9 ml of 5 M NaCl) was added. During the first 9 days of the high-PPC period (days 8-16), the same sodium intake was achieved by intravenous infusion of an average of 330 ml/day of previously collected autologous plasma.
plasma, which contained ~45 meq of sodium, and the dogs were fed 894 g/day of P/D prescription diet dog food (Hills Pet Food). On the last day of the high-PPC period (day 17), the dogs were fed 894 g of P/D dog food to which 45 meq of sodium was added, but no plasma was infused.

After the surgical recovery period, plasma was collected 5 days a week by plasmapheresis over a 3-wk period, and the plasma was stored at −20°C. This procedure has been previously described in detail (13, 14). After completion of the plasmapheresis period, a 17-day recovery period was afforded before the experiment was begun, which allowed the PPC of the dogs to return to normal levels. Then data were collected during a control period (days 1-7), a period of high PPC (days 8-16), a plasmapheresis day (day 18), and a normal-PPC period (days 19-23). The PPC of the dogs was elevated during the first 9 days of the high-protein period by infusing, by intravenous drip in 1 h, ~330 ml of previously collected autologous plasma. On day 16 during the high-protein period, the dogs were anesthetized as before, and a catheter was implanted in a prenodal lymphatic afferent to the popliteal node in the hind leg of the dog. Lymph flow was measured and lymph was collected four times the next day (day 17). In one of the dogs, the lymph initially contained red blood cells, and data obtained from lymph collection on this dog from this point in time were not used. Then, on day 18, plasmapheresis was performed to reduce PPC to normal, and after 24 h (day 19), lymph flow and lymph protein concentration were determined four times a day for the next 4 days. An additional plasmapheresis was performed on day 21 on two dogs because their PPC was slightly higher than their average value during the control period.

The lymphatic catheter was constructed with polyethylene (PE-50; Clay Adams) and Silastic (0.020 in. ID × 0.037 in. OD; Dow Corning) and was pretreated with the polymer coating material TDMAC heparin as described previously (21). The free end of the catheter was exteriorized in the inner aspect of the hind limb where it was connected to a collection vial. Next, the tip of the catheter was placed at the level of the cannulated lymphatic, and lymph from the prenodal lymphatic was collected continuously for 4 days. To prevent clotting of the lymphatic catheter, the dogs received a continuous intravenous heparin infusion (120 U·kg⁻¹·day⁻¹).

Experimental measurements and instrumentation. The dogs were housed in metabolic cages and were fitted with a backpack that held a Statham P23 AC or a P23 ID transducer at the level of the heart. The transducer wires were connected to a Grass model 7D recorder that was connected to a digital computer. Every minute throughout the day the computer sampled arterial pressure 500 times in a 3-s period, and the average was stored on a computer disk (17).

Plasma and lymph protein concentrations were measured with an American Optical refractometer, and plasma colloid osmotic pressure was determined with a colloid osmometer originally developed in this department by Prather et al. (18). During the high-PPC period, plasma protein and colloid osmotic pressures were measured before the daily plasma infusions. The PS product for the hind limb under study was calculated by an equation developed previously (2, 21).

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PS = PLF \times (L/P) [1 - (L/P)]
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where PLF is the peripheral lymph flow and L/P is the lymph-to-PPC ratio. In this calculation, the passage of protein across the microvasculature is assumed to occur only by diffusion, and any protein transport due to convection is neglected (2, 19, 21). Lymph protein transport was calculated by multiplying lymph protein concentration and lymph flow.

Statistical analysis was performed by first determining overall significance with analysis of variance for repeated measures. Second, significance on the individual experimental days was determined post hoc with Dunnett’s test for multiple comparisons with a control (5). The data were considered statistically significant if \( P < 0.05 \). All data are expressed as means ± SE.

RESULTS

Responses of PPC, plasma colloid osmotic pressure, mean arterial pressure and hematocrit to hyperproteinemia. PPC averaged 6.8 ± 0.3 g/dl during the control period and increased rapidly during the 10-day period of hyperproteinemia as shown in Fig. 1. By day 10, PPC had significantly increased and remained elevated throughout the remainder of the high-PPC period. By
day 17, PPC reached a value of 8.8 ± 0.4 g/dl, and PPC averaged 7.1 ± 0.1 g/dl during the 5-day normal-PPC period. Plasmapheresis was performed on day 18 and reduced the PPC back to values not significantly different from control.

Plasma colloid osmotic pressure also increased markedly during the high-PPC period. As seen in Fig. 1, plasma colloid osmotic pressure averaged 18.6 ± 0.8 mmHg during the normal-PPC period. During the high-PPC period, plasma colloid osmotic pressure increased to 24.2 ± 2.1 mmHg on day 17 (P < 0.05). Therefore, plasma colloid osmotic pressure increased 5.6 mmHg by day 17 compared with the average value during the normal-PPC period. During the normal-PPC period (days 19-23), the plasma colloid osmotic pressure was not significantly different from the average value during the control period (days 1-7). Also seen in Fig. 1, mean arterial pressure averaged 79 ± 3 mmHg during the control period and did not significantly change from this value throughout the experiment.

Hematocrit measured on day 17 (high PPC) was 27.3 ± 2.7 and on day 19 (normal PPC) was 27.8 ± 2.4 (P not significant). This provides evidence that blood volume did not change during hyperproteinenemia in the present experiment, which confirms our previous results (14).

Responses of lymph flow, lymph protein transport, and the PS product to hyperproteinenemia. Figure 2 shows that lymph flow averaged 3.8 ± 0.6 µl/min on day 17 in the high-PPC period and increased significantly to an average value of 12.3 ± 1.1 µl/min during the entire normal-PPC period. By day 19 when PPC had decreased to normal, the lymph flow increased to 10.6 ± 2.2 µl/min (P < 0.05) and was significantly increased throughout the remainder of the normal-PPC period. Lymph protein transport, also shown in Fig. 2, averaged 141 ± 21 µg/min on day 17 in the high-PPC period and 241 ± 24 µg/min (P < 0.05) during the entire normal-PPC period. Also, lymph protein transport significantly increased on day 20, and the value was 307 ± 63 µg/min.

Figure 2 also shows that the PS product averaged 3.0 ± 0.5 µl/min on day 17 in the high-PPC period. By day 20 in the normal-PPC period, the PS product increased to 6.0 ± 1.2 µl/min (P < 0.05), and the average value of PS during the entire normal-PPC period was 4.7 ± 0.5 µl/min, which was significantly increased compared with day 17 in the high-PPC period. The lymph-to-plasma concentration ratio was 0.44 ± 0.001 on day 17 during high PPC and averaged 0.273 ± 0.001 during the normal-PPC period (P < 0.05).

Responses of prenodal lymph protein concentration and prenodal lymph colloid osmotic pressure to hyperproteinenemia. As seen in Fig. 3, lymph protein concentration averaged 3.8 ± 0.1 g/dl on day 17 in the high-PPC period. Then plasmapheresis was performed the following day, and data for day 19 were taken the day after plasmapheresis. Plasmapheresis reduced the lymph protein concentration significantly, and on day 19 the prenodal lymph protein concentration averaged 1.9 ± 0.1 g/dl (P < 0.05 compared with day 17). The average lymph protein concentration for the entire normal-PPC period was 1.9 ± 0.1 g/dl (P < 0.05).

Figure 3 also shows that prenodal lymph colloid osmotic pressure increased markedly during the high-PPC period. Lymph colloid osmotic pressure averaged 10.4 ± 0.7 mmHg on day 17, and by day 19 the colloid osmotic pressure of the lymph had decreased to 4.7 ± 0.3 mmHg (P < 0.05) and remained significantly decreased throughout the remainder of the normal-PPC period. The average value of lymph colloid osmotic pressure during the normal-PPC period was 4.8 ± 0.2 mmHg (P < 0.05 compared with day 17). Therefore, lymph colloid osmotic pressure was 5.6 mmHg higher on day 17 than the average value during the normal-PPC period.

Relationship between lymph protein concentration and PPC. Figure 4 shows that the increased PPC was significantly associated with an increased lymph protein concentration (P < 0.05). This relationship was
plotted for dogs in this experiment with normal PPC and during hyperproteinemia caused by infusion of autologous plasma for 9 days and suggests that high PPC results in an increase in the concentration of protein in the peripheral lymphatics.

**Relationship between lymph colloid osmotic pressure and plasma colloid osmotic pressure.**

Figure 5 shows that increases in plasma colloid osmotic pressure during the period of high PPC were significantly associated with increases in lymph colloid osmotic pressure (P<0.01). The relationship was plotted for dogs in the experiment with normal PPC and during the high-PPC period caused by intravenous infusion of autologous plasma. The relationship suggests that increases in plasma colloid osmotic pressure result in increases in lymph colloid osmotic pressure.

**DISCUSSION**

This study demonstrated for the first time that increases in PPC in conscious dogs result in marked decreases in lymph flow, lymph protein transport, and the PS product. Daily intravenous infusion of previously collected autologous plasma resulted in several important changes. Compared with the average values during the normal-PPC period, the data on the last day of the high-PPC period (day 17) showed that PPC increased 1.7 g/dl to a value of 8.8 ± 0.4 g/dl, prenodal lymph flow decreased 69%, lymph protein transport decreased 42%, PS product decreased 36%, and lymph protein concentration more than doubled. Therefore, the interstitial-lymphatic system changed dramatically during chronic hyperproteinemia.

The decrease in lymph flow during hyperproteinemia may have played a major role in the increase in lymph protein concentration by removing less protein from the interstitial spaces. In fact, lymph protein transport on the last day of the high-PPC period (day 17) significantly decreased compared with the average lymph protein transport during the normal-PPC period. In further support of this hypothesis, previous studies have shown that changes in lymph flow are accompanied by inverse changes in lymph protein concentration. Increases in lymph flow during hyperproteinemia are accompanied by marked decreases in lymph protein concentration, thus “washing down the interstitium” (4, 10). On the other hand, severe decreases or cessation of lymph flow in lymphedema causes large increases in interstitial protein concentration (6).

Because we have previously shown that hyperproteinemia, produced in dogs by the same techniques used in this study, caused only a small increase in interstitial fluid volume (13, 14), the decrease in lymph flow in the present study must have been accompanied by an
approximately equal decrease in transcapillary fluid flux. However, the cause of this decreased fluid flux is not known. Starling (20) originally showed that the transcapillary flux of fluid is proportional to the CFC multiplied by the balance of hydraulic and colloid osmotic pressures of the capillary and interstitium while assuming no changes in crystalloid osmotic pressure across the capillary. In the absence of changes in the CFC, an increase in plasma colloid osmotic pressure should cause a decrease in the transcapillary flux of fluid across the microvasculature because of an increased transcapillary colloid osmotic pressure gradient. In fact, in acute experiments, an increase in blood colloids in animals (8) and humans (7) caused an increase in vascular volume. However, intravascular colloids extravasate over time and begin to appear within several hours in the interstitium. Indeed, in the present experiment, plasma colloid osmotic pressure increased 5.6 mmHg during the high-PPC period, and lymph colloid osmotic pressure increased 5.6 mmHg. Therefore, these data suggest that interstitial fluid colloid osmotic pressure increased and prevented changes in the transcapillary colloid osmotic pressure gradient, which, in turn, would prevent abnormal transcapillary fluid movement. This helps to explain why blood volume was unchanged during chronic hyperproteinemia in a previous study in our laboratory (14) and in the present study based on a lack of change in hematocrit during high PPC.

Another factor that could have decreased transcapillary fluid flux is a decrease in CFC, and several studies have shown that CFC may change during changes in PPC. Intravenous administration of albumin into cats caused an increase in lymph colloid osmotic pressure from 6.5 to 16 cmH2O in 2 h (8), and at this time the net capillary filtration pressure was at control levels, but both lymph flow and CFC had decreased 75%. This importantly suggests that decreases in the CFC may occur during hyperproteinemia, thus markedly decreasing transcapillary fluid flux and therefore decreasing lymph flow. Other evidence that the CFC may have decreased during hyperproteinemia comes from experiments that showed that perfusion of frog capillaries without protein in the perfusate caused the hydraulic conductivity of the capillaries to increase threefold (16), suggesting that an increase in PPC may result in a decrease in CFC. Other studies have shown that decreased plasma colloid osmotic pressure by plasmapheresis may cause an increase in the CFC. Efferent lymph flow from a prefemoral lymph node was elevated 24 h after plasmapheresis in spite of normalized transcapillary Starling forces (9), suggesting that transcapillary fluid flux increased during hypoproteinemia. Kramer et al. (11) found that a decrease in plasma colloid osmotic pressure by plasmapheresis was two times as effective as a rise in capillary pressure in promoting lymph flow in the lung, suggesting an increase in CFC. If the above-mentioned studies can be extrapolated to the present studies on hyperproteinemia, a decrease in CFC could have occurred and contributed to the decrease in lymph flow.

One of the factors that could have decreased capillary hydraulic conductivity, CFC, and lymph flow is an increase in viscosity of the capillary filtrate or the lymphatic fluid. Studies on hypoproteinemia showed that lymph viscosity decreases during hypoproteinemia (11). Based on linear interpolation of plasma viscosity changes with changes in PPC, calculated lymph viscosity during the normal-PPC period is 1.25 and during the high-PPC period is 1.75, a 40% increase in viscosity. Therefore, the resistance to fluid filtration at the capillary as well as resistance to lymph flow may have increased significantly because of increased viscosity of the capillary filtrate and lymph, respectively, both of which could have decreased lymph flow.

Another factor that could have decreased lymph flow during high PPC is a decrease in interstitial fluid volume. However, interstitial fluid volume likely increased in the present study, because in two previous studies in our laboratory that used the same protocol to increase PPC, extracellular fluid volume measured as sulfate space increased 12% (14) or, measured as sodium iothalamate space, increased 11% (13). Therefore, despite this likely increase in interstitial fluid volume, lymph flow decreased, possibly because of decreases in CFC.

Yet another factor that could have affected lymph flow during high PPC is a change in extracellular osmolality. However, plasma osmolality was measured previously in our laboratory during chronic hyperproteinemia in dogs, and no significant changes occurred when PPC was increased (13, 14).

Lymph protein transport decreased in the present experiment during hyperproteinemia, suggesting that the transcapillary protein flux also decreased. One factor that could have decreased this protein flux is a decrease in the PS product (2, 19, 21), which is a measure of the diffusive capacity of the capillary membrane (19). The PS product during hyperproteinemia on day 17 of the present experiment was 3.0 ± 0.5 µl/min, and this product increased significantly during the normal-PPC period to a maximum value of 6.0 ± 1.2 µl/min on day 20 and averaged 4.7 ± 0.5 µl/min for the entire normal-PPC period (P < 0.01). Therefore, decreases in either the permeability or the surface area of the capillary membrane during hyperproteinemia may have hindered transcapillary protein flux.

Reed et al. (19) recently measured the PS product with a new, precise method in the dog hind paw and compared the results to the classical Renkin estimate of PS product that we used in the present paper. Both methods gave the exact same value for PS, which, according to Reed et al. (19), "indicates that diffusional transcapillary flux of protein predominates at normal lymph flows in these experiments." Transcapillary flux of protein by diffusion likely dominated at the low to normal lymph flows that occurred in the present experiment; therefore, the calculated PS may be very close to the true value of PS. Only at high capillary hydrostatic pressures and thus high lymph flows does the transcapillary convection of protein play an important role in the transcapillary protein flux (19).
In a previous study in our laboratory, prenodal lymph acutely collected during anesthesia and with massage of the forelimb demonstrated that lymph protein concentration increased from a control value of 1.6 g/dl to 5.1 ± 0.08 g/dl during hyperproteinemia (14). The lymph protein concentration did not increase as much in the present studies because of several possible reasons: 1) PPC increased to 9.3 g/dl in the previous study compared with 8.8 g/dl in the present study, and 2) lymph collected acutely during anesthesia in the previous study may have overestimated the true lymph protein concentration because of the short collection period, forelimb massage rate, or the effects of anesthesia.

In conclusion, hyperproteinemia produced in conscious dogs by daily intravenous infusion of autologous plasma resulted in chronic decreases in lymph flow, lymph protein transport, and the PS product and chronic increases in prenodal lymph protein concentration and lymph colloid osmotic pressure. The marked decrease in PLF could have been due to a decrease in CFC, as seen in acute experiments (9, 11, 16). This decrease in lymph flow, produced by removing less protein from the interstitium, could have contributed to the decrease in lymph protein concentration (4, 6, 10, 11). In addition, a likely decrease in capillary protein permeability, as reflected in the decreased PS product, could have contributed to the decrease in lymph protein transport. This increase in lymph protein concentration and, supposedly, interstitial protein concentration, prevented any changes in the transcapillary colloid osmotic pressure gradient and thus may have helped to prevent any changes in blood volume and mean arterial pressure.

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