Vascular perfusion limits mesenteric lymph flow during anaphylactic hypotension in rats

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Short title: Mesenteric lymph flow increases in rat anaphylactic shock

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Abstract

To determine fluid extravasation in the splanchnic vascular bed during anaphylactic hypotension, the mesenteric lymph flow (Qlym) was measured in anesthetized rats sensitized with ovalbumin, along with the systemic arterial pressure (Psa) and portal venous pressure (Ppv). When the antigen was injected into the sensitized rats (n=10), Psa decreased from 125±4 to 37±2 mmHg at 10 min with a gradual recovery, while Ppv increased by 16 mmHg at 2 min and returned to the baseline at 10 min. Qlym increased 3.3-fold from the baseline of 0.023±0.002 g/min to the peak levels of 0.075±0.009 g/min at 2 min, and returned to the baseline within 12 min. The lymph protein concentrations increased after antigen, a finding indicating increased vascular permeability. To determine the role of the Ppv increase in the antigen-induced increase in Qlym, Ppv of the non-sensitized rats (n=10) was mechanically elevated in a manner similar to that of the sensitized rats by compressing the portal vein near the hepatic hilus. Unexpectedly, Ppv elevation alone produced a similar increase in Qlym, with the peak comparable to that of the sensitized rats. This finding aroused a question why the antigen-induced increase in Qlym was limited despite the presence of increased vascular permeability. Thus, the changes in splanchnic vascular surface area were assessed by measuring the mesenteric arterial flow. The mesenteric arterial flow was decreased much more in the sensitized rats (75%; n=5) than the non-sensitized Ppv elevated rats (50%; n=5). In conclusion, mesenteric lymph flow increases transiently after antigen presumably due to increased capillary pressure of the splanchnic vascular bed via downstream Ppv elevation and perfusion, and increased vascular permeability in anesthetized rats. However, this increased extravasation is subsequently limited by decreases in vascular surface area and filtration pressure.
Key Words: anaphylactic shock, mesenteric lymph, portal hypertension, vascular permeability.
Introduction

Anaphylactic hypotension is characterized in part by a decrease in effective circulating blood volume, which could be caused by peripheral pooling due to vasodilation and by a shift of intravascular fluid to the extravascular space due to increased vascular permeability (4). Actually, within 10 min after onset of systemic anaphylaxis in human, circulating blood volume was lost by 35 % due to increased fluid extravasation as evidenced by a considerable increase in hematocrit (9). The hemoconcentration, which could result from increased extravascular filtration, is also observed in animals suffering from anaphylactic hypotension (2,5,20). As a site for extravascular filtration during systemic anaphylaxis, splanchnic organs may be the most responsible vascular beds, for the following reasons: In the splanchnic vascular beds, the capillary pressure, a component of the Starling forces for fluid filtration, should be increased by the downstream hepatic venoconstriction, as observed in the anaphylactic shock models of rats (16,26), dogs (7,8), rabbits (18) and mice (21). In addition, mast cells are more prevalent in areas which come into contact with the external environment such as gastrointestinal tract (23), and there the antigen can cause the sensitized mast cells to release many chemical mediators, which increase the vascular permeability (11,22,24). These features suggest that extravascular fluid filtration may increase in splanchnic organs of experimental animals. It is well known that fluid filtrated into the interstitium is removed by the lymphatics, and the rate of transcapillary fluid filtration is reflected by the lymph flow. Taken together, it is expected that mesenteric lymph flow increases in animals with systemic anaphylaxis. However, mesenteric lymph flow as an index of splanchnic transvascular fluid movement has not been previously measured in anaphylactic hypotension. Thus the purpose of the present study is to measure the mesenteric lymph flow in anesthetized rats which suffered from anaphylactic hypotension.
Materials and methods

Animals

The experiments conducted in the present study were approved by the Animal Research Committee of Kanazawa Medical University. Forty male Sprague-Dawley rats (Japan SLC, Shizuoka, Japan) weighing 394±7 g were used in this study. Rats were maintained at 23°C and under pathogen-free conditions on a 12:12-hour dark/light cycle, and allowed food and water ad libitum.

Surgical preparation of animals and measurement of variables

Rats were anesthetized with pentobarbital sodium (50 mg•kg⁻¹, ip) and placed supinely on a heating pad (ATC-101B; Unique Medical, Tokyo, Japan) that maintained body temperature at 36-37°C. The trachea was cannulated with a stainless steel tube (2 mm ID). The adequacy of anesthesia was monitored by the stability of blood pressure and respiration under control conditions and during tail pinch. Supplemental doses of anesthetic (10% of initial dose) were given intraperitoneally if necessary. Arterial pressure (Psa) and central venous pressure (Pcv) were recorded from the right carotid artery and the right external jugular vein, respectively, using transducers (TP-400T, Nihon-Kohden, Japan), with the reference level of right atrium. The left femoral vein was catheterized for administration of antigen and saline. Following a midline incision of the abdominal wall, a polyethylene catheter (ID 0.4mm, OD 0.6mm) was advanced into the portal vein via the caecal vein with the tip of the catheter just protruding into the portal vein, for continuous measurement of Ppv. Then, the mesenteric lymph duct was cannulated with silicon tubing (ID 0.5 mm, OD 1.0 mm) and was secured with silk ligatures (6-0, Alfresa). The level of the outflow end of the lymphatic catheter was even with the site of lymphatic cannulation. Lymph was collected drop by drop in a small tube suspended from the force transducer (SB-1T, Nihon-Kohden, Japan). The lymph flow rate was expressed as the lymph weight gain per one min. After closure of the
abdomen, the baseline measurements were started. The vascular pressures and lymph weight were continuously displayed on a thermal physiograph (RMP-6008, Nihon-Kohden, Japan), and also digitally recorded at 40 Hz by PowerLab (AD Instruments, Castle Hill, Australia). As a value of a hemodynamic variable at a given time point, we adopted the average value of data obtained from 2.5 sec before to 2.5 sec after the time point, i.e., the average of 200 data points. In each group, saline was continuously infused at the rate of 10 ml•kg⁻¹•h⁻¹, and also bolusly injected with the volume same as the collected lymph at a 5 min interval through the femoral venous catheter in order to substitute the blood volume loss due to evaporation and collection of mesenteric lymph.

The total protein concentrations of the collected lymph at baseline and after antigen were determined with a commercially available kit (Wako Pure Chemical Industries, Osaka, Japan) by spectrophotometric method, following the pre-treatment of the lymph samples with Frigen II to eliminate the turbidity of lymph, which would have interfered the spectrophotometric measurement of protein concentrations.

Protocol of the experiment

The following three groups were studied: (1) The anaphylaxis group (n = 10); rats were sensitized with ovalbumin by the subcutaneous injection of an emulsion made by mixing complete Freund’s adjuvant (0.5ml) and 1 mg ovalbumin (grade V, Sigma, St. Louis, MO) -containing saline (0.5 ml) (25). Two weeks later, after the baseline measurement, the sensitized rats were intravenously injected with antigen, ovalbumin 0.6 mg. (2) The Ppv elevation group (n=10); intact non-sensitized rats were injected with the antigen and then subjected to Ppv elevation by compressing the portal vein near the hepatic hilus with a cotton bud so that Ppv increased in the manner same as that in the anaphylaxis group. (3) The control group (n=10); intact non-sensitized rats were observed for 30 min after an injection of the antigen.
In additional separate experiments, we measured the mesenteric arterial blood flow (Qa) during anaphylaxis (n=5) or Ppv elevation (n=5) to estimate indirectly the changes in splanchnic vascular surface area, which should affect the lymph flow rate. A pulsed Doppler flow probe (MC2PSS, Transonic Systems, Ithaca, NY) was placed on the mesenteric artery for continuous measurement of Qa, along with Psa, Ppv and Pcv, but not Qlym. Mesenteric vascular resistance was determined as (Psa-Ppv)/Qa.

Statistics

All results are expressed as the means ± SE. For statistical analysis, comparison of individual values within a group was made by the repeated measure analysis of variance followed by the Bonferroni post-hoc test correction method. Comparison of individual points between the three and two groups was made by the Bonferroni method and Student t-test, respectively.

Results

Figures 1 and 2 show a representative recording of the anaphylaxis group and the summarized data, respectively. When the antigen was intravenously injected into the sensitized rats of the anaphylaxis group, Psa decreased from 125±4 to 37±2 mmHg at 10 min, while Ppv increased by 16 mmHg from the baseline of 6.5±0.1 mmHg at 2 min, and returned to the baseline at 10 min. Qlym increased from 0.023±0.002 g/min of the baseline to the peak levels of 0.075±0.009 g/min (i.e., 3.3-fold baseline) at 2 min after antigen, and gradually returned to the baseline within 12 min after antigen (Figs. 1 and 2). After the antigen injection, the lymph protein concentrations increased significantly to 2.8 ± 0.1 g/dL from the baseline of 2.3 ± 0.1 g/dL in the anaphylaxis group (Fig. 3), indicating that anaphylaxis increased splanchnic vascular permeability to proteins.

To determine the influence of the increased microvascular pressure of the splanchnic vascular bed on the antigen-induced increase in Qlym, the Ppv elevation
group was examined: Ppv of the non-sensitized rats were passively elevated in a manner similar to that of the antigen-challenged sensitized rats by compressing the portal vein near the hilus (the Ppv elevation group), as shown in Figs. 2 and 4. Qlym started to increase 2-3 min after increase of Ppv, but the peak levels at 5 min were similar to that of the anaphylaxis rats (Figs. 2 and 4). As shown in Fig. 2 (the bottom), the increase in cumulative lymph weight in the Ppv elevation group tended to be smaller than that in the anaphylaxis group, although there were no significant differences as compared with the anaphylaxis group. In contrast to the anaphylaxis group, the lymph protein concentrations in the Ppv elevation group decreased significantly to 1.7 ± 0.2 from the baseline of 2.2 ± 0.1 g/dL (Fig. 3). When Ppv increased, Psa decreased presumably due to a decrease in venous return from the portal veins or the liver.

In the control group, there were no significant changes in Psa, Ppv, or Qlym throughout the experimental period of 30 min after antigen injection (Fig. 2). The lymph protein concentrations in the control group tended to decrease, but not significantly, to 2.1 ± 0.1 g/dL from the baseline of 2.3 ± 0.1 g/dL (Fig. 3). There were no significant differences in the baseline lymph protein concentrations among the three groups studied.

To estimate the change in the vascular surface area of the splanchnic vascular beds, which is one of the determinants for Qlym (26), Qa was measured during anaphylaxis or Ppv elevation in separate series of experiments. As shown in Fig. 5, Qa in the anaphylaxis group initially increased and then rapidly decreased by 75% from the baseline of 11.8±0.8 to 3.0±0.2 ml/min at 3 min after antigen, followed by a progressive decrease to the bottom of 1.9±0.3 ml/min at 13 min. In contrast, in the Ppv elevation group, Qa also decreased, but only by 52% from the baseline of 12.6±0.6 to 6.0±0.5 ml/min at 2 min after antigen, and then returned to the baseline levels. Mesenteric vascular resistance initially decreased after antigen, and then gradually increased 2-fold as Qa considerably decreased in the anaphylaxis group, while it did not change.
significantly in the Ppv elevation group.

**Discussion**

In this study, we reported that splanchnic Qlym increases during anaphylactic hypotension in anesthetized rats. This increased Qlym is characterized by high lymph protein concentrations, which is conventionally interpreted as an increase in microvascular permeability to proteins. Notably, this increase in Qlym was relatively small in magnitude, with the peak being only 3.3-fold the baseline, and was short in duration, as observed only within 12 min after antigen. The former feature was also consistent with the results of the Ppv elevation group; the peak Qlym of the Ppv elevation group was comparable to that of the anaphylaxis group, although vascular permeability was increased in the anaphylaxis group, but not in the Ppv elevation group. These findings suggest that the increase in extravascular fluid filtration of the splanchnic vascular bed is relatively small in magnitude and occurs only for a limited period of the post-antigen.

An increase in Qlym seems to be the characteristic of anaphylactic hypotension of rats, while a decrease in Qlym is reported in hemorrhagic hypotension of rats (30). The basal Qlym, 0.023 g/min, in the present study was comparable to that in another study (3). The high lymph protein concentration, as observed after antigen in the anaphylaxis group, is a hallmark of increased vascular permeability (19,27). However, there is a possibility that protein was concentrated within the lymph capillary lumen by active transport mechanisms (10) during anaphylaxis. Anaphylactic mediators might cause aquaporin-1 water channels widely distributed in lymphatic endothelia to facilitate lymph to interstitium water flux. Nonetheless, the anaphylaxis-induced increase in mesenteric vascular permeability in rats is consistent with the results of previous studies (22,24,29). Thus, the present study reinforces these findings by using mesenteric lymph flow measurement. In contrast, increased Qlym with low lymph
protein concentration in the Ppv elevation group represents the normal vascular permeability or integrity of splanchnic microvasculature of the present intact rats (19,27). Indeed, this is consistent with the finding that the lymphatic protein concentration decreased to 10% of plasma levels when venous pressure is elevated to 30 mmHg in cat intestines (15). The reason for the low lymph protein concentration during Ppv elevation is that vascular endothelial barrier is intact under purely increased microvascular pressure, and is far more restrictive to protein flow than to liquid filtration. Hence, the fluid leaving the vasculature is relatively protein free and dilutes the lymph protein concentration.

In this study, isotonic saline was infused to compensate for blood volume losses due to evaporation and collection of mesenteric lymph. However, as the mesenteric lymph contains protein (about 2 mg/dl), this isotonic saline infusion reduces the plasma protein concentration and therefore changes the protein concentration gradient from blood to extravascular tissue, an important determinant of the transvascular fluid passage. However, there were no significant changes in Qlym or the lymph protein concentrations for the control groups throughout the experimental period, although the latter tended to decrease. This suggests the dilution of plasma protein, if occurred, have not produced substantial effects on transvascular fluid movement.

The finding that the maximum level of Qlym in the anaphylaxis group was comparable to that in the Ppv elevation group (Fig. 2) was unexpected, because microvascular pressure elevation should augment extravascular filtration in the vascular bed with increased vascular permeability more significantly than that with the intact permeability: in response to an increase in capillary pressure, increased lymph flow is higher when capillary endothelium is damaged than when it is normal (28). In other words, the increase in Qlym in the Anaphylaxis group should have been much larger than that in the Ppv elevation group. The exact reasons for the small Qlym from the permeable splanchnic vascular bed in the present study are not known. One possible
explanation may be related to a decrease in the splanchnic vascular surface area, one of the determinants of filtration (a component of the filtration coefficient of the Starling equation). We here estimated the trend of the change in the vascular surface area by measuring the blood flow, although the flow parameter (ml/min) does not directly relate to a surface area measurement (cm²). When the substantial decrease in arterial flow occurred, there would be derecruitment of capillary flow, i.e., a decrease in number of open capillaries with blood flow, which should result in a decrease in vascular surface area (12). Actually, in the Anaphylaxis group, Qa much decreased to 25% of the baseline, which was contrasted with the smaller reduction of 50% in the Ppv elevation group (Fig. 5). Another plausible explanation is related to acute arterial hypotension due to anaphylaxis. Although Ppv initially increased by 16 mmHg, Psa, a determinant of the mesenteric capillary pressure, rapidly decreased to 58 mmHg at 1 min after antigen, reaching 37 mmHg at 10 min, while Psa in the Ppv elevation group remained above 90 mmHg, as shown in Fig. 2. Thus, the mesenteric capillary pressure in the anaphylaxis group might have been smaller than that in the Ppv elevation group, resulting in limited increase of fluid filtration in the former group. Furthermore, the mesenteric vascular resistance significantly increased in the anaphylaxis group, but not the Ppv elevation group, as shown in Fig. 5. If this increase in vascular resistance occurred at the level of the precapillary arterioles, as usually observed, the capillary pressure would be decreased (19).

In the anaphylaxis group, Qlym began to increase relatively soon after antigen, as compared with that in the Ppv elevation group. There should be a time-lag for the lymph flow to increase after the start of increased transcapillary fluid filtration, as observed in the Ppv elevation group. This lag time is accounted for by the following process: extravascular fluid movement initially increases the interstitial volume, and then the resultant increase in the interstitial pressure leads to the movement of accumulated fluid into the lymphatic vessel, resulting in increased lymph flow (1,19).
The mechanism for this antigen-induced initial increase in Qlym is not known. One possible explanation is that pumping of the mesenteric lymphatic vessels might be facilitated by antigen-released chemical mediators such as leukotriene (LT) B₄, LT C₄, LT D₄, thromboxane A₂, all of which could exert a positive contractive activity on the lymphatic vessels (6,17). Another explanation is related to the dramatic increase in mesenteric blood flow as observed transiently and immediately after antigen in the anaphylaxis group (Fig. 5). This short-lasting increment of mesenteric blood flow would increase the vascular surface area and the capillary pressure, which could result in increased transcapillary filtration, and thus account for in part the initial increase in Qlym after antigen.

It is known that at acutely elevated venous pressures, transvascular fluid flux is much greater than Qlym in the gut. Under these circumstances of acutely elevated venous pressures, intestinal villi secrete fluid into the gut lumen, which is called filtration secretion. Granger et al. (13) described this phenomenon, demonstrating that interstitial fluid pours into the gut lumen under circumstances of mesenteric venous hypertension via the tips of the villi. However, the venous pressure level at which filtration secretion occur was higher than 20 mmHg (14). In the present study, the peak levels of Ppv in the anaphylaxis group (18 mmHg) were apparently lower than that threshold pressure. Moreover, filtration secretion did not develop until 30 min after the start of venous pressure elevation (14). Thus, it is unlikely that filtration secretion affects Qlym in the present study.

**Perspectives and Significance**

Anaphylactic hypotension, a potentially fatal circulatory disorder, probably results from a mixture of reduced venous return to the heart due to venodilation and hypovolemia via fluid extravasation, cardiac dysfunction, and arterial dilation (4). In this study, to explore how the fluid extravasation in splanchnic vascular beds is
involved in anaphylactic hypotension, we measured mesenteric lymph flow rate in anesthetized rats with anaphylaxis. We here demonstrated that the transcapillary fluid filtration is increased, as evidenced by an increase in protein rich Qlym. Previous studies reported the increased permeability of mesenteric vessels, but not the transient increase in lymph flow during anaphylaxis. We further showed that the increase in lymph flow was caused by transient increases in vascular permeability, blood flow and filtration pressure. The subsequent decrease in vascular pressure and more gradual decrease in vascular perfusion limited the increase in Qlym and presumably the overall filtration. In future studies, measurements of edema or organ weight gain would be required to determine what extent the overall tissue filtration rate is reflected by Qlym. In addition, the experiment on mesenteric lymph capillary pumping may provide more mechanistic information on mesenteric transvascular fluid movement during anaphylactic hypotension.
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Figure legends

Figure 1. A representative recording of the response to ovalbumin antigen (0.6 mg) in an anesthetized rat. Psa, systemic arterial pressure; Ppv, portal vein pressure; CVP, central venous pressure; Lymph weight, weight of collected mesenteric lymph.

Figure 2. The summary of time course changes in the systemic arterial pressure, portal venous pressure, lymph flow and cumulative lymph flow of the anaphylaxis group (n=10, closed circle), Ppv elevation group (n=10, open triangle) and control group (n=10, open circle). Means ± SE; *P<0.05 vs. baseline; #P<0.05 vs. the anaphylaxis group.

Figure 3. The lymph protein concentrations at baseline (open bars) and after an injection of the antigen (closed bars) in the control group (n=10), anaphylaxis group (n=10), and Ppv elevation group (n=10). Means ± SE; *P<0.05 vs. baseline; #P<0.05 vs. the control group.

Figure 4. A representative recording of the response in an anesthetized rat of the Ppv elevation group.

Figure 5. The summary of time course changes in systemic arterial pressure, portal venous pressure, mesenteric arterial blood flow and mesenteric vascular resistance in the anaphylaxis group (n=5, closed circles) and the Ppv elevation group (n=5, open squares). Means ± SE; *P<0.05 vs. baseline; #P<0.05 vs. the Ppv elevation group.
Fig. 2

Systemic arterial pressure (mmHg)

- Control
- Anaphylaxis
- Ppv elevation

Portal venous pressure (mmHg)

Lymph flow (g/min)

Cummulative lymph weight (g)

Time after antigen (min)
Fig. 3

Lymph protein concentration (g/dl)

Control  Anaphylaxis  Ppv elevation

* baseline  
# after antigen
Fig. 5

Systemic arterial pressure (mmHg)

Portal venous pressure (mmHg)

Mesenteric arterial flow (ml/min)

Mesenteric vascular resistance (mmHg/ml/min)

Time after antigen (min)