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Adaptation of Mesenteric Collecting Lymphatic Pump Function Following Acute Alcohol Intoxication

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Abstract

Objective—Acute alcohol intoxication increases intestinal lymph flow by unknown mechanisms, potentially impacting mucosal immunity. We tested the hypothesis that enhanced intrinsic pump function of mesenteric lymphatics contributes to increased intestinal lymph flow during alcohol intoxication.

Methods—Acute alcohol intoxication was produced by intragastric administration of 30% alcohol to conscious, unrestrained rats through surgically-implanted catheters. Time-matched controls received either no bolus, vehicle, or isocaloric dextrose. Thirty minutes after alcohol administration, rats were anesthetized and mesenteric collecting lymphatics were isolated and cannulated to study intrinsic pumping parameters. In separate experiments, mesenteric lymphatics were isolated to examine direct effects of alcohol on intrinsic pump activity.

Results—Lymphatics isolated from alcohol-intoxicated animals displayed significantly decreased contraction frequency (CF) than the dextrose group, elevated stroke volume index (SVI) versus all other groups, and decreased myogenic responsiveness compared to sham. Elevating pressure from 2 to 4 cm H₂O increased the volume flow index 2.4-fold in the alcohol group versus 1.4-fold for shams. Isolated lymphatics exposed to 20 mM alcohol had reduced myogenic tone, without changes in CF or SVI.

Conclusions—Alcohol intoxication enhances intrinsic pumping by mesenteric collecting lymphatics. Alcohol directly decreases lymphatic myogenic tone, but effects on phasic contractions occur by an unidentified mechanism.

Keywords

splanchnic lymphatics; lymphatic smooth muscle; lymphangion; myogenic tone

INTRODUCTION

The lymphatic system is important for removing excess interstitial fluid and returning it to the systemic circulation, which helps maintain fluid homeostasis [20]. The lymphatics of the digestive tract transport approximately two-thirds of all lymph produced in the body and have a key role in mucosal immunity [23]. These lymphatics traffic lymphocytes from the digestive tract to the mesenteric lymph nodes which are at a pivotal position in immune anatomy, forming the border between mucosal immunity and the remainder of the immune system that normally maintains systemic ignorance to nonpathogenic microbes in the intestine [34,51]. In addition, mesenteric lymphatics transport lipids, lipid-soluble vitamins,

antigens, and a variety of other compounds absorbed in the small intestine to these lymph nodes and larger collecting lymphatics, eventually reaching the systemic circulation [26,36,41].

Alcohol intoxication is associated with marked alterations in host defense mechanisms and has been shown to increase susceptibility to infections as well as to impair host responses involved in clearing infectious processes. Alcohol-induced immune suppression exacerbates coexisting suppressive effects of other drugs of abuse, superimposed infection, or cancer [50]. In addition, alcohol intoxication is a major risk factor for traumatic injuries [12,35] that are frequently complicated by secondary infections during the recovery period [44]. Excess alcohol at the time of burn injury or prior to a surgical intervention is associated with impaired host defense response to infections [13,47]. While evidence suggests that altered cytokine profiles are directly related to an impaired host defense, investigators have also shown a contribution of barrier dysfunction to the post-injury increase in infections in the alcohol-intoxicated host [14]. However, current knowledge of how alcohol consumption affects the complex functions of immune function in the digestive tract remains very rudimentary.

Understanding how alcohol affects lymphatic function and how it contributes to immunity in the digestive tract is of importance considering that most of the body's lymphocytes and antibody production are within the gut. Very little is known about how alcohol affects lymphatic function, although studies with rats have shown significantly accelerated mesenteric lymph flow when diets containing alcohol are administered intragastrically as a bolus [5] or as a continuous infusion into the duodenum [27] versus control diets lacking alcohol. Similarly, another study showed that consumption of alcohol in humans causes an increase in thoracic duct lymph flow [6]. This alcohol-induced increase in lymph flow from the gut may function to accelerate delivery of pathogens and other antigens to the lymph nodes for immunosurveillance, but could also be potentially harmful if the gut barrier is compromised and an overwhelming number of pathogens are present.

In rats, collecting lymphatic vessels are composed of an inner layer of endothelial cells and a single layer of smooth muscle cells, which form segments called lymphangions, that are separated by unidirectional valves [21]. Each lymphangion can generate both tonic and phasic contractions. They expand and contract cyclically, pumping fluid against a pressure gradient and their output is sensitive to transmural pressure and flow. Due to its functional similarities with the heart, the lymphangion cycle is typically characterized in a similar fashion as the cardiac cycle [7,9].

While the phasic contractions of collecting lymphatics resemble heart function, their tonic contractile nature resembles the myogenic response in arterioles, defined as constriction in response to elevated luminal pressure and relaxation when pressure is decreased [19,31,38]. A number of intrinsic adaptations of the lymphatic pump to changes in luminal pressure and flow have provided evidence of myogenic activity [4,37]. However more recently, myogenic constriction and dilation of isolated mesenteric collecting lymphatic vessels was more definitively described [15].

In the current study, we examined whether acute alcohol intoxication changes the intrinsic, contractile function of mesenteric lymphatic vessels. Using isolated, perfused mesenteric collecting lymphatics, we observed that acute gastric administration of alcohol causes an adaptive functional response in mesenteric lymphatics, favoring facilitated transport of lymph. Subsequently, we examined the direct effects of alcohol on functional responses in isolated lymphatics to better understand the mechanisms involved.

MATERIALS AND METHODS

Animals

All procedures were approved by the Institutional Animal Care and Use Committee at the Louisiana State University Health Sciences Center and were performed in accordance with the guidelines of the National Institute of Health (NIH publication No. 85-12, revised 1996). Male Sprague-Dawley rats (270–350 g body wt) were housed in a controlled temperature (22 °C) and controlled illumination (12:12 h light dark cycle) environment. After arrival, the rats were submitted to a one week acclimation period and were provided standard rat chow (2018 Teklad Global 18% Protein Rodent Diet, Harlan) and water *ad libitum*.

Gastric and venous catheter placement

A previously described procedure was used [43]. Briefly, rats were anesthetized with ketamine and xylazine (90 and 9 mg/kg, respectively), and a sterile catheter was aseptically placed into the antrum of the stomach. The catheter was routed subcutaneously through a trocar and exteriorized at the nape of the neck. After cannula placement, animals returned to individual cages and were allowed 24–48 hours to recover from surgery, with food and water provided *ad libitum*.

Collecting lymphatic isolation

Rats were anesthetized (ketamine and xylazine at 90 and 9 mg/kg, respectively), a midline laparotomy was performed, and the small intestine and mesentery was exteriorized and excised, and placed in ice-cold albumin physiological salt solution (APSS: NaCl, 120 mM; KCl, 4.7 mM; CaCl₂·2H₂O, 2 mM; MgSO₄·7H₂O, 1.2 mM; NaH₂PO₄, 1.2 mM; Na pyruvate, 2 mM; glucose, 5 mM; EDTA, 0.02 mM; MOPS, 3 mM and purified BSA 1 g/100ml). A blood sample (~0.5 ml) was withdrawn from the aorta to determine serum alcohol concentration. Animals were euthanized with an overdose of ketamine/xylazine followed by cervical dislocation. In each experiment, a section of mesentery was pinned in a dissection chamber, and a collecting lymphatic vessel (80–200 µm internal diameter and 1–2 mm of length) was carefully dissected from surrounding adipose and connective tissue with the aid of a stereomicroscope. The isolated lymphatic was transferred to an isolated vessel chamber (Living Systems Instrumentation, Burlington VT) and was mounted onto two resistance-matched glass micropipettes. Isolated vessels with only one valve were used to ensure optimal pressure control in the entire segment [15]. The chamber was transferred to a Nikon Eclipse TS100 inverted microscope, equipped with a halogen lamp, 10X working distance objective, and CCD camera for video image acquisition. The intravascular pressure was controlled by a servo-controlled feedback pressure myograph system (Living Systems).

Pressure Control Protocol

Initially the intraluminal pressure was set at 2 cm H₂O during 45–60 min for equilibration and development of lymphatic spontaneous contractions. The luminal diameter was continuously tracked by a video dimension analyzer (Living Systems, Inc.) attached to a personal computer for data acquisition and to a DVD recorder for video storage [24]. Vessels were used for study if both the following criteria were met within the equilibration period: 1) the vessel developed regular, spontaneous contractions at 2 cm H₂O, and 2) the spontaneous contractions that were reasonably uniform across the length of the vessel. To evaluate myogenic responsiveness of the isolated lymphatics, a pressure step procedure was used in all the protocols. This consisted of abrupt, stepwise increases of the intraluminal pressure (to 4, 6, 8, 10, and 12 cm H₂O) from the baseline level (2 cm H₂O), holding the elevated pressure during at least 25 lymphatic contractions, and then a step decrease to the baseline pressure. After completion of the pressure step series the bath solution was changed

to a Ca^{2+} free APSS at 37 °C, which abolished phasic and tonic contraction, and was used to measure passive diameter (MaxD) of the same collecting lymphatic at the same pressures. The MaxD was used as the denominator to normalize diameter data, due to varying diameters of the lymphatics isolated for experiments.

Lymphatic Contractile Parameters

The following parameters were determined from the lymphatic luminal diameter measurements: Contraction frequency (CF), end diastolic diameter (EDD), end systolic diameter (ESD), amplitude of contraction ($\text{AMP} = \text{EDD} - \text{ESD}$), the maximal passive diameter at each pressure (MaxD; determined in Ca^{2+} -free conditions), initial change in EDD following a pressure step increase = $\text{EDD}_1 - \text{EDD}_{\text{baseline}}$, where EDD_1 is the EDD associated with the first contraction after the pressure step, normalized change in EDD following a pressure step increase = $100 * (\text{EDD} - \text{EDD}_1) / \text{MaxD}$, tone = $100 * (\text{MaxD} - \text{EDD}) / \text{MaxD}$, normalized AMP = AMP / MaxD , stroke volume index ($\text{SVI} = \pi(\text{EDD}^2 - \text{ESD}^2)$), Ejection Fraction ($\text{EF} = \text{SVI} / (\pi \text{EDD}^2)$), Volume Flow Index ($\text{VFI} = \text{CF} \times \text{SVI}$) [10,15].

Alcohol Treatment Protocols

In Vivo Alcohol Intoxication Model—Conscious and unrestrained animals received an intragastric bolus of 30% ethyl alcohol (2.5 g/kg) via the previously implanted gastric catheters. This mode of alcohol administration reflects a human binge drinking episode, described as the consumption of five or more drinks over a period of time sufficient to elevate blood alcohol levels above intoxicating levels [40]. Blood alcohol levels, determined using an Analox GM7 micro-stat (Lunenburg, MA) averaged 276 ± 31 mg/dl at the time of excision of mesenteric lymphatics. Three time-matched control groups were used: 1) Sham, in which animals received no intragastric administration via the catheter, 2) administration of vehicle (water) at the same volume as the alcohol group, and 3) administration of an isovolumic, isocaloric bolus of 52% dextrose (12.2 g/kg). The mesentery was removed for lymphatic dissection 30 min. after the alcohol administration. Vessel dissection and mounting on the glass micropipettes took approximately 20 minutes to complete, and was followed by a 20 minute stabilization period during which the lymphatic established phasic contractions. After this stabilization period, diameter tracking was initiated and baseline contractions were recorded for 30 min. prior to the beginning of the pressure step protocol. Thus, the time from the initial, gastric bolus administration of alcohol, dextrose, or vehicle to the beginning of the step pressure protocol was approximately 100 min. for all the lymphatic vessels in the gastric administration groups.

Direct Application of Alcohol to Isolated Lymphatics—Collecting lymphatics were isolated from rats not subjected to the gastric/vascular catheter surgery. The isolated lymphatics were mounted and pressurized, and diameter was measured continuously during the pressure control protocol outlined above, first with APSS as the bath solution, and then with Ca^{2+} -free APSS. The bath solution was then changed back to APSS containing calcium to restore tone and phasic contractions. After stabilization, the bath solution was changed to APSS containing 20 mM or 40 mM ethanol, and approximately 6 min. later the pressure control protocol was repeated. The ethanol bath solution was used for the duration of the experiment prior to the switch to Ca^{2+} -free APSS. In addition, two experiments were performed in which no ethanol was added during the second part of the experiment, to verify that there were no effects due to tachyphylaxis (data not shown).

Data Analysis

Data are presented as mean $\pm$ SE. For baseline data, the mean was calculated using the 5 contractions prior to a pressure step. For elevated pressures, the steady state mean was calculated using data from contractions 13–25 after the pressure step. Multigroup comparisons were performed using repeated measures ANOVA followed by Bonferroni *t*-tests where appropriate. Significance was accepted at $P < 0.05$.

RESULTS

Lymphatic Myogenic Response Characteristics

Isolated, mesenteric collecting lymphatics, perfused at a luminal pressure of 2 cm H₂O, displayed typical phasic changes in diameter (Fig. 1). The application of an abrupt increase in pressure (+2 to +10 cmH₂O) caused a rapid increase in both EDD and ESD, followed by slow decrease in EDD to a new steady state 12–13 contractions after the pressure step increase, coupled with a more rapid decrease in ESD (and thus an increase in AMP) over the same time period (Fig. 1). A recent report termed this decrease in EDD in response to elevated pressure as “lymphatic myogenic constriction” and also described the compensatory AMP increase in response to acute pressure elevation [15].

Impact of Alcohol Intoxication on Phasic Contraction Frequency

Fig. 2 shows the time course of changes in normalized EDD for each group, in the 25 contractions following step increases of +2, +4, +6, +8, and +10 cm H₂O. In panels A (sham), B (vehicle), and C (dextrose), the larger step increases in pressure caused a decrease in normalized EDD over time and the establishment of a new steady state during the time course. In addition, at higher pressure the overall time for 25 contractions to occur was shorter. In contrast, lymphatics from alcohol treated rats (D) did not display the decrease in EDD over time after abrupt upward pressure steps, and the time required for 25 contractions was longer.

Noting these differences, we summarized the diameter and phasic contraction data. Fig. 3 shows the steady state contraction frequency (CF) at each pressure. The CF in lymphatics isolated from alcohol-intoxicated rats was lower than CF for the three controls at each pressure. This difference was significant between the alcohol and dextrose groups. These data indicate that alcohol intoxication inhibits phasic contraction frequency in mesenteric lymphatics.

Impact of Alcohol Intoxication on Tonic Contractile Properties of Mesenteric Lymphatics

Isolated mesenteric lymphatics from the sham, vehicle, and dextrose groups displayed a myogenic response, however lymphatics from the alcohol intoxicated rats did not. Fig. 4 summarizes the tonic contractile properties of mesenteric lymphatics obtained from alcohol-intoxicated rats and the three control groups. The mean baseline EDD and ESD was similar among all the treatment groups (Fig. 4A and B). Fig. 4C shows the initial change in lymphatic EDD following abrupt upward pressure steps of +2 to +10 cm H₂O, with no significant differences between groups. The steady state changes in normalized EDD achieved in contractions 13–25 following the upward pressure steps as shown in Fig. 2 are summarized in Fig. 4D. Negative values reflect myogenic constriction. The mean tone of vessels in each group was plotted as a function of pressure (Fig. 4E). Lymphatics from alcohol-intoxicated rats displayed lower tone values than the control groups at each pressure. This difference was significant between the alcohol and dextrose groups. These data suggest that alcohol intoxication suppresses the myogenic response in mesenteric lymphatics.

Impact of Alcohol Intoxication on Mesenteric Lymphatic Phasic Contractions and Pumping Efficiency

In addition to tone, we evaluated parameters related to the magnitude of phasic contractions. Fig. 5A shows the steady state normalized AMP as a function of pressure for lymphatics isolated from each treatment group. Lymphatics isolated from alcohol intoxicated rats had a significantly higher AMP at 2–8 cm H₂O compared to those from the sham. However, the AMP values for the vehicle group were also elevated above sham, but not as high as for alcohol, suggesting that the increase in AMP compared to sham could be due in part to gastric distention. In contrast, lymphatics from the dextrose group, which served as a control for both gastric distention and caloric intake, had relatively low AMP values at each pressure, that were significantly different from the alcohol group at 2–8 cm H₂O and from the vehicle group at 2 and 4 cm H₂O. These data suggest that alcohol intoxication may increase the magnitude of contraction. Alternatively, it is possible that dextrose may have an opposite effect.

This data was clarified when evaluating stroke volume index (SVI; Fig. 5B), which represents a change in cross sectional area of the lumen rather than diameter. The plots of SVI versus pressure for the sham and vehicle groups were virtually identical, while the SVI of lymphatics from alcohol intoxicated rats was significantly higher at 4 and 6 cm H₂O. For lymphatics from dextrose-treated rats, the SVI was slightly lower than the sham and vehicle groups at all pressures, and was significantly lower than the alcohol group at 2–8 cm H₂O. The SVI data suggest that alcohol intoxication increases the magnitude of contraction in mesenteric collecting lymphatics that is not due to gastric distention or its caloric impact.

At baseline pressure (2 cm H₂O), mesenteric lymphatics from the rats treated with alcohol or vehicle had a slightly higher ejection fraction (EF) than sham, and a significantly higher EF than the dextrose group. At higher pressures, lymphatics from the alcohol treated rats tended to have a slightly higher EF than the sham and vehicle groups, and remained significantly higher than the dextrose group up to 8 cm H₂O. Lymphatic pump efficiency is expressed as EF plotted versus pressure for each treatment group (Fig. 5C). This data suggested that alcohol causes a slight enhancement of lymphatic pumping efficiency.

To determine whether the changes in lymphatic contractile behavior translate into an overall change in pump function, we calculated the VFI, an index of lymphatic pumping output over time. While the VFI for lymphatics from alcohol-intoxicated rats was slightly higher than for all the control groups, this difference was not significant. It is worth noting, however, that the increase in pressure from 2 to 4 cm H₂O caused a 2.4-fold (± 0.4) increase in VFI in lymphatics from the alcohol group, but only a 1.4-fold (± 0.2) increase in VFI in the sham group ($P < 0.05$). The increases in the vehicle and dextrose groups were not significantly different from the alcohol or sham groups. (It is worth noting that in the alcohol group the SVI increased when pressure was raised from 2 to 4 cm H₂O, while in the sham group it remained at about the same value; Fig. 5B.) These data suggest that alcohol intoxication causes a modest overall increase in the intrinsic pump activity of mesenteric collecting lymphatics.

Direct Effects of Alcohol on Isolated Mesenteric Lymphatics

To determine the direct effects of alcohol, isolated mesenteric lymphatics excised from naive rats were studied. Isolated lymphatics were evaluated before and after changing the bath solution to APSS containing alcohol. The results indicate that alcohol produces dose-dependent effects. At 40 mM alcohol in the bath solution, lymphatic pumping stopped and no further studies were performed using this alcohol concentration (data not shown). Subsequent studies were performed using a 20 mM alcohol solution. Fig. 6 shows the time

course of changes in normalized EDD in lymphatics without alcohol and then with 20 mM alcohol, for the 25 contractions following step increases of +2, +4, +6, +8, and +10 cm H₂O from a baseline of 2 cm H₂O. Like the three control groups shown in Fig. 2, the larger upward pressure steps caused a decrease in normalized EDD to a new steady state. After the addition of alcohol this response was attenuated, but not completely inhibited. In addition, unlike the *in vivo* administration of alcohol, direct application of alcohol to the isolated lymphatic did not drastically lengthen the time for 25 contractions to occur (Fig. 6B). Likewise, direct application of alcohol to isolated lymphatics did not significantly alter CF (data not shown), and also did not affect AMP, SVI, EF, or VFI (data not shown). However, direct application of alcohol to isolated lymphatics did affect myogenic tone. Fig. 7A shows the initial change in lymphatic EDD following abrupt upward pressure steps of +2 to +10 cm H₂O from a baseline pressure of 2 cm H₂O. The increase in EDD following upward pressure steps was slightly lower after alcohol was added to the bath, however this was due to a slight increase in EDD at baseline pressure (Fig. 7B). As seen with alcohol intoxication *in vivo*, direct application of alcohol to isolated lymphatics inhibited the myogenic response to step increases in pressure (Fig. 7C). This was due to the inability of these lymphatic vessels to develop an increased steady-state tone at higher pressures after alcohol was introduced (Fig. 7D). These data show that the alcohol intoxication-induced inhibition of myogenic constriction is a direct effect of alcohol on mesenteric collecting lymphatics.

DISCUSSION

We examined the impact of acute alcohol intoxication on mesenteric lymphatic function. Our results showed that alcohol intoxication reduced contraction frequency, tone, and lymphatic myogenic response, and increased stroke volume. We also observed that in lymphatics isolated from alcohol-intoxicated rats, raising the luminal pressure from 2 to 4 cm H₂O, which approximates the increase in mesenteric lymphatic luminal pressure during a period of enhanced lymph formation in the gut [9], causes a greater increase to a new steady state VFI, an indicator of a lymphangion's output [9,10], compared to control. These data suggest that mesenteric collecting lymphatics have an adapted intrinsic pump function during alcohol intoxication that facilitates lymph flow. In addition, we observed that the inhibition of lymphatic myogenic tone can be reproduced by direct application of alcohol to isolated mesenteric collecting lymphatics, suggesting that alcohol directly impacts the tonic aspect of lymphatic contractile function.

To our knowledge, this is the first study to investigate how alcohol intoxication affects mesenteric collecting lymphatic function. Intragastric administration of alcohol was previously shown to cause a 6-fold increase in lymph flow from the superior mesenteric lymph duct, while isocaloric dextrose caused a ~2.5-fold increase [5]. A different study in rats showed that continuous intraduodenal infusion of alcohol plus dietary lipids significantly increased mesenteric lymph flow versus the control lipid meal not containing alcohol [27]. In addition, a study with fasting male humans showed that oral consumption of alcohol caused a significant increase in lymph flow from the thoracic duct [6]. Such increases in flow generally occur during nutrient absorption as a result of elevated transcapillary filtration in the gut and lymph formation in lacteals [22,32]. In the current study, we compared the responses of isolated lymphatics from rats administered alcohol and isocaloric dextrose to an isovolumic bolus of vehicle only (water) because of the observation that dextrose administration consistently caused a reduction in AMP compared to previously published results [15]. We also used a sham (surgery only) group to assess whether gastric distention may also have a significant influence on intrinsic pumping of lymphatics. The finding that alcohol intoxication can enhance the increase to a new steady state VFI caused by a small upward step in intraluminal pressure shows that intrinsic collecting lymphatic pump function is affected by alcohol intake in a way that favors increased lymph flow

during periods of elevated transcapillary filtration and lymph formation. Considering the role of lymph in host-defense, the alcohol-induced increase may function to accelerate delivery of pathogens and other antigens to the lymph nodes for immunosurveillance. However, during traumatic injuries involving gut ischemia, when the gut barrier is compromised, elevated lymph flow would be harmful if the mesenteric lymph nodes become overwhelmed, and post-nodal lymph carries pathogens to the central circulation, where they infect the lung and other organs [17]. This may be a factor contributing to the increased morbidity and mortality observed in alcohol-intoxicated trauma victims.

In the current study, alcohol caused a decrease in mesenteric lymphatic vessel tone. The molecular mechanisms of tone generation in collecting lymphatics appear to resemble that of arterioles [15,39,49]. Alcohol causes dilation of gastric and mesenteric arterioles [25,46,52]. Studies of mesenteric arterioles showed that this response occurs irrespective of route of administration and appears to be due to direct interaction of the arterioles with alcohol as its metabolites acetaldehyde and acetate fail to produce the vasodilation response [1,2]. Our finding that alcohol inhibits myogenic tone of mesenteric collecting lymphatics appears to resemble, at least in part, the alcohol-induced decrease in tone of arterioles found in the gut. The alcohol-induced attenuation of myogenic tone and the inhibition of the myogenic response in lymphatics appear to contribute in part to the increased steady state SVI. Because smooth muscle tone is related to cytosolic calcium levels, it is possible that alcohol may cause decreased tone by lowering calcium in the saroplasm of the lymphatic smooth muscle layer during lymphatic diastole. Calcium-sensitive mechanisms like myosin light chain (MLC) kinase (MLCK)-mediated phosphorylation of MLC are important for lymphatic tonic contraction strength [49]. The literature shows conflicting results regarding how alcohol affects cytosolic calcium levels in vascular smooth muscle cells [45,54]. It is possible that both the decreased tone and the increased AMP caused by alcohol may be directly related to altered changes in cytosolic calcium. Alternatively, signaling pathways like Rho kinase, which enhance calcium sensitization and also have been shown to be involved in tone generation in lymphatics [28], could also be responsible for the changes. Another possibility is that a calcium-independent mechanism may be involved in the alcohol-induced decrease in lymphatic myogenic responsiveness.

Our data also show topical application of alcohol to isolated mesenteric collecting lymphatics does not reproduce the *in vivo* alcohol intoxication-induced decrease in steady state CF, and elevated lymphatic steady state AMP and SVI. These parameters represent the “cardiac-like” nature of collecting lymphatic vessels, for which the molecular mechanisms are poorly understood [11,48]. Thus these effects on lymphatic pumping caused by *in vivo* intoxication could be due to a wide variety of neurogenic, endocrine, and inflammatory signals mediators that are released in the gut when alcohol enters the stomach and later the small intestine. For example, enteric neurons containing substance P, calcitonin gene related peptide (CGRP), and vasoactive intestinal peptide all innervate mesenteric lymphatics [30,42]. Substance P causes constriction and increased contraction frequency in rat mesenteric lymphatics [3,16], while CGRP has been shown to decrease CF in isolated mesenteric lymphatics from guinea pig [29]. Lymphatic pump function is also influenced by the baroreceptor reflex and oxidative stress, and can be modulated by inflammatory stimuli like endotoxin and fMLP [8,18,33,53]. The presence of recently ingested food in the stomach may also impact lymphatic pumping, as indicated the drop in AMP we observed with intragastric dextrose administration, and how this may impact alcohol’s effects remains to be explored. These potential mechanisms will be taken into consideration in future studies directed at understanding how alcohol intoxication affects the “cardiac-like” aspects of mesenteric collecting lymphatic function.

In summary, the current study demonstrates that alcohol intoxication impacts the pump function of mesenteric collecting lymphatics by inhibiting the lymphatic myogenic response, decreasing CF, and increasing SVI at a given pressure. In response to a pressure step within the physiological range, the enhanced increase in SVI in lymphatics from the alcohol-treated rats contributes to a greater increase in VFI than in sham rats, indicating that acute alcohol intake can facilitate intrinsic lymphatic contractile mechanisms for enhancing lymph flow through collecting lymphatics. While alcohol directly inhibits the lymphatic myogenic response, the effect of alcohol intoxication on phasic contractile properties is indirect and the mechanism remains elusive.

Acknowledgments

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Fig. 1.

Lymphatic myogenic constriction and increased AMP in response to an abrupt increase in pressure. The time course of diameter changes in an isolated perfused mesenteric collecting lymphatic. The top tracing shows the pressure reading over time (baseline of 2 cm H₂O and step increase to 10 cm H₂O) and the bottom tracing shows lymphatic diameter. End diastolic diameter (EDD) and end systolic diameter (ESD) are determined from the tracing, and the contraction amplitude (AMP) is calculated as EDD-ESD. During the elevated, constant pressure, there is a time-dependent decrease in EDD, termed myogenic constriction, and also a compensatory increase in AMP resulting from ESD declining more rapidly than EDD.

Fig. 2.

Effect of alcohol on the time course of lymphatic EDD in response to abrupt increases in pressure. Each panel shows the change in normalized EDD for the first 25 contractions over time following pressure steps of +2, +4, +6, +8, and +10 cm H₂O. Baseline pressure was 2 cm H₂O. In panels *A* (sham group) *B* (vehicle), and *C* (dextrose), higher pressures caused a decrease in EDD (shown normalized to MaxD) and the establishment of a new steady state during the time course. In addition, at higher pressure the contraction frequency was increased, so that the overall time for 25 contractions to occur was shorter. In contrast, lymphatics from alcohol treated rats (*D*) did not display the decrease in EDD over time after abrupt upward pressure steps, and the time for 25 contractions was longer. The error bars are S.E. N=6 for the sham group. N=5 for all other groups.

Fig. 3. Decreased contraction frequency (CF) in collecting lymphatics isolated after gastric administration of alcohol. Steady state CF is shown for each pressure. [†]P<0.05 and ^{††}P<0.01, alcohol versus dextrose. N=6 for the sham group. N=5 for all other groups.

Fig. 4.

Myogenic properties of isolated, collecting lymphatics in rats after gastric administration of alcohol and in controls. *A*. Baseline mean EDD and ESD (*B*) for the sham, vehicle, dextrose, and alcohol bolus groups. *C*. The initial change in lymphatic EDD following abrupt upward pressure steps of +2 to +10 cm H₂O. Baseline pressure was 2 cm H₂O. *D*. Myogenic constriction, expressed as the steady state change in normalized EDD, following abrupt upward pressure steps. Negative values represent myogenic constriction. **P*<0.05, alcohol versus sham. *E*. Tone of lymphatic vessels, plotted as a function of pressure. †*P*<0.05, alcohol versus dextrose. N=6 for the sham group. N=5 for all other groups.

Fig. 5. Gastric administration of alcohol increases the amplitude of contraction, the stroke volume index (SVI), and the ejection fraction (EF) of isolated mesenteric lymphatics. **A.** Normalized AMP (AMP/MaxD), **B.** SVI, **C.** EF, and **D.** the volume flow index (VFI) are plotted as functions of pressure. * $P < 0.05$ and ** $P < 0.01$, alcohol versus sham; † $P < 0.05$ and †† $P < 0.01$, alcohol versus dextrose; ‡ $P < 0.05$, alcohol versus vehicle; § $P < 0.05$ and §§ $P < 0.01$, dextrose versus vehicle. $N = 6$ for the sham group. $N = 5$ for all other groups.

Fig. 6.

Effect of direct application of alcohol on the time course of lymphatic EDD in response to abrupt increases in pressure in isolated mesenteric collecting lymphatics. Each panel shows the change in normalized EDD for the first 25 contractions over time following pressure steps of +2, +4, +6, +8, and +10 cm H₂O. Baseline pressure was 2 cm H₂O. In panels A (no alcohol) higher pressures caused a decrease in EDD (shown normalized to MaxD) and the establishment of a new steady state during the time course. In addition, at higher pressure the contraction frequency was increased, so that the overall time for 25 contractions to occur was generally shorter. After alcohol (20 mM final concentration) was added to the bath, the lymphatics displayed less of a decrease in EDD over time after abrupt upward pressure

steps, and the time for 25 contractions about the same after the different pressure steps. The error bars are S.E. N=4 lymphatics studied.

Fig 7.

Myogenic properties of isolated collecting lymphatics prior to the addition of alcohol (control) and with alcohol in the bath (final concentration 20 mM). *A*. The initial change in lymphatic EDD following abrupt upward pressure steps of +2 to +10 cm H₂O. Baseline pressure was 2 cm H₂O. *B*. Mean CF in isolated collecting lymphatics before and after addition of alcohol to the bath solution. Steady state CF is shown for each pressure. *C*. Myogenic constriction, expressed as the steady state change in normalized EDD, following abrupt upward pressure steps (baseline was 2 cm H₂O). Negative values represent myogenic constriction. *D*. Tone of isolated collecting lymphatics before (control) and after addition of alcohol, plotted as a function of pressure. **P*<0.05, no alcohol versus alcohol. N=4 lymphatics studied.