Protein washdown as a defense mechanism against myocardial edema

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Received 15 October 1999; accepted in final form 24 April 2000

Myocardial edema occurs in many pathological conditions. We hypothesized that protein washdown at the myocardial microvascular exchange barrier would change the distribution of interstitial proteins from large to small molecules and diminish the effect of washdown on the colloid osmotic pressure (COP) of interstitial fluid and lymph. Dogs were instrumented with coronary sinus balloon-tipped catheters and myocardial lymphatic cannulas to manipulate myocardial lymph flow and to collect lymph. Myocardial venous pressure was elevated by balloon inflation to increase transmicrovascular fluid flux and myocardial lymph flow. COP of lymph was measured directly and was also calculated from protein concentration. Decreases occurred in both protein concentration and COP of lymph. The proportion of lymph protein accounted for by albumin increased significantly, whereas that accounted for by β-lipoprotein decreased significantly. The change in the calculated plasma-to-lymph COP gradient was significantly greater than the change in the measured COP gradient. We conclude that the change in the distribution of interstitial fluid protein species decreases the effect of protein washdown on interstitial fluid COP and limits its effectiveness as a defense mechanism against myocardial edema formation.

interstitial heart disease; colloid osmotic pressure

gradients that regulate transmicrovascular filtration as represented in the Starling-Landis equation

\[ J_V = L_p A \left[ (P_{cap} - P_{int}) - \sigma_d (\Pi_p - \Pi_{int}) \right] \]

where \( J_V \) is the rate of microvascular filtration, \( L_p \) is hydraulic conductivity, \( A \) is the surface area available for microvascular fluid exchange, \( P_{cap} \) and \( P_{int} \) are the hydrostatic pressures within the capillary and interstitial space, respectively, \( \sigma_d \) is the osmotic reflection coefficient with a value between 0 and 1, and \( \Pi_p \) and \( \Pi_{int} \) are the COPs exerted by plasma and interstitial fluid, respectively (10). \( L_p \) represents the ease with which water traverses the microvascular barrier. The \( \sigma_d \) represents the effectiveness with which the colloid osmotic gradient is exerted across the microvascular barrier (10).

The phenomenon termed “protein washdown”, the decrease in protein concentration of lymph following an increase in \( J_V \), has been demonstrated to occur in the myocardium (6). This process, when combined with an increase in lymph flow, decreases the protein concentration and the COP of interstitial fluid. As can be seen from Eq. 1, this change acts to decrease \( J_V \), thus moderating edema formation.

The relationship between \( J_V \) and the protein concentration of lymph has been modeled as

\[ C_L/C_P = [(1 - \sigma_r) + PS/J_V]/(1 + PS/J_V) \]

where \( C_L \) and \( C_P \) are protein concentrations in lymph and plasma, respectively, \( \sigma_r \) is the solvent-drag reflection coefficient with a value between 0 and 1, and \( PS \) is the microvascular protein permeability-surface area product (21). Equation 2 demonstrates that an increase in \( J_V \) will act to decrease \( PS/J_V \) and, thereby, decrease \( C_L \) with respect to \( C_P \) such that \( C_L/C_P \) decreases and approaches \( 1 - \sigma_r \).

Plasma contains a wide variety of protein species that differ in size and charge as well as function. The effectiveness of the microvascular membrane as a barrier to movement of these protein species differs as a function of these physical properties. Therefore, the
terms $\sigma_f$ and $P$ from Eq. 2 will be different for each species of protein molecule. Because interstitial fluid is an ultrafiltrate of plasma, the distribution of proteins found in interstitial fluid is a function of the distribution found in plasma as well as the ease with which each protein species crosses the microvascular barrier. This distribution would be expected to change following an increase in $J_V$, because the relationship between $C_{f}/C_P$ and $J_V$ is different for each protein species.

The refractive index of a biological solution is a reliable and accurate indicator of protein concentration (16). Because of the documented relationship between COP and protein concentration (7, 14), the ease of measuring refractive index, and the difficulty associated with sampling interstitial fluid, $\Pi_{int}$ is often estimated by measuring $C_{f}$. However, $\Pi_{int}$ is dependent on both the concentration and the type of proteins within that fluid. It has been clearly demonstrated that albumin generates a higher COP per unit mass than larger protein molecules (17). This is because albumin has more particles per unit mass and because it induces a greater redistribution of ions via the Donnan ion effect (17).

In a study utilizing coronary sinus occlusion to induce myocardial edema, Laine and Granger (6) observed a substantial decrease in lymph protein concentration but a relatively small decrease in myocardial lymph COP. They further noted a shift in the distribution of lymph protein after washdown in favor of smaller protein species. We hypothesized that an increase in $J_V$ within the myocardium would cause a significantly greater decrease in the interstitial fluid concentration of large protein molecules than small ones, resulting in a shift in the distribution of lymph proteins to smaller molecules. We further hypothesized that, because of this distribution change, the effect of increased $J_V$ on the measured COP of lymph would be significantly less than the COP calculated from $C_{f}$, thereby moderating the effectiveness of protein washdown as a defense mechanism against myocardial edema.

MATERIALS AND METHODS

Animal preparation. All procedures were approved by the University of Texas Animal Welfare Committee and were consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Six mongrel dogs with body mass exceeding 15 kg and of either sex were used. Anesthesia was induced with thiopental sodium (20 mg/kg) and maintained with 1.0 to 2.5% halothane. After intubation, the dogs were ventilated with a respirator (Harvard Apparatus, South Natick, MA) set to deliver room air at a volume of 25 ml/kg and at a rate appropriate to maintain arterial $PCO_2$ between 35 and 40 mmHg. We introduced a Swan-Ganz catheter (5-Fr) into the cranial (superior) vena cava via the right jugular vein. A fluid-filled catheter connected to a pressure transducer was introduced into the femoral artery and the tip advanced to the abdominal aorta. All data from the transducer were recorded through a transducer amplifier and analog-digital converter (MacLab, Division of ADInstruments, Milford, MA) directly to a computer (Macintosh Quadra 950, Apple Computer, Cupertino, CA) executing Chart software (MacLab). Arterial pressure was monitored as an indicator of depth of anesthesia. A fluid-filled catheter was placed via the femoral vein into the caudal (inferior) vena cava for fluid or drug administration and for blood collection. After median sternotomy, we advanced the previously inserted Swan-Ganz catheter into the coronary sinus and sutured it to the free wall of the sinus so that it did not compromise coronary sinus flow. The prenodal lymphatic trunk draining the left ventricle was identified and cannulated as previously described (6, 19). This lymphatic vessel has been estimated to drain ~85% of total cardiac lymph (9). We administered heparin sodium intravenously (300 U/kg body wt). The lymph cannula was connected via fluid-filled tubing to a graduated pipette set at a height equal to that of the cannulation site.

Physiological measurements. The myocardial lymphatic cannulation allowed assessment of lymph flow and collection of lymph for determination of COP and the concentrations of total protein, albumin, and $\beta$-lipoprotein. Blood was collected from the femoral vein catheter and centrifuged to produce plasma. COPs of lymph and plasma were measured directly using a colloid osmometer (model 4400, Wescor, Logan, UT). Total protein concentrations of lymph and plasma were determined by refractometry (AO TS Meter, American Optical, Buffalo, NY). To facilitate measurement of concentrations $<$2.5 g/dl, a plot was constructed from the scales on the refractometer relating protein concentration to refractive index. Because this relationship is linear, we measured the refractive index for each sample and used the derived relationship to calculate the protein concentration. COP values were also determined by calculation using the curvilinear relationship between COP and plasma protein concentration (PC) reported by Landis and Pappenheimer (7).

$\text{COP} = 2.1 \text{PC} + (0.16 \text{PC})^2 + (0.009 \text{PC})^3$ (3)

Protein fractions were determined by electrophoresis as previously described (6). Plasma and lymph samples were combined with a 40% sucrose solution and Bromphenol blue tracking dye. Polycrylamide gradient gels (PAA4/30, Pharmacia) were electrophoresed in a Tris-barbital/sodium barbital buffer (Gelman) at a constant voltage of 70 V for 30 min followed by 100 V for 1 h. The gels were fixed in 10% sulfosalicylic acid solution and then stained in a Ponceau S and 7.5% trichloroacetic acid solution. Destaining, utilizing the Destainer GC-411 and 10% acetic acid, was performed in a 7% acetic acid solution and then stained in a Ponceau S and 7.5% trichloroacetic acid solution. Destaining, utilizing the Destainer GC-411 and DPS power supply (Pharmacia), was performed in a 7% acetic acid solution at 25 V for 1–2 h or until the background was clear. The gels were then scanned on a scanning densitometer (model R-112, Beckman) for protein densities at a wavelength of 520 nm.

Experimental protocol. Lymph and plasma samples were obtained for the determination of baseline values for COP and concentrations of total protein and for electrophoresis. Baseline values for lymph flow rate were also determined. The Swan-Ganz catheter balloon was then inflated with oil so that the coronary sinus flow was reduced and myocardial microvascular pressure was increased. This technique reliably induces acute myocardial edema formation and increased cardiac lymph flow (4, 6, 15). One hour after balloon inflation, lymph flow was measured and lymph and plasma samples were collected for determination of COP and total protein concentrations and for electrophoresis.

Data analysis. All values are reported as means ± SE unless otherwise noted. Albumin and $\beta$-lipoprotein concen-
Concentrations were calculated as fractions, determined by electrophoresis, of the total protein concentration. We calculated plasma-to-lymph gradients (the difference between the plasma and lymph) for measured and calculated COP values and compared them using a paired *t*-test. Baseline and postocclusion values for each variable were also compared using a paired *t*-test. We performed data analysis with a computer executing SigmaStat (SSPS, Chicago, IL). A value of *P*, 0.05 was considered significant.

RESULTS

Partial occlusion of the coronary sinus resulted in a 5.6 ± 0.7 (SD)-fold increase in lymph flow. These data were not further analyzed because the magnitude of both lymph flow and the change in lymph flow are affected by cannula height (5). Sinus occlusion caused no significant change in COP or protein concentrations in plasma but caused significant decreases in COP and concentrations of total protein, albumin, and β-lipoprotein in lymph (Table 1). The measured and calculated plasma-to-lymph gradients for COP increased significantly following coronary sinus occlusion (Table 2). The increase in the COP gradient was significantly greater for calculated values (8.3 ± 0.7 mmHg) than for measured values (2.7 ± 1.0 mmHg) (*P < 0.01). The proportion of the total protein in lymph accounted for by albumin increased significantly following occlusion, whereas that accounted for by β-lipoprotein decreased significantly (Table 2).

DISCUSSION

Coronary sinus occlusion has been demonstrated to be a reliable model for induction of myocardial edema. Coronary sinus occlusion results in a decrease in cardiac lymph protein concentration and increases in the following variables: coronary venous and capillary pressures, myocardial microvascular filtration rate, myocardial interstitial water content and pressure.

Table 1. Effect of partial coronary sinus occlusion on colloid osmotic pressure and protein concentrations in cardiac lymph and plasma

<table>
<thead>
<tr>
<th></th>
<th>COP, mmHg</th>
<th>Protein, g/dl</th>
<th>Albumin, g/dl</th>
<th>β-Lipoprotein, g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lymph</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>13.3 ± 0.5</td>
<td>5.1 ± 0.1</td>
<td>2.5 ± 0.2</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>Postocclusion</td>
<td>10.4 ± 0.5*</td>
<td>2.7 ± 0.1*</td>
<td>1.8 ± 0.1*</td>
<td>0.0 ± 0.0*</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>21.0 ± 0.5</td>
<td>6.2 ± 0.1</td>
<td>2.9 ± 0.2</td>
<td>0.5 ± 0.0</td>
</tr>
<tr>
<td>Postocclusion</td>
<td>20.8 ± 0.6</td>
<td>6.1 ± 0.1</td>
<td>2.8 ± 0.2</td>
<td>0.5 ± 0.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. COP, colloid osmotic pressure. *P < 0.01 compared with baseline value.

Table 2. Effect of partial coronary sinus occlusion on the plasma-to-lymph gradient for measured and calculated COP and relative proportions of albumin and β-lipoprotein

<table>
<thead>
<tr>
<th></th>
<th>Measured COP, mmHg</th>
<th>Calculated COP, mmHg</th>
<th>β-Lipoprotein, %</th>
<th>Albumin, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td>7.7 ± 0.6</td>
<td>5.5 ± 0.6</td>
<td>50.0 ± 4.2</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td><strong>Postocclusion</strong></td>
<td>10.4 ± 0.9*</td>
<td>13.8 ± 0.7†</td>
<td>67.5 ± 5.3†</td>
<td>0.5 ± 0.3†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Albumin (%) and β-lipoprotein (%) represent the percentage of total protein in cardiac lymph accounted for by albumin and β-lipoprotein, respectively. *P < 0.05 compared with baseline value; †P < 0.01 compared with baseline value.

Fig. 1. Colloid osmotic pressure (COP) of lymph samples plotted as a function of total protein concentration (TP; baseline, closed circles; postocclusion, open circles). Linear regression of the baseline and postocclusion data area represented as a solid line. Additionally, previously derived relationships between plasma protein concentrations and COP are shown. The curves reported by Landis and Pappenheimer (7) for albumin (A), globulin (G) and TP (TPL) are represented as dotted lines. The curve reported by Navar and Navar (14) for TP (TPN) is represented as a dashed line.

Fig. 2. COP of plasma samples calculated from the plasma protein concentration using Eq. 3 derived by Landis and Pappenheimer (7) plotted as a function of the measured COP for the same samples superimposed on a line of unity.
cardiac lymph flow, and fluid transudation across the epicardium (4, 6, 15, 18).

Our data demonstrate that the protein washdown that results from increased microvascular filtration in the myocardium is accompanied by a significant change in the distribution of protein species in lymph. This change is characterized by a proportional increase in small protein molecules, primarily represented by albumin (Stokes-Einstein radius of 37 Å; 69,000 mol wt), and a decrease in large protein molecules represented by β-lipoprotein (Stokes-Einstein radius of 120 Å; >2,000,000 mol wt) (7, 21). As a result of the shift to smaller, more osmotically active protein molecules, the plasma-to-lymph gradient for measured COP increased by only 35%, whereas the COP gradient calculated from protein concentration increased by over 150%. This reduced response of lymph COP to protein washdown limits the effectiveness of protein washdown as a defense mechanism against edema in the myocardium.

Figure 1 demonstrates COP of myocardial lymph plotted as a function of protein concentration from baseline and postocclusion data. As a reference, the relationships between COP and the concentrations of total protein, albumin, and globulin calculated by Landis and Pappenheimer (7) and by Navar and Navar (14) are also included. Figure 1 demonstrates that the washdown-induced change in protein distribution causes the lymph COP-protein concentration relationship to move across rather than down the traditional curves. Extrapolation of this relationship to the zero-zero intercept is not helpful because further increases in the microvascular filtration rate would drive the lymph protein concentration to some filtration independent value rather than zero (21). The washdown-induced change in lymph protein distribution should not affect the accuracy of refractive index as a measure of protein concentration because refractive index is affected by protein mass rather than particle number (16). These results further suggest that protein concentration of myocardial lymph is an unreliable indicator of COP and any assessment of lymph COP should be made by direct measurement.

In contrast to the results of our analysis of lymph, a comparison of measured and calculated values for plasma COP (Fig. 2) demonstrates a highly predictive relationship. This finding is expected because the equation for calculating COP reported by Pappenheimer and Landis (Eq. 3) (7) was derived using plasma values for protein concentration and COP.

Complete assessment of the effect of protein washdown on the transmicrovascular COP gradient (\(\Pi_p - \Pi_{int}\)) must include the effect of the \(\sigma_d\). The value for \(\sigma_d\) is dependent on the permeability of the barrier to protein such that it equals “zero” when the barrier is completely permeable to protein and equals “one” when the barrier is completely impermeable. The \(\sigma_d\) value for total protein has not been determined for the myocardial microvasculature; however, estimated values of 0.75 and 0.96 have been reported for albumin and β-lipoprotein, respectively (1, 6).

Protein washdown functions as a protective mechanism against edema formation by decreasing the interstitial protein concentration and, therefore, the COP of interstitial fluid. This change, in turn, moderates \(J_V\) by decreasing the total transmicrovascular pressure gradient (Eq. 1).

A clearer understanding of the relative importance of protein washdown as an antiedema mechanism can be gained by using the evaluation method described by Taylor (20), where the response of each protective mechanism to an edematogenic stress is calculated in terms of transmicrovascular pressure gradient. We performed this analysis using the following assumptions: the myocardial microvascular reflection coefficient was equal to one, and the observed increase in lymph flow was representative of the actual physiological response with the understanding that the magnitude of the lymph flow increase through a cannulated lymphatic vessel is dependent on the height of the outflow cannula (5). Furthermore, we modified Taylor’s approach to include the change in epicardial transudation as well as myocardial lymph flow in the estimation of steady-state microvascular filtration (18). Using data from the current study and previously published information, we calculated the following relative contributions of antiedema mechanisms following coronary sinus occlusion: increased myocardial interstitial hydrostatic pressure represented 86%, increased lymph flow and transepidermal transudation represented 6%, and increased COP gradient represented 8%. These estimates correspond closely to previous estimates for the heart (20, 22).

The data presented here demonstrate that, during protein washdown associated with increases in microvascular filtration, the distribution of protein species in lymph changes and the lymph protein concentration-COP relationship moves from a curve describing large protein molecules to one describing smaller molecules. The effectiveness of protein washdown in reducing the COP of the myocardial interstitial fluid under these conditions is diminished, and the decline in the interstitial fluid COP will be overestimated if the change in lymph protein concentration is used as a guide.

This study was supported by National Heart, Lung, and Blood Institute Grants HL-36115 and HL-01999 and the American Heart Association.

REFERENCES


