Artículo:

Effects and behaviour of polidisperse macromolecules in low pressure pulmonary edema secondary oleic acid infusion

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Effects and behaviour of polidisperse macromolecules in low pressure pulmonary edema secondary oleic acid infusion

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ABSTRACT. Previously in low-pressure pulmonary edema (LPPE) induced with oleic acid (OA) in isolated lobes, we achieved edema reabsorption with 6% Hetastarch (HTS) a colloidal plasma expander.1,2 In this study, this approach was tested in intact animals. Severe LPPE was produced in 19 dogs with intravenous OA (0.075 mg/kg). Two hours later, dogs were randomized into a control (n = 9) and a treatment group (n = 10). In the treatment group, HTS was infused to maintain oncotic pressures (COP) at COP > 30 mmHg. This required ultrafiltration of excess fluid to sustain pulmonary wedge pressure (PWP) at baseline levels. To control for the effects of ultrafiltration, non-treated HTS dogs (controls) were infused normal saline and were ultrafiltered to maintain comparable PWP. In the HTS treated animals, COP rose to 34.7 ± 10, compared to 5.1 ± 3.2 mmHg in controls. There were no differences in PWP. Although significant HTS extravasations occurred suggested by dry weight of treated lungs 2.84 ± 0.29 compared to controls 2.33 ± 0.3 g/kg,dog.weight, (p < 0.05), edema was significantly improved in HTS treated animals, as deduced from a significant reduction in pulmonary wet weight 24.9 ± 5.8 g/kg dog weight in comparison with control group 34.3 ± 11.8 (p < 0.05). The administration of HTS reduced the edema formation in severe LPPE secondary to OA but not enough to significantly improve gas exchange.

Key words: Pulmonary edema, oleic acid, Hetastarch.

INTRODUCTION

As summarized in the Starling equation, the rate and direction of transvascular fluid exchange (Jv) are determined by the hydrostatic and oncotic gradients across exchange capillaries by the conductance of the membrane (KF). The reflection coefficient σ of the membrane to proteins corrects the predicted oncotic gradient (intravascular oncotic pressure niv-interstitial oncotic pressure nis) for the fact the membrane is not ideally semi permeable.

Jv = Kf ([Piv-Pis] − σ [niv-nis])

Improvement in LPPE has been achieved by decreasing in capillary pressure,3 however, this is not always possible in a clinical situation. Others treatments should be sought.
Previously we achieve edema reabsorption with plasma expander colloid infusion (6% Hetastarch) (HTS) in low pressure pulmonary edema (LPPE) induced with oleic acid (AO) in isolated lobes. In this study, after oleic acid pulmonary edema was produced, the same approach was tested in intact animals. We anticipated different problems in this model: firstly, HTS can be diluted with reabsorption fluid from all tissues with consequent loss in oncotic pressure and secondly the metabolic breakdown of these large molecules may also serve to decrease their effectiveness by decreasing the reflection coefficient of smaller particles.

METHODS

Nineteen mongrel dogs (weight between 15-25 kg) were anaesthetized (pentobarbital, 30 mg/kg), intubated with a No 9 endotracheal tube, and ventilated at a tidal volume of 15 mL/kg with 100% O\textsubscript{2}. The ventilator frequency adjusted (12-18 min) to maintain the arterial partial pressure of CO\textsubscript{2} (PcO\textsubscript{2}) near 35 Torr (Harvard animal respiratory with Douglas bag reservoir). Anaesthesia was maintained with intermittent doses of pentobarbital 30 to 60 mg every one or two hours.

The right jugular vein was exposed, and a thermistor-tipped, balloon-tipped catheter connected to a pressure transducer was inserted under continuous pressure monitoring in to the pulmonary artery for pulmonary artery pressure (PaP) measurement. With the balloon inflated, PWP could be obtained, as well as providing samples of mixed venous blood.

Gas exchange analysis. Qs/Qt is calculated by \((\text{CO}_2 - \text{CaO}_2)/(\text{CcO}_2 - \text{CvO}_2)\), where CvO\textsubscript{2}, where CcO\textsubscript{2}, CaO\textsubscript{2}, and CvO\textsubscript{2} are the O\textsubscript{2} contents of the end-capillary, arterial and mixed venous blood, respectively. CaO\textsubscript{2} and CvO\textsubscript{2} are measured directly using a CO scrubbing technique, and CcO\textsubscript{2} is calculated according to \(\text{CcO}_2 = (\text{PB} - 47 - \text{PaCO}_2 - \text{Pa}_0) 0.003 + \text{CaO}_2\). Where PB is barometric pressure and PaO\textsubscript{2} and PaCO\textsubscript{2} are the partial pressure of O\textsubscript{2} in arterial and venous blood respectively. In the case of PaO\textsubscript{2} decrease under 120 mmHg, arterial oxygen saturation (SaO\textsubscript{2}) was often less than 100%, and the corresponding saturation of blood exposed to ideal alveolar gas (SaO\textsubscript{2}) were estimated using PaO\textsubscript{2}, Ph and temperature, alveolar oxygen tension (PaO\textsubscript{2}), and Cain’s monogram; where \(\text{CcO}_2 = (\text{CaO}_2 - 0.003 \times \text{PaO}_2) \text{ SaO}_2/\text{SaO}_2 + 0.003 \times \text{PaO}_2\). And PaO\textsubscript{2} = \((\text{Pb} - 47 - \text{PaCO}_2)\).

Experimental protocol

Baseline hemodynamic measurements Qt, Ppa, PWP, and BP measurements were obtained. After time was allowed for these variables to stabilize, mixed venous and arterial blood samples were drawn, and PO\textsubscript{2}, PCO\textsubscript{2}, and Ph were measured with appropriately calibrated electrodes. Drugs and fluids were administered using a 20 cm catheter placed in the left femoral vein. The oleic acid (0.075 mg/kg) was then slowly infused into the inferior vena cava over 5 min. The following 2 hrs we maintained the baseline PWP with infusion of saline 0.9%. After two hours measurements were obtained Qt, systemic and pulmonary pressure, venous and arterial blood gases and O\textsubscript{2} content; furthermore, oncotic pressure (COP) was determined with a membrane oncometer (Wescor colloid osmometer model 4400) and hemocrit (Hct) was obtained with microhemocrit technic. After anticoagulation with heparin (initially 400 units/kg, supplemented with 50 u/kg every 2 hours, except if excessive bleeding incision sites occurred), we dissected the right femoral artery and vein, and dialysis catheters were introduced and an ultrafilter (fibro hem filter gambo) was installed. Dogs were randomized into a control (n = 9) and treatment (n = 10) group.

Experimental groups

Treatment Group (T). In ten dogs we infused hetastarch (6%). For 4 h, we measured hourly Qt, Ppa, PWP, BP, COP, Hct and gas exchange. In the first two hours we calculated the amount of hetastarch with the following formula: \((\text{Hct} - 1) \times 70 \text{ mL} \times \text{kg})\) which is an estimation of plasma volume. During the third and fourth hours, HTS infusion was continued to maintain COP above 30 mmHg. Due to reabsorption of liquid from all tissues, ultrafiltration of excess fluid was required to maintain PWP at baseline levels.

Control group (C). Nine dogs were similarly instrumented. Repeated measurements were made at the same intervals. The PWP was maintained at baseline values throughout the remainder of the experiment with infusion of saline 0.9%. To reproduce any possible effects of ultrafiltrated throughout the 4 hours, replacing the ultrafiltered volume with saline 0.9%.

Gravimetric analysis. After the last set of measurements was taken, all the animals were exsanguinated and then sacrificed, their chest were rapidly opened, the trachea dissected and clamped, the vascular pulmonary hilar was dissected and the lungs excised, the hila vessels were not clamped. After obtaining the wet weight (WW), the lungs were dried with flow air for 48 hours, and them put in an oven at 40° to dry to a constant weight, and dry weight was obtained (DW). Some of the variation in lobar weights could be due to variation in size of the dogs. To facilitate comparisons, the wet and dry weights in grams were expressed as a fraction of body weight (BW) in kilograms. Statistical analysis. Analysis of variance (ANOVA two-way) was applied to compare the groups during each phase of the study. This was followed by multirange test to compa-
the groups individually; furthermore, we used students test to analyze the difference in wet weight, and dry weight between groups.

RESULTS

Tables 1 and 2 summarize the effect of 0.075 mg/kg oleic acid on hemodynamic measurements and gas exchange in 19 dogs, separated in two groups. Two hours after injection of oleic acid, PaO$_2$, PvO$_2$, Ph and Qt fell and Qs/Qt rose significantly in both groups in comparison with the baseline measurement (P < 0.001); analysis of variance showed no significant difference among groups; furthermore, PaCO$_2$, mean pulmonary pressure (PPX), and PWP did not change.

After the groups were randomized, PaO$_2$ and PvO$_2$ increased and Qs/Qt decreased in the HTS group during the four hours of treatment suggesting some improvement, however no significant difference between treatment and control groups were obtained (Figure 1). The CO decreased progressively during the experiment in both groups at similar ratio (p = NS). At the fourth hour of HTS infusion we achieved to increase COP in this group from 7.5 ± 3.3 to 34.7 ± 10 mmHg, compared to control group in which COP drops from 7.3 ± 3.3 to 5.1 ± 3.2 mmHg. There were no differences in PWP during all the experiments (Figure 2).

Mean wet weight / body weight, wet weight / dry weight (WW/BW and WW/DW in g/kg.dog.weight of treated lung are shown in table 3, figures 3, 4 and 5 show significantly

<table>
<thead>
<tr>
<th>PaO$_2$ Torr (T)</th>
<th>503 ± 30</th>
<th>232 ± 143</th>
<th>200 ± 136</th>
<th>191 ± 136</th>
<th>215 ± 150</th>
<th>262 ± 180</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO$_2$ Torr (C)</td>
<td>507 ± 55</td>
<td>188 ± 146</td>
<td>153 ± 149</td>
<td>125 ± 118</td>
<td>135 ± 129</td>
<td>169 ± 137</td>
</tr>
<tr>
<td>PvO$_2$ Torr (T)</td>
<td>74 ± 9.6</td>
<td>51 ± 6.4</td>
<td>48 ± 5.6</td>
<td>43 ± 4.2</td>
<td>48 ± 14</td>
<td>53 ± 27</td>
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<tr>
<td>PvO$_2$ Torr (C)</td>
<td>77 ± 17</td>
<td>47 ± 10</td>
<td>39 ± 8</td>
<td>38 ± 12</td>
<td>38 ± 11</td>
<td>39 ± 10</td>
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<tr>
<td>PaCO$_2$ Torr (T)</td>
<td>36 ± 3.8</td>
<td>37 ± 7.1</td>
<td>40 ± 7.6</td>
<td>40 ± 4.7</td>
<td>38 ± 3.6</td>
<td>38 ± 7.7</td>
</tr>
<tr>
<td>PaCO$_2$ Torr (C)</td>
<td>38 ± 4.9</td>
<td>38 ± 8.2</td>
<td>37 ± 5.5</td>
<td>37 ± 4.6</td>
<td>37 ± 4.8</td>
<td>39 ± 8.5</td>
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<td>Pha (T)</td>
<td>7.37 ± .03</td>
<td>7.24 ± .12</td>
<td>7.22 ± .11</td>
<td>7.34 ± .07</td>
<td>7.34 ± .10</td>
<td>7.36 ± .11</td>
</tr>
<tr>
<td>Pha (C)</td>
<td>7.32 ± .07</td>
<td>7.23 ± .12</td>
<td>7.29 ± .6</td>
<td>7.23 ± .6</td>
<td>7.22 ± .07</td>
<td>7.21 ± .08</td>
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<tr>
<td>Qs/Qt (T)</td>
<td>19.9 ± 8.1</td>
<td>33.4 ± 21</td>
<td>44.1 ± 22</td>
<td>35.3 ± 19</td>
<td>31.1 ± 25</td>
<td>32.7 ± 21</td>
</tr>
<tr>
<td>Qs/Qt (C)</td>
<td>17.0 ± 6</td>
<td>36.3 ± 23</td>
<td>42.6 ± 25</td>
<td>48.4 ± 28</td>
<td>45.3 ± 27</td>
<td>42.4 ± 30</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>C.O. LTS/M (T)</th>
<th>5.0 ± .9</th>
<th>3.56 ± 1.7</th>
<th>3.54 ± .81</th>
<th>3.52 ± .83</th>
<th>3.22 ± .96</th>
<th>2.8 ± .43</th>
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</thead>
<tbody>
<tr>
<td>C.O. LTS/M (C)</td>
<td>5.22 ± 1.22</td>
<td>3.6 ± .6</td>
<td>3.27 ± .52</td>
<td>3.85 ± 1.4</td>
<td>3.27 ± 1.1</td>
<td>3.49 ± 1.3</td>
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<td>PPX mmHg (T)</td>
<td>10.7 ± 2.2</td>
<td>11.7 ± 2.1</td>
<td>12.5 ± 4.2</td>
<td>19.5 ± 5.1</td>
<td>13.5 ± 3.8</td>
<td>14.4 ± 3.9</td>
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<td>PPX mmHg (C)</td>
<td>11.1 ± 2.2</td>
<td>10.8 ± 2.3</td>
<td>10.8 ± 1.7</td>
<td>17.2 ± 2.7</td>
<td>12.9 ± 2.1</td>
<td>14.5 ± 2.6</td>
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<td>PWP mmHg (T)</td>
<td>4.2 ± 1.5</td>
<td>4.9 ± 2.2</td>
<td>4.8 ± 2.4</td>
<td>4.9 ± 2.7</td>
<td>4.8 ± 2.2</td>
<td>4.6 ± 2.3</td>
</tr>
<tr>
<td>PWP mmHg (C)</td>
<td>5.1 ± 1.2</td>
<td>3.8 ± 2.0</td>
<td>3.8 ± 1.8</td>
<td>4.3 ± 1.6</td>
<td>4.4 ± 1.9</td>
<td>5.2 ± 2.0</td>
</tr>
<tr>
<td>COP mmHg (T)</td>
<td>7.5 ± 3.3</td>
<td>15.3 ± 4.8</td>
<td>20.4 ± 7.5</td>
<td>27.5 ± 11.3</td>
<td>34.7 ± 10.2</td>
<td></td>
</tr>
<tr>
<td>COP mmHg (C)</td>
<td>7.0 ± 3.3</td>
<td>6.4 ± 3.2</td>
<td>5.7 ± 3.5</td>
<td>5.2 ± 3.3</td>
<td>5.1 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>HcT % (T)</td>
<td>35.2 ± 4.6</td>
<td>30.4 ± 4.2</td>
<td>29.3 ± 5.5</td>
<td>28 ± 4.2</td>
<td>29.6 ± 4.9</td>
<td></td>
</tr>
<tr>
<td>HcT % (C)</td>
<td>35.4 ± 5.9</td>
<td>34.3 ± 6.9</td>
<td>34.3 ± 6.9</td>
<td>33.0 ± 5.6</td>
<td>33.0 ± 5.6</td>
<td></td>
</tr>
</tbody>
</table>

Tables 1 and 2 summarize hemodynamic and gas exchange between treatment group (T) and control group (C)
Effects and behavior of polidisperse macromolecules in low pressure pulmonary edema

GAS EXCHANGE

Table 3.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HTS</th>
<th>NO O.A.**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet weight/BW</td>
<td>34.3 ± 11.8</td>
<td>24.9 ± 5.8*</td>
<td>8.61 ± 1.3</td>
</tr>
<tr>
<td>Dry weight/BW</td>
<td>2.33 ± 0.3</td>
<td>2.84 ± 0.29*</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Wet W/Dry W</td>
<td>14.5 ± 3.5</td>
<td>8.9 ± 1.7*</td>
<td>4.6 ± 0.2</td>
</tr>
</tbody>
</table>

* Denote differences (p < 0.05) from control group.
** Data from Prewitt’s study.

Table 4.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HTS</th>
<th>P =</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrafiltration</td>
<td>1,047 ± 257</td>
<td>3,459 ± 593</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Diuresis</td>
<td>1,973 ± 724</td>
<td>274 ± 138</td>
<td>= 0.0001</td>
</tr>
<tr>
<td>Balance</td>
<td>1,154 ± 1,325</td>
<td>-36.4 ± 951</td>
<td>= 0.0001</td>
</tr>
</tbody>
</table>

Figure 1. Gas exchange between treatment group (HTS) and control group during 6 hours of experiment.

This is a study model of acute pulmonary edema. Two hours after intravenous injection of oleic acid, intrapulmonary shunt increased and PaO₂ decreased significantly, secondary to alveolar flooding. The severity of injury, analyzed with gas exchanges and lung weights were comparable to similar studies. In this study all the gas exchange parameters in the treated animals showed some improvement after 3 hour of HTS infusion. The DW/BW of the HTS group was higher.
than control group, and the difference was found to be significant. This difference reflects an increase in no evapora-
table substances; we used to interpreted that the increase in
dry weight is due to extra vascular blood; however, in this
particular experiment increased of DW/BW of the treated
lungs suggested HTS extravasations, related to the pro-
portion of HTS which cross the circulation and the coeffi-
cient reflection of HTS after O.A. Nevertheless, we can not
excluded more haemorrhagic edema in HTS treated group.
We found that pulmonary edema was significantly impro-
ved in HTS treated animals as deduced from a significant
reduction in lung wet weight. We considered these gravi-
metric measurements as the gold standard.
In isolated lobe preparation with a stepwise pressure
elevation technique, Oppenheimer and cols observed
that administration of 25 grams of albumin resulting in
an increase of COP by 24 mmHg, produced a change
in Pc from 12.2 to 23 cm H₂O; Pc is commonly
interpreted as the Pc at which the forces across the
microvasculature are in balance. After the addition of
albumin, Pc < Pc induces constant weight loss along
the same rate of filtration to the Pc relationship. During
edema reabsorption the non-convective transport of pro-
teins from plasma to the interstitial space tend to equi-
librate the osmotic gradient, send the loss of water from
interstitial to intravascular could raise the protein con-
centration in the interstitial space. As a result, a de-
crease in the transmembrane oncotic gradient oc-
curred, the rate of reabsorption might decrease progress,
and with time, some filtration might reassumed at a
lower rate. In this study we do not know if the improve-
ment in lung wet weight was secondary to edema reab-
sorption or only a decrease in the rate of filtration during
the 4 hours of treatment.
In a study in whole dog preparation, Prewitt and cols found a decrease in pulmonary edema formation by reducing the PWP by 5 mmHg; however, they found no improvement in a group treated with a 50 gram albumin infusion resulting in increases in COP by 6.0 mmHg. They assumed that albumin infusions in vivo did not change Kf, σ, or nis, and they concluded that increased intravascular oncotic pressure niv did not reduce edema, because oleic acid reduced σ and the protein concentration difference (niv - nis). The main difference between the Prewitt study and ours was due to the fact that they used albumin, which has lower σ in comparison with HTS. The difference in σ is likely due to a lower molecular weight (mw 68,000 for albumin and mw > 450,000 for HTS), different molecular shape, and different rigid properties. Reeking and cols, in dog paw preparation found, for proteins σ increases regularly with increasing molecular size, but σ for dextran is greater than for protein of comparable effective diffusion radius (cre). It is possible that the negative charge of the proteins facilitates their transport relative to uncharged dextran or that permeation of the long-chain dextran molecule is hindered relative to the more compact rigid protein molecules. Although, OA decreased σ for HTS, the oncotic gradient between intravascular and interstitial spaces could have been enough to decrease the rate of filtration or produce edema reabsorption in our treated dogs. Another possible explanation for the differences between Prewitt’s and our results might be the amount of oncotic agent administered and the changes in niv in our work were larger than those in his experiment.

To maintain COP > 30 mmHg and PWP at baseline level and to prevent the loss of effectiveness of HTS secondary to reabsorption of liquid from all tissues due high COP, ultra filtration of excess fluid was required. Ultra filtration could reduce pulmonary edema by itself by removing some toxic substance. In dog preparation Sivak and cols demonstrated in pulmonary edema induced with OA, improvement in extravascular lung water measurements in a group treated with ultrafiltration. However, these improvements were not different compared to a group treated with furosemide. To control for the effects of ultrafiltration, the control group was infused with normal saline and was ultrafiltered to maintain comparable PWP. The control group was ultrafiltered during the same period of time, but the amount of ultra filtrated fluid required to maintained the baseline PWP was significantly less, secondary to decreases in intravascular volume consequent to low oncotic pressure and very high filtration in all tissues. The total balance of fluid in HTS group was slightly negative at the end of the experiment –36 mL versus a positive balance for control (Table 4). This balance is related as mention before to an increase in ultrafiltration required in the treated group, the Hct during HTS infusions and at the end of the experiment were lower in the HTS group reflecting the immense amount of fluid reabsorbed from all the tissues and the dilution of the red cells, even though the enormous amount of ultrafiltrated fluid obtained in this group.

**CONCLUSIONS**

HTS reduces the edema formation in severe LPPE secondary to OA but not enough to significantly improve gas exchange.

**REFERENCES**