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Original research article

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

Andrés Palomar,[1] Luis Oppenheimer[2]

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 ultra filtration 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 $\pm$ 10, compared to 5.1 $\pm$ 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 $\pm$ 0.29 compared to controls 2.33 $\pm$ 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 $\pm$ 5.8 g/kg dog weight in comparison with control group 34.3 $\pm$ 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.*

RESUMEN. Previamente en edema pulmonar de bajas presiones inducido con ácido oleico (AO) en lóbulos aislados, logramos producir reabsorción de edema con el uso de Hetastarch (HTS) al 6% que es un agente coloide expansor del plasma. En este estudio, este abordaje fue probado en animales intactos. Importante edema pulmonar de bajas presiones fue producido en 19 perros con la infusión de AO intravenoso (0.075 mg/kg). Dos horas más tarde, los perros fueron separados en forma aleatoria en grupo control (n = 9) y en grupo de tratamiento (n = 10). En el grupo de tratamiento, HTS fue infundido para mantener una presión oncótica plasmática (COP) mayor de 30 mmHg. Esto requirió ultrafiltración del exceso de líquido para mantener una presión capilar pulmonar (PCP) a niveles basales. Para controlar los efectos de la ultrafiltración, los perros no tratados con HTS (control) fueron infundidos con solución salina normal y fueron ultrafiltrados para mantener una comparable PCP. En el grupo tratado con HTS, la COP se elevó a 34.7 ± 10, comparado con 5.1 ± 3.2 mmHg del grupo control. No existieron diferencias entre los grupos en cuanto al nivel de PCP. Aunque existió una significante extravasación del HTS sugerido por el incremento en el peso seco de los pulmones tratados 2.84 ± 0.29 comparado con controles 2.33 ± 0.3 g/kg/peso del perro, ($p < 0.05$), el edema fue significativamente reducido en el grupo tratado con HTS, deducido de una significativa reducción en el peso húmedo pulmonar final 24.9 ± 5.8 g/kg/ peso del perro. En comparación con el grupo control 34.3 ± 11.8 ($p < 0.05$). La administración de HTS reduce la formación de edema pulmonar secundaria a infusión de AO, pero no lo suficiente para producir mejoría significativa en el intercambio gaseoso.
Palabras clave: *Edema pulmonar, ácido oleico, 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.

$$J_v = Kf([Piv-Pis) - \sigma [niv-nis])$$

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.

[1] Clínica Lomas Altas México, D.F
[2] Departments of Sugery and Critical Care, University of Manitoba, Winnipeg, Manitoba R3E OZ3 Canada.

Address for correspondence:
Andrés Palomar MD, FCCP. Clínica Lomas Altas Reforma 2608-10 C.P. 11950. Tel: 5081-8100, Fax. 5081-8295, México D.F. E-mail apalomarl@hotmail.com.

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_2. The ventilator frequency adjusted (12-18 min) to maintain the arterial partial pressure of CO_2 (PCO_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 (CCO_2–CaO_2)/(CcO_2 – CvO_2), where CvO_2, where CcO_2, CaO_2, and CvO_2 are the O_2 contents of the end-capillary, arterial and mixed venous blood, respectively. CaO_2 and CvO_2 are measured directly using a CO scrubbing technique, and CcO_2 is calculated according to CcO_2 = (PB – 47 –$PaCO_2$-$Pa0_2$) 0.003 + CaO_2. Where PB is barometric pressure and PaO_2 and $PaCO_2$ are the partial pressure of O_2 in arterial and venous blood respectively. In the case of PaO_2 decrease under 120 mmHg, arterial oxygen saturation (SaO_2) was often less than 100%, and the corresponding saturation of blood exposed to ideal alveolar gas (SaO_2) were estimated using PaO_2, Ph and temperature, alveolar oxygen tension (PaO_2), and Cain's monogram; where CcO_2 = (CaO_2 – 0.003 x PaO_2) SaO_2/SaO_2 + 0.003 x PaO_2. And PaO_2 = (P_B-47-$PaCO_2$).

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_2, PcO_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_2 content; furthermore, oncotic pressure (COP) was determined with a membrane oncometer (Wescor colloid osmometer model 4400) and hematocrit (Hct) was obtained with microhematocrit 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 gambro) 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: (Hct-1 * 70 mL * 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 reabsortion 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 hilia 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-

re 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 1.

	Base	Ac. oleic 2 h	1 h ULTF	2 h ULTF	3 h ULTF	4 h ULTF
PaO_2 Torr (T)	503 ± 30	232 ± 143	200 ± 136	191 ± 136	215 ± 150	262 ± 180
PaO_2 Torr (C)	507 ± 55	188 ± 146	153 ± 149	125 ± 118	135 ± 129	169 ± 137
PvO_2 Torr (T)	74 ± 9.6	51 ± 6.4	48 ± 5.6	43 ± 4.2	48 ± 14	53 ± 27
PvO_2 Torr (C)	77 ± 17	47 ± 10	39 ± 8	38 ± 12	38 ± 11	39 ± 10
$PaCO_2$ Torr (T)	36 ± 3.8	37 ± 7.1	40 ± 7.6	40 ± 4.7	38 ± 3.6	38 ± 7.7
$PaCO_2$ Torr (C)	38 ± 4.9	38 ± 8.2	37 ± 5.5	37 ± 4.6	37 ± 4.8	39 ± 8.5
Pha (T)	7.37 ± .03	7,24 ± .12	7.22 ± .11	7.34 ± .07	7.34 ± .10	7.36 ± .11
Pha (C)	7.32 ± .07	7.23 ± .12	7.29 ± 0.6	7.23 ± 0.6	7.22 ± .07	7.21 ± .08
Qs/Qt (T)	19.9 ± 8.1	33.4 ± 21	44.1 ± 22	35.3 ± 19	31.1 ± 25	32.7 ± 21
Qs/Qt (C)	17.0 ± 6	36.3 ± 23	42.6 ± 25	48.4 ± 28	45.3 ± 27	42.4 ± 30

Table 2.

	Base	Ac oleic 2 h	1 h ULTF	2 h ULTF	3 h ULTF	4 h ULTF
C.O. LTS/M (T)	5.0 ± .9	3.56 ± 1.7	3.54 ± .81	3.52 ± .83	3.22 ± .96	2.8 ± .43
C.O. LTS/M (C)	5.22 ± 1.22	3.6 ± .6	3.27 ± .52	3.85 ± 1.4	3.27 ± 1.1	3.49 ± 1.3
PPX mmHg (T)	10.7 ± 2.2	11.7 ± 2.1	12.5 ± 4.2	19.5 ± 5.1	13.5 ± 3.8	14.4 ± 3.9
PPX mmHg (C)	11.1 ± 2.2	10.8 ± 2.3	10.8 ± 1.7	17.2 ± 2.7	12.9 ± 2.1	14.5 ± 2.6
PWP mmHg (T)	4.2 ± 1.5	4.9 ± 2.2	4.8 ± 2.4	4.9 ± 2.7	4.8 ± 2.2	4.6 ± 2.3
PWP mmHg (C)	5.1 ± 1.2	3.8 ± 2.0	3.8 ± 1.8	4.3 ± 1.6	4.4 ± 1.9	5.2 ± 2.0
COP mmHg (T)		7.5 ± 3.3	15.3 ± 4.8	20.4 ± 7.5	27.5 ± 11.3	34.7 ± 10.2
COP mmHg (C)		7.0 ± 3.3	6.4 ± 3.2	5.7 ± 3.5	5.2 ± 3.3	5.1 ± 3.2
HcT % (T)		35.2 ± 4.6	30.4 ± 4.2	29.3 ± 5.5	28 ± 4.2	29.6 ± 4.9
HcT % (C)		35.4 ± 5.9	34.3 ± 6.9	34.3 ± 6.9	33.0 ± 5.6	33.0 ± 5.6

Tables 1 and 2 summarize hemodynamic and gas exchange between treatmet group (T) and control group (C)

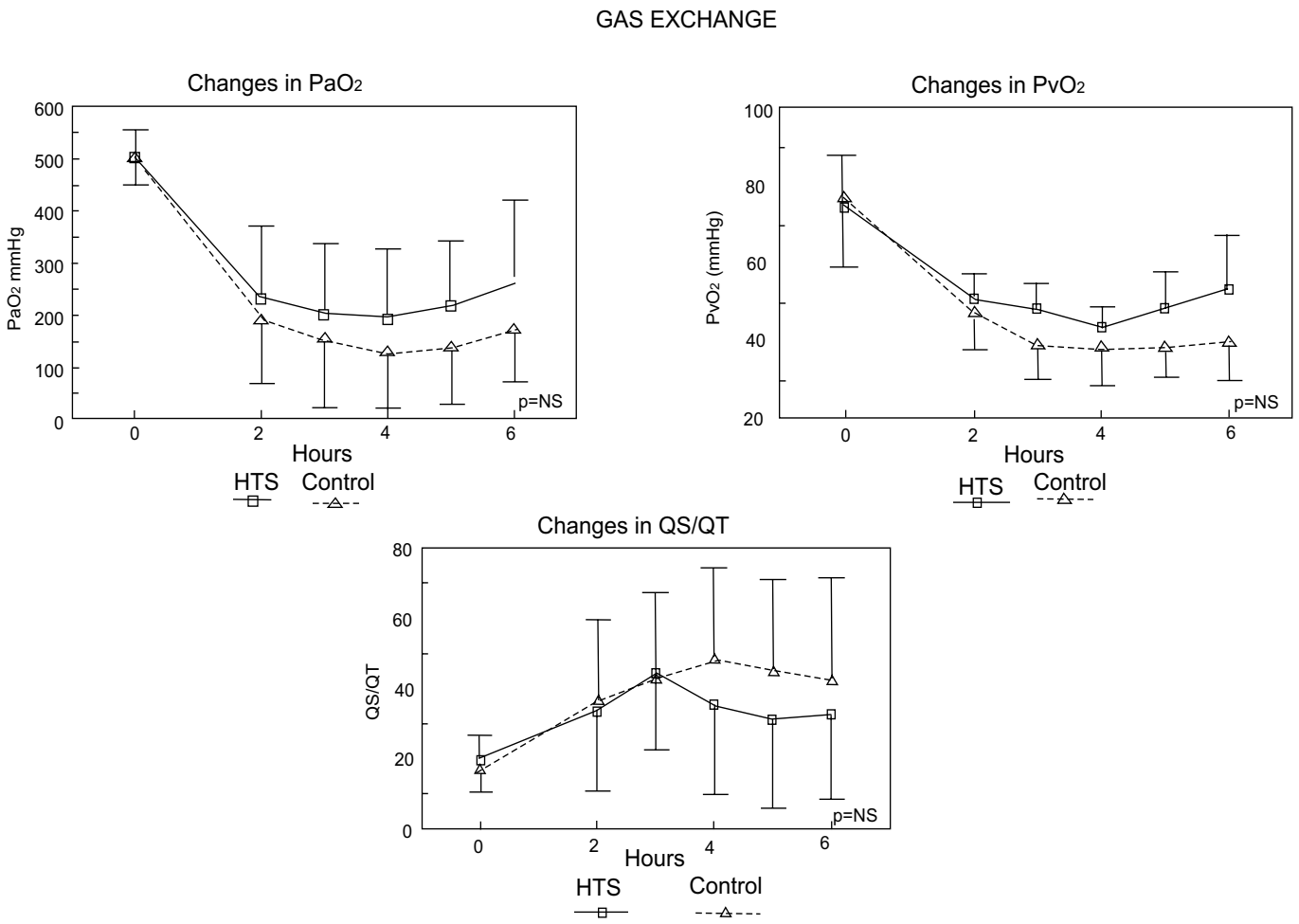

Figure 1. *Gas exchange between treatment group (HTS) and control group during 6 hours of experiment.*

Table 3.

	Control	HTS	NO O.A.**
Wet weight/BW	34.3 ± 11.8	24.9 ± 5.8*	8.61 ± 1.3
Dry weight/BW	2.33 ± 0.3	2.84 ± 0.29*	1.8 ± 0.3
Wet W/Dry W	14.5 ± 3.5	8.9 ± 1.7*	4.6 ± 0.2

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

Table 4.

	Control	HTS	P =
Ultrafiltration	1,047 ± 257	3,459 ± 593	$p < 0.0001$
Diuresis	1,973 ± 724	274 ± 138	$p = 0.0001$
Balance	1,154 ± 1,325	-36.4 ± 951	$p = 0.0001$

improvement in pulmonary edema in treated group. Dry weight corrected by dog weight of treated lung was 2.84 ± 0.29 in table compared to controls 2.33 ± g/kg dog weight ($p < 0.05$). For comparison with our groups, corresponding values of lung excised from dogs in our laboratories, anaesthetized and ventilated in a similar manner, but not given oleic acid, are also presented.

DISCUSSION

This is a study model of acute pulmonary edema. Two hours after intravenous injection of oleic acid, intrapulmonary shunt increased and PaO_2 decreased significantly, secondary to alveolar flooding. The severity of injury, analyzed with gas exchanges and lung weights were comparable to similar studies.[3] 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

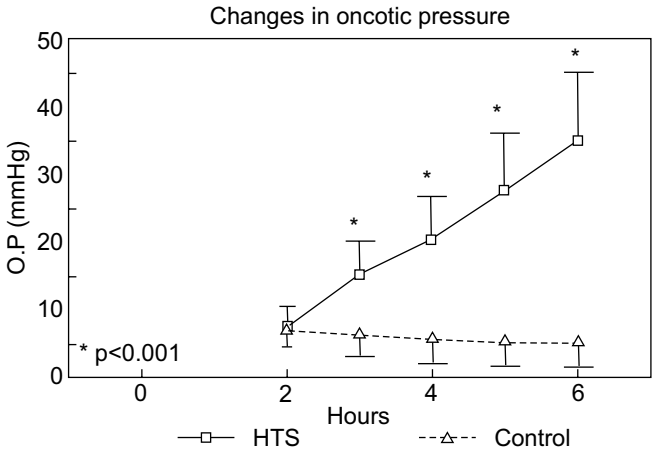

Figure 2.

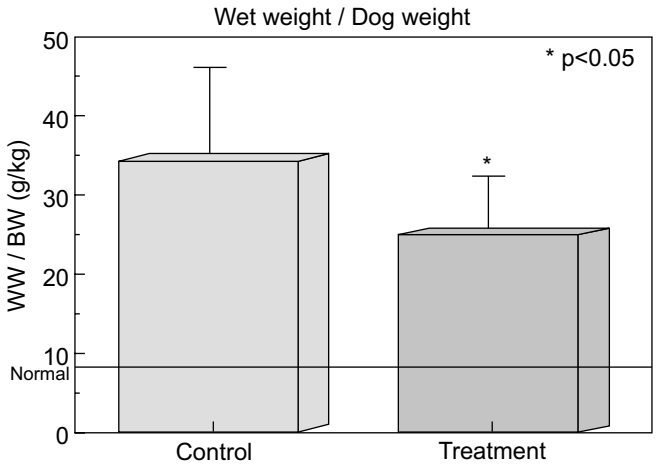

Figure 3.

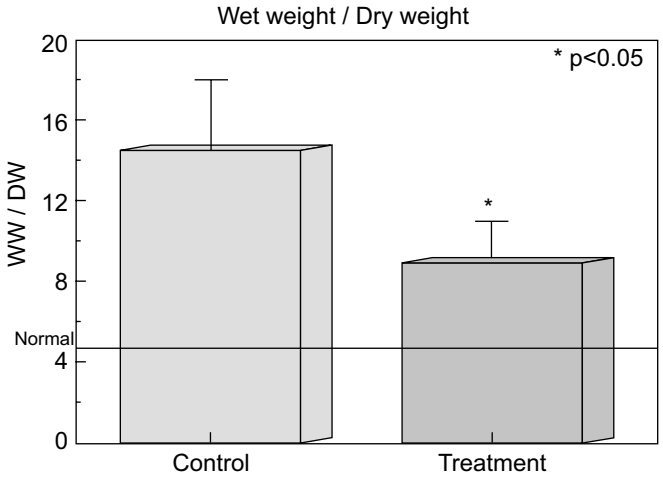

Figure 4.

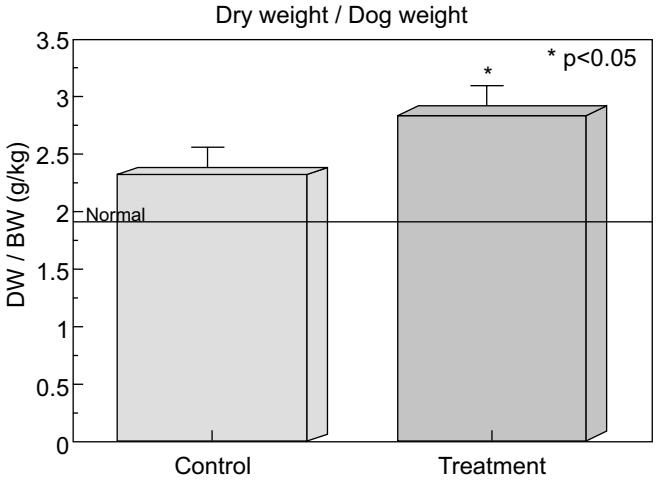

Figure 5.

than control group, and the difference was found to be significant. This difference reflects an increase in no evaporable substances; we used to interpreted that the increase in dry weight is due to extra vascular blood;[4] however, in this particular experiment increased of DW/BW of the treated lungs suggested HTS extravasations, related to the proportion of HTS which cross the circulation and the coefficient 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 improved in HTS treated animals as deduced from a significant reduction in lung wet weight. We considered these gravimetric 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 Pcrit from 12.2 to 23 cm H_2O; Pcrit is commonly interpreted as the Pc at which the forces across the microvasculature are in balance. After the addition of albumin, Pc < Pcrit induced constant weight loss along the same rate of filtration to the Pc relationship.[5] During edema reabsortion the non-convective transport of proteins from plasma to the interstitial space tend to equilibrate the osmotic gradient, send the loss of water from interstitial to intravascular could raise the protein concentration in the interstitial space. As a result, a decrease in the transmembrane oncotic gradient occurred, the rate of reabsortion might decrease progress, and with time, some filtration might reassumed at a lower rate. In this study we do not know if the improvement in lung wet weight was secondary to edema reabsortion 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).[3] 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,[6] 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 (αe). 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.[7] 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 reabsortion 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 reabsortion 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[8] 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 ultrafiltrated to maintain comparable PWP. The control group was ultrafiltrated 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.

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