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Aspirin effect on early and late changes in acute lung injury in sheep

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Abstract. *Objective:* There have been several studies that have already explored the potential beneficial role of cyclo-oxygenase (CO) inhibitors on oleic acid (OA)-induced lung injury in different species. These studies report no significant effect of CO inhibition, though thromboxane B_2 (TxB_2) was effectively blocked. However, recent studies indicate that pre-treatment with aspirin (ASA) preserve gas exchange in OA lung injury in dogs. Aim of our study has been to evaluate the potential beneficial effects of the pre-treatment with low doses of ASA on gas exchange, hemodynamics, respiratory mechanics, prostanoids and lung histology in OA-induced lung injury in sheep.

Design: 0.09 ml/kg of OA was administered into the right atrium of 14 anaesthetized sheep. Six received a bolus of ASA (10 mg/kg i.v.) 30 min before OA, the others saline as placebo.

Measurements and results: Pulmonary and tissue gas exchange, pulmonary and systemic hemodynamics, respiratory system mechanics, TxB_2 and 6-keto-PGF1α, leukocytes and platelets concentrations were measured throughout the subsequent 3 h and lung histology was effected at end-experiment. The principal findings of our study are: 1) ASA reduces OA-induced early pulmonary vasoconstriction and bronchoconstriction, parallelled by a suppression of TxB_2 generation; 2) the late increase in pulmonary artery pressure and airway resistance due to OA is not inhibited by ASA; 3) the early disturbance in pulmonary gas exchange is reduced by ASA, whereas the late severe deterioration is exaggerated by ASA; 4) the stability of tissue exchange ratio (R) at ≈ 1 in ASA-group compared to its fall to ≈ 0.7 in controls.

Conclusion: Our findings suggest that ASA: 1) is only effective to treat the very transient TxB_2-induced pulmonary vasoconstriction resulting in hydrostatic edema, and it is ineffective, even accentuates, the subsequent major pulmonary endothelial cell injury leading to alveolar flooding that is unrelated to TxB_2; 2) has a transient protective effect on the TxB_2-induced early bronchospasm; 3) has a biphasic behaviour on gas exchange, with a benefit which lasts only one hour and then results in a worse gas exchange; 4) has an immediate, stabilizing, persisting effect on R, contrasting with its transient effect on pulmonary hemodynamics and PaO_2.

Key words: Adult respiratory distress syndrome – Pulmonary hypertension – Pulmonary and tissue gas exchange – Aspirin – Acute lung injury

Acute diffuse lung injury is associated with pulmonary hypertension and reduction of blood flow to injured areas due to pulmonary vasoconstriction, and/or vascular obliteration of the pulmonary bed caused by thrombosis or other mechanisms [1]. Injection of oleic acid (OA) into the pulmonary vasculature produces pulmonary edema and a copious outpouring of fluid into the airways [2, 3]. Many experimental data suggest that the early increase of pulmonary vascular resistance associated with OA-induced lung injury is largely due to reversible vasoconstriction [4–6]. Several mediators, eg. cyclo-oxygenase (CO) metabolites of arachidonic acid (AA), released by activated platelets, leukocytes or by pulmonary tissue itself, may be responsible at least partially for OA-induced pulmonary arterial hypertension and edema [5, 7, 8].

There have been several studies that have already explored the potential beneficial role of reversible, short-acting cyclo-oxygenase inhibitors (with dose-dependent action) (indomethacin, meclofenamate, ibuprofen) on OA-induced lung injury in different species including sheep, evaluating the pulmonary hemodynamics and vascular permeability with sophisticated techniques [5, 7, 9–11]. These studies report no significant effect of cyclo-oxygenase inhibition, though thromboxane B_2 (TxB_2) was effectively blocked. However, recent studies indicate that pretreatment with aspirin (30 mg/kg i.v.) largely preserves gas exchange in OA lung injury in dogs [12]. These data differ from previous reports in which prophylactic ibuprofen in sheep [5] had no effect on the evolution of OA lung injury. On the other hand, ibuprofen pretreatment limited hypoxemia and the rise in extravas-

cular lung water in dogs with OA pulmonary edema [7]. Nevertheless, the different effects on gas exchange and hemodynamics of these agents could be explained by the different mechanism of action of aspirin (ASA), i.e., irreversible (dose-independent) cyclo-oxygenase inhibition, long-lasting because of its biochemical mechanism of acetylation, able to permanently block cyclo-oxygenase.

It is difficult to reconcile the controversial literature results obtained after blockade of cyclo-oxygenase. Moreover, despite the great number of animal studies on the role of the products of the AA cascade during acute lung injury, few studies have been published on cyclo-oxygenase product levels in human ARDS.

In addition, we cannot overlook the fact that the same pharmacological agent may have different effects depending on the species of the animals studied. Indeed, unlike other common laboratory animals like dogs and rats, and unlike also humans, the sheep is particularly reactive to produce thromboxane in response to foreign particles injected into the pulmonary vasculature because of the presence of pulmonary intravascular macrophages in this species (such as in goat, ox, calf and pig) [13].

The aim of the present study has been to evaluate the potential beneficial effects of the pre-treatment with low doses of aspirin (ASA) on pulmonary and tissue gas exchange, hemodynamics, respiratory system mechanics, prostanoids, and lung histology in an ovine model of lung injury infusing i.v. oleic acid.

Methods

Animal preparation

Fourteen adult sheep (30–42 kg, mean 36±6 kg) were studied. The animals were premedicated with ketamine (10 mg/kg i.m.), atropine (0.03 mg/kg i.m.) and diazepam (0.28 mg/kg i.m.). Anesthesia was then induced with ketamine (1 mg/kg i.v.). Paralysis was obtained with pancuronium bromide (0.1 mg/kg i.v.) to eliminate possible fighting against the ventilator and assure a complete muscle relaxation for the mechanical measurements. The same doses of ketamine and pancuronium were repeated every 30 min throughout the experiment. The sheep were ventilated in the supine position with a constant flow ventilator (Ohio CPU 1, Medical Products, Madison, Wisconsin) via a cuffed endotracheal tube. FiO_2 was 0.4; respiratory rate (14/min) and tidal volume (10–15 ml/kg) were adjusted to maintain baseline arterial PCO_2 between 35 and 40 mmHg during the initial control period. No positive end-expiratory pressure was used. Heparin (5000 IU i.v.) was administered before catheterization. Sheep received 10–15 ml/kg/h of intravenous Ringer solution during anesthesia to maintain stable hematocrit.

An indwelling large bore catheter (16G, DV i.v. catheter) was placed in the femoral vein for i.v. infusions and venous blood sampling. A thermistor-tipped Swan-Ganz catheter (7F, Edwards Labs.) inserted via the left external jugular vein was positioned under pressure monitoring in a branch of the pulmonary artery. The catheter was connected to a Novatrans Mx 807 pressure transducer (Medex Inc.) for measurements of mean pulmonary arterial (P_{pa}) and wedge (P_{wp}) pressures and allowed mixed venous ($\bar{v}$) blood sampling. A polyethylene catheter (18G) (Cavafix MT, Braun) inserted into the abdominal aorta via the right femoral artery and connected by low compliance tubing to a Novatrans Mx 807 pressure transducer (Medex Inc.) was used for measurement of systemic arterial mean pressure (P_{sa}) and sampling of arterial (a) blood. Pulmonary, systemic arterial pressures were recorded on an oscillographic recorder (Honeywell RM 300 recorder). The hydrostatic zero reference was at midchest level, and vascular pressures were measured at end-expiration. Heart rate was determined from a continuously monitored electrocardiographic lead. Cardiac output ($\dot{Q}t$) was obtained by thermodilution using a cardiac output computer (Model 9520, Edwards Laboratories, Santa Anna, CA) and injections of 5 ml of 5% glucose solution (1 °C); four consecutive determinations were averaged. We calculated cardiac index (CI = $\dot{Q}t$ divided by body weight) and pulmonary [$PVR = (P_{pa} - P_{wp}) \cdot 80/\dot{Q}t$] and systemic ($SVR = P_{sa} \cdot 80/\dot{Q}t$) arterial resistance ($dyne \cdot s \cdot cm^{-5}$).

Blood gases and pH were measured with a tonometered automated gasanalyzer (ABL2 Radiometer, Copenhagen, Denmark) immediately after sampling and were corrected for temperature. Venous admixture (% of total cardiac output) was calculated with the equation $\dot{Q}s/\dot{Q}t = (Cc'O_2 - CaO_2)/(Cc'O_2 - C\bar{v}O_2)$, where $Cc'O_2$ is end-capillary O_2 content, calculated from the alveolar gas equation and blood O_2 saturation, CaO_2 and $C\bar{v}O_2$ are arterial and mixed venous O_2 content, respectively. O_2 consumption ($\dot{V}O_2$) was calculated as $\dot{Q}t \cdot (CaO_2 - C\bar{v}O_2)$. CO_2 production ($\dot{V}CO_2$) was calculated as $FetCO_2 \cdot \dot{V}A$, where $\dot{V}A$ is alveolar ventilation. Calculation of $\dot{V}A$ required determination of physiological dead space ($\dot{V}_D/\dot{V}_T$), according to standard formula [$(PaCO_2 - P\bar{E}CO_2)/PaCO_2$]. Expired air was collected in a Douglas bag over a period of 2 min to allow measurement of mixed expiratory CO_2 tension. End-tidal CO_2 concentration ($FetCO_2$) was measured with the infrared absorption analyser of the CPU 1 ventilator and then transformed in its corresponding partial pressure value [taken as representative of alveolar gas ($PACO_2$)].

Respiratory exchange ratio (R) was computed as $\dot{V}CO_2/\dot{V}O_2$ ratio. Alveolar PO_2 (PAO_2) was obtained as $PAO_2 = PiO_2 - PaCO_2/R + PaCO_2 \cdot FiO_2 \cdot [(1-R)/R]$, where Pi, Pa, R, Fi are inspired and arterial partial pressures, respiratory exchange ratio and fractional concentration in inspired gas, respectively.

Airway opening pressure was measured with the pressure transducer built in the ventilator. Insufflation volume (Vt) was obtained by integration of the flow signal. Static compliance of the respiratory system (Cst, rs) was computed as Vt, corrected for compression losses, divided by end-inspiratory plateau pressure, i.e. the elastic recoil pressure of the respiratory system at end-inflation and at zero end-expiratory pressure [14]. Flow resistance of the airways (R_{aw}) (endotracheal tube included) was computed from the difference between peak inflation pressure and end-inflation plateau pressure divided by constant insufflation flow rate.

Peripheral blood leukocytes (WBC) and platelets (PLT) were counted with automated devices (S Plus, Coulter Electronics, Hialeah, FL, and Baker Instr. 810 Platelet Analyzer, Baker Instr., respectively). Plasma concentrations of thromboxane B_2 (TxB_2) and 6-keto-prostaglandin F1 alpha (6-keto-PGF1α), the stable degradation products of TxA_2 and prostacyclin, were measured by radioimmunological methods in blood samples collected in cold polypropylene syringes containing phenoprofen in 0.037 M EDTA to a final concentration of 1 µg/ml. TxB_2 radioimmunoassay [15] was performed with commercial kit (ABT Esslingen, FRG) in platelet poor plasma obtained by centrifugation at 3800 g for 30 min at 4 °C. The detection limit of the method was 10 pg/ml, intra- and inter-assay variation coefficients were 8.7 and 10.4% respectively, and cross reactivity with other AA-metabolites was 1%. 6-keto-PGF1α was assayed according to Patrono et al. [16]. Only 20 µl of plasma were employed to minimize aspecific interferences with antigen-antibody binding. The antibody was diluted 1 : 100000 in a final volume of 1.5 ml phosphate buffer 0.02 M at pH 7.40. Free antibody was separated from that bound to 6-keto-PGF1α by addition of 10 mg of charcoal (Norit A) and further centrifugation. The antibody was kindly supplied by Dr. A. Peskar (Department of Pharmacology, University of Bochum, FRG). Detection limit was 7.5 pg/ml, intra- and inter-assay variation coefficients were 5.5 and 9.6% respectively, and cross reactivity with other AA-metabolites was 1%.

Experimental protocol

Each experimental period consisted of hemodynamic ($\dot{Q}t$, P_{pa}, P_{wp}, P_{sa}) and blood gas measurements and simultaneous sampling of (a) and ($\bar{v}$) blood for WBC and PLT counts, and for measurements of TxB_2 and 6-keto-PGF1α levels in plasma.

After steady-state conditions had been present for 30 min as assessed by stable heart rate, systemic and pulmonary blood pressures,

baseline measurements were made (BL_1). Immediately thereafter, one group of sheep ($n = 6$) received a bolus of ASA (10 mg/kg i.v.) whereas the control group ($n = 8$) received an equal volume of saline solution as placebo. A second set of measurements was obtained 30 min after ASA or placebo (BL_2). Immediately thereafter, right atrial infusion of OA (cis-9-octadecenoic acid, 1 ml = 0.9 g) began in all animals. The total dose of OA solution was 0.09 ml/kg and was injected over about 20 min at a rate of 0.2 ml/min, with an automated infusion pump. All measurements were repeated immediately after OA administration (T_0), and at T_{30}, T_{60}, T_{120} and T_{180} min later. At the conclusion of the experiments (3 h), the sheep were killed with a bolus injection of KCl. The chest was opened and the lungs removed for microscopic examination.

Statistics

All data are expressed as means (±SD). Comparison within groups was carried out by a one-way analysis of variance for repeated measures (ANOVA), followed by multiple comparisons using the Tukey testing procedure. Comparisons between groups were performed with the non-parametric Wilcoxon's Rank Sum test. We rejected the null hypothesis for $p<0.05$. Linear regression analysis was performed when appropriate with the least-squares method.

Results

Mean values (±SD) of all measured and derived variables for the two groups of sheep are shown in Tables 1, 2 and Figs. 1–3. There were no significant differences in any baseline value between the two groups and in the experimental group between pre- and post-ASA values.

Hemodynamics (Table 1)

P_{wp}, HR and mean P_{sa} were similar for all stages between ASA and control group and did not change over time in either group. In control sheep (C) CI which had fallen by about 60% at the end of OA infusion, rose slightly during the following half hour to about 80% of baseline, then declined slowly throughout the rest of the study. The pattern of changes in P_{pa} was a mirror image of that of CI with a marked early rise from 2.0 kPa (15 mmHg) to 4.4 kPa (33 mmHg) on average, followed by a partial return towards baseline at 30 min [3.3 kPa (25 mmHg)] and did not further change later on. Since P_{wp} did not change, PVR almost quadrupled at the end of OA infusion, fell to about twice baseline PVR at 30 and 60 min post-infusion and sustained a slow delayed rise later on. SVR showed the same pattern: early peak at T_0, followed by partial return towards baseline and delayed rise later on.

In ASA-pretreated sheep (A) there was no change over time in CI and the early peak of P_{pa}, from 1.9 kPa (14 mmHg) to 2.4 kPa (18 mmHg) on average, and PVR (from 171 to 277 dyne·s·cm^{-5}) was blunted and not significant. There was a delayed rise in P_{pa} and PVR so that both P_{pa} (C = 3.3±0.9 kPa (25±7 mmHg), A = 3.6±0.8 kPa (27±6 mmHg)), and PVR(C = 691±470, A = 518±182 dyne·s·cm^{-5}) were similar to the control group at 3 h post-infusion. SVR early decreased by about 75% and did not change over 3 h.

Prostanoids concentration and blood cells (Fig. 1)

In control sheep (C) plasma TxB_2 reached exceedingly high values, that were not significantly higher in ($\bar{v}$) than (a) blood, after OA administration (T_0). At T_0 peak plasma TxB_2 was positively correlated ($r = 0.76$, $p<0.01$) with P_{pa}. There was a gradual decline of blood TxB_2 throughout the 3 h post-infusion period, during which time both PVR and P_{pa} remained elevated and there was the decrease in the correlation coefficient ($r = 0.36$, p = NS, at T_{180}). R^2 coefficient showed a value of the data variability explained by the linear regression equals to 58% in the early phase (T_0) but progressively reduced later on (31% at T_{30}–T_{60}, 13% at T_{120}–T_{180}). 6-keto-PGF1α also rose at the end of the OA infusion and returned toward baseline during the following 3 h: its higher level at T_0 in (a) (100 pg/ml, on average) than in ($\bar{v}$) (59 pg/ml, on average) blood ($p<0.05$) suggests that prostacyclin was produced in the lung. WBC and PLT counts profoundly decreased at all times after OA administra-

Table 1. Effects of OA infusion on hemodynamics in ASA-pretreated sheep (A) ($n = 6$) and in untreated controls (C) ($n = 8$)

		OA (0.09 ml·kg^{-1} i.v.)				
	Baseline	T_0	T_{30}	T_{60}	T_{120}	T_{180}
CI, ml·min^{-1}·kg^{-1}	A 105±25	105±23[a]	104±23	106±18	117±31	116±41
	C 131±25	79±22[b]	102±36[b]	96±23[b]	98±36[b]	85±37[b]
P_{pa}, mmHg	A 14±5	18±5[a]	18±4[a]	17±4[a]	19±4[b]	27±6[b]
	C 15±6	33±11[b]	25±9[b]	23±8[b]	24±8[b]	25±7[b]
PVR, dyne·s·cm^{-5}	A 171±72	277±138[a]	301±131[b]	245±118	286±36	518±182[b]
	C 203±149	814±421[b]	464±232[b]	454±235	511±317	691±470[b]
P_{wp}, mmHg	A 6±5	6±4	6±4	6±5	7±5	7±5
	C 4±3	7±3	6±3	5±3	5±3	5±3
HR, beats·min^{-1}	A 132±20	116±23	106±21	102±19	127±44	134±49
	C 146±13	106±25	117±16	131±42	148±50	155±36
P_{sa}, mmHg	A 104±13	87±22	89±15	85±9	88±26	89±30
	C 86±21	80±19	84±26	79±23	83±20	76±25
SVR, (×100) dyne·s·cm^{-5}	A 26±11	21±9[b]	19±10[b]	17±6[b]	17±10[b]	17±12[b]
	C 16±8	25±13[b]	21±11	19±9	22±11	26±18[b]

Values are means±SD. Significant differences ($p<0.05$) between the two groups ([a], Wilcoxon's rank sum test) and within the group from baseline ([b], ANOVA) are shown

Table 2. Effects of OA infusion on pulmonary and tissue gas exchange in ASA-pretreated sheep (A) ($n = 6$) and in untreated controls (C) ($n = 8$)

		Baseline	OA (0.09 ml·kg^{-1} i.v.)				
			T_0	T_{30}	T_{60}	T_{120}	T_{180}
PaO_2, mmHg	A	148±33	90±44^b	71±25^{ab}	55±14^b	46±13^b	49±16^{ab}
	C	137±36	61±23^b	51±13^b	56±11^b	62±16^b	66±17^b
$\dot{Q}s/\dot{Q}t$, %	A	9±6	33±6^b	35±23^{ab}	48±12^b	59±18^b	61±18^{ab}
	C	11±7	41±18^b	53±13^b	54±10^b	48±21^b	40±16^b
$PaCO_2$, mmHg	A	33±5	37±4	36±4^a	38±4^a	48±10^b	50±15^b
	C	34±7	39±8	46±7^b	48±10^b	52±16^b	51±15^b
a-$APCO_2$, mmHg	A	2±2	6±2^b	6±3^{ab}	9±5^{ab}	16±10^b	18±16^b
	C	3±1	9±6^b	15±5^b	18±9^b	20±14^b	19±15^b
pHa	A	7.42±0.05	7.39±0.07	7.42±0.05^a	7.37±0.04	7.25±0.01^b	7.23±0.02^b
	C	7.41±0.01	7.36±0.09	7.30±0.09^b	7.27±0.01^b	7.24±0.02^b	7.24±0.02^b
$P\bar{v}O_2$, mmHg	A	49±7	42±8	33±7^b	31±8^b	28±10^{ab}	29±10^b
	C	56±14	35±11^b	35±10^b	37±8^b	39±8^b	32±19^b
$P\bar{v}CO_2$, (mmHg)	A	39±4	42±5	42±5^a	44±5^a	55±9^b	56±17^b
	C	38±7	46±9^b	52±9^b	55±12^b	59±17^b	62±20^b
$C(a-\bar{v})O_2$ vol%	A	3.5±0.4	3.4±0.4	4.3±0.7	3.4±0.6	2.8±1.2	2.7±0.9^a
	C	2.4±1.0	4.8±1.9^b	4.6±2.1^b	3.9±1.7	4.0±1.8	4.8±1.7^b
$\dot{V}O_2$, (ml/min)/kg	A	3.4±1.6	3.2±1.5	3.2±1.4	3.2±1.2	2.9±1.2^b	2.8±1.5^b
	C	3.1±1.3	3.6±1.4	4.3±1.5	3.6±1.1	3.5±0.8	3.7±1.2
$\dot{V}CO_2$, (ml/min)/kg	A	3.3±1.5	2.9±1.3	2.9±1.4	2.9±1.3^b	2.8±1.4^b	2.6±1.4^b
	C	3.1±0.8	2.3±1.1	2.8±1.2	2.4±1.0	2.5±1.0	2.5±0.9
R,	A	0.97±0.44	0.91±0.41^a	0.91±0.44^a	0.90±0.41^a	0.96±0.48^a	0.93±0.50^a
	C	1.00±0.26	0.64±0.31^b	0.65±0.28^b	0.67±0.28^b	0.71±0.29^b	0.68±0.24^b

Values are means±SD. Significant differences ($p<0.05$) between the two groups (^{a}, Wilcoxon's rank sum test) and within the group from baseline (^{b}, ANOVA) are shown

tion in both (a) and ($\bar{v}$) blood, with PLT slightly lower in the former.

ASA pre-treatment caused a significant fall in plasma TxB_2, fully abrogated the OA-induced rise in TxB_2 and lowered 6-keto-PGF1α to below the detection threshold of 7.5 pg/ml. Pre-treatment with ASA did not prevent the OA-induced decrease of circulating PLT and WBC.

Respiratory system mechanics (Fig. 3)

Cst,rs decreased about 50% below baseline at the end of OA-infusion (T_0) (from 34.6±11.6 to 16.4±6.7 ml/cmH_2O) ($p<0.01$) whereas at the same experimental time R_{aw} increased about 50% above baseline, from 1.3±0.4 kPa·l^{-1}·s to 2.0±0.3 kPa·l^{-1}·s (13.4±4.2 to 20.3±2.5 cmH_2O·l^{-1}·s) ($p<0.05$). Cst, rs and R_{aw} did not change significantly over the 3 h following.

ASA-pretreatment did not modify the prolonged fall in respiratory system compliance observed after OA injury (from 36.8±10.3 of baseline to 20.7±7.8 ml/cmH_2O at T_0). Moreover, Cst,rs showed a further decrease for the last 2 hours: 17.5±6.9 at T_{120}, 14.2±4.1 ml/cmH_2O at T_{180}. Instead, ASA-pretreatment significantly blunted ($p<0.05$) the early (T_0) increase of R_{aw} from 1.2±0.3 kPa.l^{-1}.s to 1.6±0.4 kPa.l^{-1}.s (11.7±2.6 to 16.0±3.9 cmH_2O.l^{-1}.s). R_{aw} showed a late, significant increase vs. base ($p<0.05$) so that R_{aw} was similar in the two groups of sheep at 2 h (C = 2.1±0.7, A = 2.2±0.9 kPa·l^{-1}·s) (C = 20.5±7.1, A = 22.8±8.7 cmH_2O·l^{-1}·s) and at 3 hours (C = 2.2±0.9, A = 2.1±0.6 kPa·l^{-1}·s) (C = 22.1±9.2, A = 21.0±5.6 cmH_2O·l^{-1}·s) post-infusion.

Blood gases and gas exchange (Table 2, Fig. 2). In control sheep(C) PaO_2 fell from 18.2 kPa (137 mmHg) to 6.8 kPa (51 mmHg) on average at 30 min and remained low throughout the rest of the study. $\dot{Q}s/\dot{Q}t$ rose from 11 to 53% on average at 30 min and slowly declined throughout the rest of the study. $PaCO_2$, a-$APCO_2$ and $P\bar{v}CO_2$ showed a marked rise at 30 min and rose slightly later on while pHa decreased at the same experimental times. $P\bar{v}O_2$ decreased by about 40% from baseline to end of OA-infusion and remained significantly lower throughout the duration of the experiment. $C(a-\bar{v})O_2$ doubled at T_0 and remained higher later on. $\dot{V}O_2$ and $\dot{V}CO_2$ levels increased and decreased (even though non-significantly), respectively, across time during the study. R significantly decreased at T_0 ($p<0.01$) and did not change later on.

In ASA-pretreated sheep (A), the fall in PaO_2 was more gradual so that PaO_2 was higher than in control animals at the end of OA infusion, A = 12.0, C = 8.1 kPa (A = 90, C = 61 mmHg) on average, and at 30 min, A = 9.4, C = 6.8 kPa (A = 71, C = 51 mmHg) on average, and lower later on, A = 6.5, C = 8.8 kPa (A = 49, C = 66 mmHg) on average, at 3 h. $\dot{Q}s/\dot{Q}t$ was lower than in controls at 30 min after OA infusion (A = 35, C = 53%, on average) and was higher later on (A = 61, C = 40%, on average at 3 h). The early rise of $PaCO_2$, a-$APCO_2$, $P\bar{v}CO_2$ and decrease of pHa were blunted. There was a delayed rise in $PaCO_2$, a-$APCO_2$, $P\bar{v}CO_2$

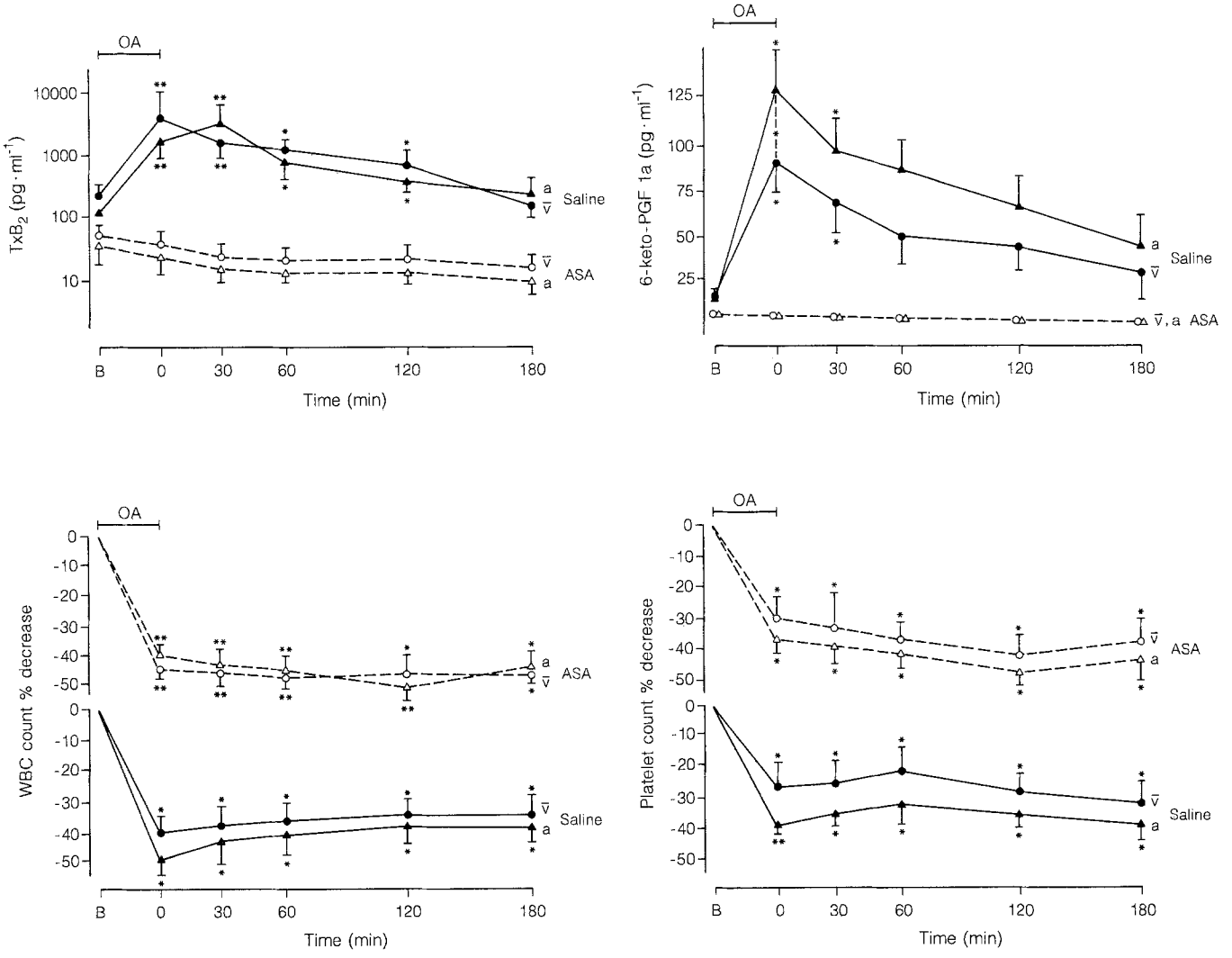

Fig. 1. Effects of OA infusion on arterial (a) and mixed venous ($\bar{v}$) blood prostanoids (TxB_2, 6-keto-PGF1α) (*above*) and blood cells (WBC, platelets) (*below*) concentrations in saline-pretreated sheep (*continuous line*) (▲ : a; ● : $\bar{v}$) and in ASA-pretreated sheep (*dotted line*) (△ : a; ○ : $\bar{v}$). Values are means±SD. Note the difference in scale which is logarithm for TxB_2. *Asterisks* above and below curves and asterisks between curves indicate significant differences within the group from baseline and between the two groups, respectively (*, $p<0.05$; **, $p<0.01$)

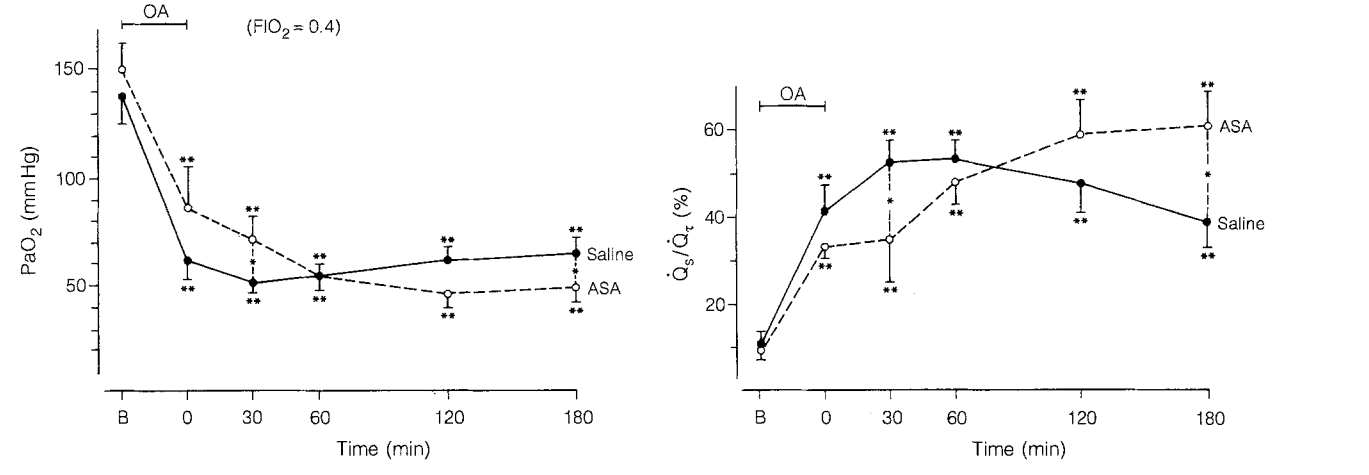

Fig. 2. Effects of OA infusion on PaO_2 and venous admixture ($\dot{Q}s/\dot{Q}t$) in saline-pretreated sheep (*continuous line*) and in ASA-pretreated sheep (*dotted line*). Values are means±SD. For further explanations of symbols, see legend of Fig. 1

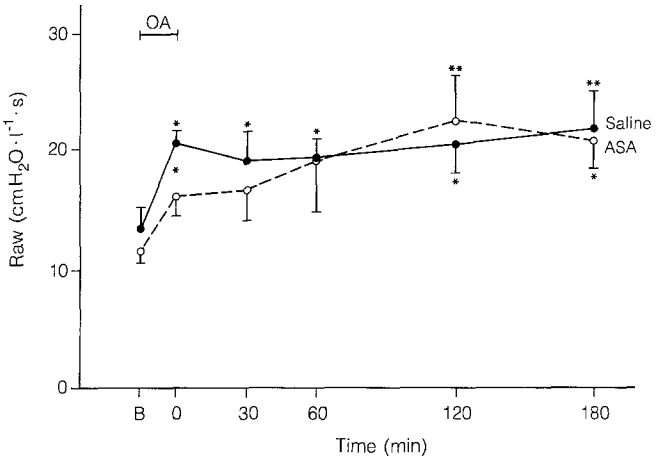

Fig. 3. Effects of OA on airways resistance (R_{aw}) in saline-pretreated sheep (*continuous line*) and in ASA pretreated sheep (*dotted line*). Values are means ± SD. For further explanations of symbols, see legend of Fig. 1

and decrease of pHa such that they were similar in ASA-pretreated and control sheep at 3 h post-infusion. Similarly, the early decrease of $P\bar{v}O_2$ (T_0) was blunted but delayed changes brought it to similar values as in control sheep at 3 h post-infusion. $C(a-\bar{v})O_2$ maintained stable at 1 h then decreased at T_{120} and T_{180} ($p<0.05$). $\dot{V}CO_2$ fell at 1 h ($\dot{V}O_2$ later on) and remained below baseline until the end of the study. The early decrease of $R(T_0)$ was blunted and the respiratory exchange ratio remained stable during the rest of the experiment.

Morphology

The lungs were heavy, with areas of hemorrhage which were often confluent, and their cut surfaces showed exudation of edematous fluid and hemorrhage. Microscopic examination revealed diffuse patchy alveolar edema with focal hemorrhage of irregular distribution sometimes extending into the perivascular regions in both control and ASA-sheep. Septal pulmonary vessels were distended and contained microthrombi and fat microemboli. Alveolar septa showed some cellular infiltration (polymorphonuclear leukocytes, erythrocytes) and evidence of interstitial edema, causing septal thickening and enlarged spaces between cells and components of the basal membrane. These spaces often contained granular or membranous material. On transmission electron microscopy lungs of both groups of sheep contained granular electron-dense material in alveoli. The alveolar space was filled with erythrocytes, polymorphonuclear leukocytes, macrophages and eosinophilic proteinaceous exudate, sometimes also found in small bronchioles. The lesions were qualitatively similar in the two groups of sheep and were interpreted as ultrastructural evidence of both interstitial and alveolar edema.

Discussion

The principal findings of our study are that ASA-pretreatment profoundly modified responses to OA-infusion in sheep: 1) ASA-pretreatment reduces OA-induced early pulmonary vasoconstriction and bronchoconstriction, paralleled by a suppression of thromboxane generation; 2) the late increase in pulmonary artery pressure and airway resistance due to OA-injection is not inhibited by ASA: 3) the early disturbance in pulmonary gas exchange is reduced in the presence of ASA, whereas the late severe deterioration of gas exchange is even exaggerated in the presence of this CO inhibitor; 4) the immediate, persisting effect of ASA on tissular exchange ratio, contrasting with its transient effect on pulmonary hemodynamics and PaO_2.

Our hemodynamic findings contribute to a better understanding of the mechanisms of pulmonary hypertension induced in sheep by OA-infusion. The marked rise in P_{pa} and PVR present in the early phase seems mostly dependent on functional pulmonary vasoconstriction, probably due to local release of thromboxane, largely reversible since both P_{pa} and PVR peaked immediately after OA infusion and fell halfway back towards baseline 30 min later. Two principal arguments are in favour of the role of CO-metabolites of AA in the early pulmonary vasoconstriction: 1) P_{pa} was fully abrogated by ASA-pretreatment; 2) plasma levels of TxB_2 peaked when P_{pa} was maximal and diminished at the same time as P_{pa}. Moreover, also the good correlation (largely expected) of TxB_2 levels with the early (T_0) increase in P_{pa}, suggests almost 2/3 of the initial pulmonary hypertension could be attributed to the thromboxane increase.

We believe that neither acute hypoxic pulmonary vasoconstriction nor mechanical obstruction of pulmonary vessels can account for the early rise in P_{pa}. Furthermore, the presumed mechanism of compression of arteries by the interstitial lung edema could be excluded, as pointed out by Michel et al. [17].

Diffuse alveolar hypoxia was prevented through use of a high FiO_2: indeed, PaO_2 was maintained at high levels at T_0 (165 ± 17 in controls, 192 ± 9 mmHg in ASA-sheep), not changing significantly from its baseline values (175 ± 20 in controls, 193 ± 10 mmHg in ASA-group). However, since $P\bar{v}O_2$ was lower (T_0) in controls compared to ASA-sheep and being evidence that a low $P\bar{v}O_2$ may contribute to hypoxic pulmonary vasoconstriction, focal alveolar hypoxia cannot be completely excluded in lung units with low ventilation/perfusion ratio. However, since at this early stage $\dot{Q}s/\dot{Q}t$ was not different between controls and ASA-sheep, focal alveolar hypoxia and hypoxic vasoconstriction probably were similar in animals that develop early pulmonary hypertension and in those who did not.

It is unlikely that mechanical obstruction of pulmonary vessels [5, 18] differed in controls and in ASA-sheep since the amount of OA infused was the same (3 – 3.5 ml) and far too small to cause significant vascular obstruction. Moreover, the fall in blood cells count in (a) and in ($\bar{v}$) blood, which probably reflected the lung trapping of WBC and PLT, was also the same in the two groups of animals (Fig. 1).

Pulmonary gas exchange was less impaired at an early stage of OA-induced lung injury and more impaired at later stages in ASA-sheep, with a cross over point at T_{60}. This late deterioration with ASA is quite interesting and

the contention is based upon the lesser $\dot{Q}s/\dot{Q}t$ and higher PaO_2 during the first hour and the higher $\dot{Q}s/\dot{Q}t$ and lower PaO_2 at later stages in ASA-sheep compared to controls (Fig. 2). Thus modifications of pulmonary gas exchange in ASA-pretreated compared to control sheep were clearly biphasic, with an early protective effect of ASA and a late deleterious one. The higher PaO_2 at early stages of OA-induced lung injury probably resulted from the combined effect of a higher CI and $P\bar{v}O_2$ [19]. We cannot rule out, however, that at the early stage of OA-induced lung injury preferential reduction in pulmonary vasoconstriction in well ventilated lung zones took place [10, 20]. Indeed, we consider thromboxane-induced vasomotion increase determined a diffuse but not homogeneous change in the flow-resistive properties of the entire vascular bed, with a predominant local vasoconstriction in diseased areas of the lung (where it associates with other important factors responsible for the irreversible vascular obstruction, i.e. vascular thrombosis [1]), and redistribution of blood flow from shunt pathways toward normal $\dot{V}A/\dot{Q}$ lung units. Consequently, early reduction of $\dot{Q}s/\dot{Q}t$ and hypoxemia could be attributed to reduction of ventilation/perfusion ($\dot{V}A/\dot{Q}$) mismatching.

We have two arguments in favour of this hypothesis: 1) a purely geometric, passive factor, related to the flow resistance-vessel diameter curvilinear, and concave toward the y-axis (resistance), relationships. Indeed, if ASA-inhibition of increased vascular tone were homogeneous and diffuse in the entire pulmonary bed, it would proportionally induce more important modifications in the diseased vasoconstricted zones (because of shift of the curve to the left) than in the residual normal ones [21]. If so, $\dot{Q}s/\dot{Q}t$ should be increased whereas, on the contrary, it reduces. Consequently, we suggest that vascular tone inhibition by ASA determines an important reduction of the flow-resistive properties predominantly in normal lung areas; 2) a metabolic mechanism [10]. Indeed, since vascular tone did not increase uniformly in the pulmonary bed, but it was augmented primarily in the shunt pathways or low $\dot{V}a/\dot{Q}$ units, local inhibition of a vasodilator may be presumed. Abolition of this vasodilator by ASA infusion would be expected then to increase local resistance and diminish shunt flow with a resulting increase in perfusion of normal lung areas. This response is consistent with our observations.

Conversely, protracted inhibition of thromboxane-induced pulmonary vasoconstriction was probably responsible for the late aggravation of pulmonary gas exchange (later rise of $\dot{Q}s/\dot{Q}t$ and fall of PaO_2) in ASA-pretreated sheep. This gas exchange impairment was suggested by the mismatching between the progressive rise in $PaCO_2$ (equivalent in both groups) and the parallel lower $\dot{V}CO_2$ in ASA-pretreated sheep. Since both tidal volume and respiratory rate were adjusted to maintain baseline $PaCO_2$ in a normal range only during the initial control period, the $PaCO_2$ rise was best explained by the combined effects of the higher $P\bar{v}CO_2$ and increased $\dot{Q}s/\dot{Q}t$ (Table 1).

Whereas the early changes in pulmonary hemodynamics were prevented by ASA-pretreatment, the late increase in P_{pa} and PVR was not, so that hemodynamic changes of this late phase are attributable to other mechanisms, not related to TxA_2-release [22]. Indeed at 3 h post-infusion P_{pa} and PVR were higher than corresponding baseline values in ASA-pretreated and controls, with no difference between the two groups. Therefore, it means that ASA is only effective to treat the very transient thromboxane-induced pulmonary vasoconstriction (essentially post-capillary) resulting in hydrostatic edema, and that it is ineffective, even accentuates, the subsequent major pulmonary endothelial cell injury leading to alveolar flooding that is unrelated to thromboxane. Indeed, in control sheep TxB_2 decreased towards baseline although P_{pa} did not fall and in ASA-pretreated sheep the delayed rise in P_{pa} took place although plasma TxB_2 remained low.

Aggravation of hypoxemia does not appear to play a role in the late pulmonary hypertension, since PaO_2 tended to increase and FiO_2 was maintained at 0.4. Therefore, progressive aggravation of the pulmonary damage is probably responsible for the late hemodynamic modifications. Furthermore, this was suggested by the presence of similar histologic findings in both control and ASA-pretreated sheep.

Although we have no direct evidence that prostacyclin was synthetized in the lung either by lung tissue itself or by blood cells, the higher concentration of 6-keto-FGF1α in arterial than in mixed venous blood at T_0 in control sheep [100 pg/ml vs. 59 pg/ml, respectively, on average] ($p < 0.05$) suggests that prostacyclin was indeed produced in the lung itself. Since prostacyclin escapes pulmonary inactivation, the increase in arterial concentration could occur by the locally increased synthesis and release of prostacyclin in response to OA-infusion to counteract by its vasodilating activity the vasoconstrictor action of TxA_2, so that the net hemodynamic effect depends on the balance between the opposite actions of these vasoactive agents [23]. However, it cannot be ruled out that the early increase of prostacyclin levels may occur as a result of the extensive endothelial damage observed in experimental lung injury.

The pulmonary vasodilator effect of prostacyclin is well documented [24]. In our study, it was fully masked in control sheep by the concomitant and more important increase in thromboxane production so that its role in adjusting ventilation/perfusion ratios ($\dot{V}A/\dot{Q}$) in OA-induced lung injury cannot be assessed. Moreover, also the generation in this model of nitric oxide (NO), an endothelium derived relaxing factor (EDRF), could play a role to explain the systemic and pulmonary vasodilation which is not counteracted by thromboxane in ASA treated animals. As compared with the systemic circulation, studies of endothelium-dependent relaxation mediated by NO in the pulmonary vascular bed are relatively scarce. Nevertheless, sufficient evidence has emerged in recent years to allow a preliminary assessment of the role of NO in the modulation of pulmonary vascular tone in health and during acute alveolar hypoxia [25]. Recent studies [26] suggest that EDRF is released during canine OA lung injury and attenuates pulmonary hypertension. Moreover, preliminary results of other Authors suggest that inhaled NO is both an effective and selective pulmonary vasodilator [27]. However, whether EDRF impairs the

hypoxic regulation of gas exchange (dilating vessels to both ventilated and unventilated alveoli) in OA lung injury is uncertain.

Respiratory system mechanics

ASA-pretreatment did not improve the maintained reduction of respiratory system static compliance observed after OA. The possibility that hemodynamic changes following administration of ASA were secondary to acute alterations in respiratory system mechanics could so be excluded. However, the fact the ASA-pretreatment early decreased the peak airway pressure and, consequently, the early increase of airways resistance (R_{aw}), with no change of Cst,rs, suggests that TxA_2 constricted predominantly large airways. Later increases of R_{aw} could be attributable to other mechanisms, not related to TxA_2-release. We only speculate on lipoxygenase pathway mediators and oxidant by-products could play a role in this late phase.

Tissue gas exchange

Our findings suggest that the protracted inhibition of the thromboxane-induced systemic vasoconstriction results in a systemic vasodilation in ASA-pretreated sheep. This vasodilation, in turn, may impair the mechanisms that modulate the functional distribution of capillary perfusion (controlled by the precapillary sphincter), modifying the surface area available for the blood-tissue exchange and the capillary-to-cell diffusion distance. This modified vaso-regulation could result, at the later stage of OA lung injury, in a peripheral shunting of blood to organs with low O_2 requirements [28], as indicated by the reduction of the arterial-mixed venous O_2 content difference despite the unchanged cardiac output and the low SVR (Table 2). Moreover, the presence of a vascular mechanism of tissue maldistribution, reducing blood flow disproportionately for an effective blood-tissue exchange, with consequent abnormal O_2 extraction, is confirmed by the progressively decrease of $\dot{V}O_2$ and $\dot{V}CO_2$, significantly reduced at the later stage in ASA-pretreated sheep (Table 2).

Moreover, in analyzing any change in tissular exchange ratio it is important to separate out the cause; a rise in $\dot{V}O_2$ has different implications than a fall in $\dot{V}CO_2$. Our data show that the change in tissue R mainly arises from an abrupt rise in $\dot{V}O_2$; this likely represents a marked increase in hypermetabolism due to organ inflammation. Such a process is likely to be accompanied by a fall in tissue R as hypermetabolism generally is accompanied by a fuel shift to lipids. In our mind the most intriguing aspect of the study is this rise in $\dot{V}O_2$ in the untreated group; which does not occur in the treated. Can OA-induced injury be mediated by prostaglandin as the primary mediator? A second issue is to determine whether respiratory exchange ratio (R) in fact equals tissue exchange ratio. Under circumstances where there is a change in acid-base status or a change in CO_2 stores the R value may not equal tissue R hence no assessments of fuel utilization can be made. However, since arterial shunting does not modify tissue exchange ratio and providing R of entire organism represents tissue R, the stability in R at ≈ 1 in ASA-pretreated sheep compared to its fall to ≈ 0.7 in untreated ones, could suggest shift to preferential utilization of lipids by tissues in controls (Table 2). Shift to anaerobic metabolism is unlikely since $P\bar{v}O_2$ is as low as in ASA-group (Table 1). How can these changes be related to the altered pulmonary capillary permeability in this experimental pulmonary edema? It is generally agreed that the main site of injury in OA lung injury is the capillary endothelium: moreover, it is now clear that pulmonary capillary endothelial tissue has a metabolic and endocrine function, so that it inactivates or activates certain circulating compounds while synthesizing and releasing others [29]. Even though it is well known that O_2 tissue delivery decreases when blood flow decreases, it is important to point out that, in addition to requiring oxygen, lung tissue requires continued supplies of glucose, because both pulmonary artery endothelial cells and lung epithelial cells show high rates of aerobic glycolysis[30]. This finding could confirm the above mentioned metabolic shift toward lipids in control sheep. Furthermore, it has been suggested that aspirin may affect the fatty acid composition pattern in certain phospholipids found in lung surfactant [31]. However, the mechanism of this observed decrease in linoleic acid content and its meaning is not very clear. In summary, it seems that the role of the lung on whole-body metabolism in this experimental pulmonary edema deserves more attention.

In conclusion, we found that ASA-pretreatment in a sheep model of OA pulmonary edema profoundly modified pathophysiological responses: indeed, unlike Julien's results in OA lung injury in sheep [5] indicating no effect of ibuprofen, we found an effective protective action of low-doses of ASA against early acute pulmonary hypertension and bronchoconstriction, and on increase of TxB_2, typical hallmarks of the first phase of lung injury. Differences in mechanisms of action of the two drugs, amount of OA administered (their dose was 30% less than our dose was) and their duration of OA-infusion (1 h, about 1/3 the rate of our infusions), anesthetics used (barbiturates vs. our ketamine) might account for these differences. Thus, it is likely that lung injury was considerably greater in our sheep than it was in the others. This probably explains why they were able to carry out their experiments for 8 h, whereas we stopped at 3 h. However, this is a sufficient time to clearly develop both the most important early cardio-respiratory changes and the functional late picture, completely defined [32]. Our contention that the effects of drugs may be different is supported by a study of Fuhrman et al. [7]: it showed in dogs given about the same amount of OA over the same length of time, that ibuprofen only attenuated the increase of plasma TxB_2 and the early pulmonary hypertension and OA-induced early hypoxemia.

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