External detection of pulmonary accumulation of indium-113m labelled transferrin in the guinea pig

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Abstract

Accumulation of radioisotope labelled transferrin in the lungs of guinea pigs was determined with an external detection system. The method is based on the intravascular and extravascular distribution of indium-113m labelled transferrin compared with the intravascular distribution of technetium-99m labelled red blood cells. Guinea pigs were given iloprost, a prostacyclin analogue and potent pulmonary vasodilator, and noradrenaline, a pulmonary vasoconstrictor, in an attempt to increase and decrease respectively the blood volume in the lungs. Neither agent altered transferrin accumulation in the lung by comparison with a saline infusion. Iloprost infused before and after oleic acid infusion reduced macromolecular leakage when compared with oleic acid alone. These data suggest that the double isotope method can distinguish between hydrostatic and injury induced pulmonary oedema.

The basis of external radioflux detection of isotope labelled plasma protein in the pulmonary circulation was described in sheep.¹ Modifications of this method in large experimental animals have been reported³⁻⁵ but are not applicable to many laboratories. Clinical studies using this method have also been reported.³⁻⁸ We have developed a modification of the double isotope method for use in the guinea pig.² Two methodological questions regarding this model are whether changes in lung blood pool volume affect the detection of protein flux, and whether the method can distinguish between protein poor and protein rich pulmonary oedema.

Procedures previously used to answer these questions,^{1-9,10} such as whole blood transfusion and left atrial balloon inflation, are not applicable in the guinea pig because of its size. We therefore used pharmacological methods. Noradrenaline, a pulmonary vasoconstrictor that causes pulmonary hypertension,¹¹ increases pulmonary capillary pressure,¹² and reduces pulmonary vascular volume by causing predominant postcapillary vasoconstriction,¹³⁻¹⁵ was used to induce hydrostatic oedema. Iloprost, a prostacyclin analogue and a potent pulmonary vasodilator, was used to increase lung blood volume.^{11,16} The effect of iloprost on pulmonary oedema induced by oleic acid was studied to show whether the

external detection of radioactivity could show changes in plasma protein accumulation in the lung.

Materials and methods

Thirty five male inbred albino guinea pigs (Dunkin-Hartley, conventionally bred) were used (Sahlins Farm, Malmö, Sweden). They weighed 895 (SD 145) g and were kept under a 12 hour daylight cycle, in 30-50% humidity, at 20-22°C. The animals were housed two to a cage (19 × 34 × 56 cm), and they had free access to food (EWOS, Södertälje, Sweden) and drinking water.

EXPERIMENTAL PROCEDURES

Guinea pigs were anaesthetised with ketamine (Ketalar 30 mg/kg body weight subcutaneously) and xylazine (Rompun 3 mg/kg intramuscularly) and maintained on ketamine 4 mg/ml Ringer solution infused at 3.0 ml/h. A single injection of xylazine (3 mg/kg intramuscularly) was administered 2.5 hours after the start of anaesthesia. The animals breathed spontaneously during the experiments. Body temperature was kept at 36-38°C by means of a rectal thermistor and a heating pad. Polyethylene catheters (diameter 0.96 mm outside, 0.58 mm inside) were introduced into the left and right femoral arteries. A catheter was placed in the right internal jugular vein for the infusion of drugs. Carotid artery blood pressure was recorded with a Micro-Switch 156 PC transducer (Honeywell, USA) zeroed to the heart level. When needed, mepivacain (Carbocain 5 mg/ml) was added drop by drop to prevent vascular spasm during catheterisation. The catheters were flushed with heparinised saline (50 IE/ml saline).

RADIOACTIVITY MEASUREMENTS

Red blood cells were labelled *in vivo* with technetium-99m (half life 6.0 hours) and transferrin (molecular weight 76 000) was labelled *in vivo* with indium-113m (half life 100 min). An external dual probe scintillation detector system was used to sample the entire photon energy spectrum for both isotopes continuously. One scintillation probe was placed vertically over the heart at midsternal level and the other in the horizontal plane just caudal to the right shoulder. Both probes were collimated with lead (fig 1) and registered activity from two conically shaped volumes in the chest. The collimators reduced radiation from surrounding tissues by 68-94%. The efficiency of sampling decreased with increas-

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Figure 1 Structure of the collimator used in the experiments (measurements in millimetres).

ing distance from the detector (> 60% reduction 2.0 cm into the thorax). The base of the “sampling cone” in the thorax was 15 mm in diameter 2 cm below the opening of the collimator for the energy level of ^{99m}Tc. The calculated cone volume was smaller with the energy level of ^{113m}In. Thus the heart probe mainly sampled activity from the heart and the other probe mainly the right lung with a very small contribution from the heart. The areas under the four energy peaks were integrated and used for further calculations. Counts were collected for three minute periods for 3.5 hours.

To calculate the contribution of ^{113m}In radiation to the ^{99m}Tc peak, ^{113m}In was injected 15 minutes before ^{99m}Tc. Thirty minutes were allowed for distribution and equilibrium of the radionuclides after the ^{99m}Tc injection. The latter part of this period was used for baseline measurements.

PROTOCOL

Treatment groups are given in table 1. Oleic acid (0.03 ml/kg/10 min intravenously; Sigma chemical, St Louis, USA) or saline infusions were started at time zero. In the combined experiments iloprost (supplied by E Schilling, Schering AG Research, Berlin) infusion (5 ng/min/kg) was started 60 minutes before oleic acid administration and lasted for a total

Table 1 Treatment of the five groups

No of animals	Treatment	Dose
6	Iloprost	40 ng/min/kg, 120 min
6	Noradrenaline	1 µg/min/kg, 120 min
10	Saline	0.03 ml/kg, 10 min
7	Oleic acid	0.03 ml/kg, 10 min
6	Iloprost + oleic acid	5 ng/min/kg, 180 min + 0.03 ml/kg, 10 min

of 180 minutes with a break for the oleic acid infusion. In the single agent experiments noradrenaline (1 µg/min/kg) or iloprost (40 ng/min/kg) infusions were started at time zero and continued for 120 minutes. The drugs were diluted in saline and given to the animals in a total volume of 4 ml.

At the end of the experiment, the animals were killed with 1 ml of pentobarbital sodium (60 mg/ml). The lungs were dissected and weighed immediately. Pieces of lung for wet-dry weight analysis were dried to a constant weight at 80°C. Lung water was expressed as a percentage of wet weight (wet weight – dry weight)/wet weight × 100.¹⁷

BLOOD ANALYSES

Blood cell counts were performed on 200 µl whole blood taken from the femoral artery into EDTA microtainer tubes at 0, 15, 30, 60, 90, and 120 minutes. White blood cell and platelet counts were determined in a Twincounter 187 (Analys Instrument, Stockholm) and expressed as percentages of the initial values. Differential white blood cell counts were performed on blood films stained with Giemsa-May-Grünwald at Medi-Lab, Stockholm. For determination of arterial oxygen tension (Pao₂) 400 µl whole blood was taken anaerobically from the femoral artery in heparinised syringes at 0, 15, 30, 60, 90, and 120 minutes and analysed with an OSM Hemoximeter (Radiometer, Copenhagen) and an ABL 3 Bloodgas Analyser set at 38°C. The results were expressed as percentages of the initial values.

As a measure of free oxygen radical activity, plasma concentrations of uric acid were measured spectrophotometrically (Abbott Biochromatic Analyser 100; A-GENTTM Uric Acid, Abbott Diagnostica Division). Whole blood (500 µl) was collected in 50 µl 3.8% citrate buffer at 0, 15, 30, 45, 60, 90, and 120 minutes in the group of animals having oleic acid and iloprost. The samples were immediately centrifuged and the plasma was frozen for subsequent analyses. The red blood cells of the same samples were washed three times with saline and kept at 4°C for up to 72 hours before determination of catalase activity.¹⁸ Catalase activity was measured in red cell lysates by observing the decrease in absorbance at 240 nm of 30 mM H₂O₂. The decrease of absorbance per time unit is a direct measure of the catalase activity, which is expressed as the rate constant/g haemoglobin (K/g Hb).

DATA HANDLING

All counts from ^{99m}Tc and ^{113m}In were corrected for background radiation, physical decay, and channel overlap. Loss of activity through blood sampling was considered to be almost constant. The integrals of the four energy peaks were calculated by the multichannel analyser. Each data point was then calculated with the use of these integrals as follows:

$$\frac{{}^{113m}\text{In lung cpm}}{{}^{99m}\text{Tc lung cpm}} \\ \frac{{}^{113m}\text{In labelled heart cpm}}{{}^{99m}\text{Tc heart cpm}}$$

The activity ratios were plotted against time

Table 2 Baseline data (mean (SD) values) on the anaesthetised guinea pigs before the infusions

Group	Saline	Oleic acid	Noradrenaline	Iloprost	Iloprost + oleic acid
White blood cells ($10^9/l$)	7.1 (1.0)	5.7 (3.0)	7.6 (4.1)	8.6 (1.3)	11.0 (0.9)
Neutrophils ($10^9/l$)	3.2 (0.9)	2.6 (2.3)	—	—	6.6 (1.5)
Platelets ($10^9/l$)	268 (86)	315 (67)	273 (25)	310 (63)	321 (49)
Arterial oxygen tension (mm Hg)	61 (13)	74 (16)	67 (13)	67 (12)	65 (4)
Mean arterial blood pressure (mm Hg)	54 (5)	57 (7)	52 (3)	58 (7)	62 (9)
Heart rate (beats/min)	177 (27)	181 (26)	179 (16)	206 (29)	180 (12)
Plasma uric acid ($\mu\text{mol/l}$)	32 (18)	37 (10)	56 (23)	59 (32)	64 (25)
Red blood cell catalase (K/g Hb)	180 (44)	—	—	—	292 (259)

Conversion to SI units: 1 mm Hg $\approx$ 0.133 kPa.

and by linear regression (method of least squares) of the steepest part of the curve the regression line slope (RLS) and the intercept of the regression line were derived. To avoid artefacts from uneven mixing of activity or failure to reach equilibrium between intravascular and extravascular spaces the first 30 minutes were excluded from this analysis.

STATISTICS

Mean values and standard deviations are given. Statistical evaluation was performed with the F test for equality of variance, Student's *t* test, and one way analysis of variance (ANOVA). Probability values below 0.05 were considered statistically significant.

Results

Baseline haematological, biochemical and haemodynamic measurements are shown in table 2.

EFFECT OF NORADRENALINE AND ILOPROST

There were no significant differences in transferrin accumulation (expressed as RLS $\times$

10^{-3} min^{-1}) in the lung after infusion of noradrenaline (mean RLS 0.52) or iloprost (1.69) and after saline infusion (1.01) (table 3).

Mean arterial blood pressure increased to a maximum value that was 190% (SD 8%) of the initial value four minutes after the start of the noradrenaline infusion and fell to 49% (9%) within two minutes of the end of the infusion. Iloprost caused a substantial decrease in mean arterial blood pressure, which reached 64% (10%) of the initial value six minutes after the start of the infusion, remained unchanged throughout the rest of the experiment, and rose to 81% (15%) of the initial value after the infusion had stopped. Saline infusion did not change blood pressure. Heart rate did not alter significantly with any infusion, though it was somewhat higher in the noradrenaline group at 15 minutes (131% (17%) of baseline).

Noradrenaline caused the white blood cell count to increase by 82% (13%) at 15 minutes and it remained raised until the end of the infusion (fig 2, table 4), returning to near basal values (120% (25%)) 30 minutes after the infusion stopped. The white cell count did not change with the iloprost infusion. The platelet count did not change significantly in any group. There was no change in PaO_2 in any group. Lung water measurements after the infusion showed no difference between noradrenaline (81.3% (0.7%)), iloprost (81.2% (1.5%)), and saline (79.9% (1.4%)).

EFFECT OF OLEIC ACID WITH AND WITHOUT ILOPROST

When iloprost was given with oleic acid transferrin accumulation (expressed as RLS $\times 10^{-3} \text{ min}^{-1}$) in the lung was less (RLS 1.59) than after oleic acid alone (3.86; $p = 0.026$); see table 3. The first 60 minutes of infusion were used to control for the effects of iloprost alone. The changes in transferrin accumulation did not differ from those seen with saline. During the oleic acid infusion period when iloprost was stopped temporarily transferrin accumulation increased significantly more (RLS 13.0) than in the group receiving oleic acid alone (3.86; $p = 0.011$).

The changes in blood cell counts and PaO_2 did not differ significantly between the animals receiving iloprost and oleic acid and the group having oleic acid only except that the maximum fall in white blood cell count seen at 15 minutes was greater in the iloprost plus oleic acid group (58% (30%)) of the initial value) than in the

Figure 2 White blood cells (WBC), as percentages of initial values, in saline ($n = 10$), noradrenaline ($n = 6$; $1 \mu\text{g/min/kg}$), or iloprost ($n = 6$; 40 ng/min/kg) treated guinea pigs: mean values versus time. Noradrenaline versus saline, iloprost versus saline: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Student's *t* test).

Table 3 Regression line slope (RLS) values for transferrin accumulation ($\times 10^{-3} \text{ min}^{-1}$) from individual animals in the five treatment groups

Saline	Oleic acid	Noradrenaline	Iloprost	Iloprost + oleic acid
1.20	5.59	-0.42	0.81	0.96
-1.34	3.00	-0.37	1.49	1.93
2.40	3.57	1.62	2.73	-0.24
1.14	4.38	0.25	1.45	0.22
1.99	3.85	1.21	1.25	5.48
1.33	2.20	0.86	2.39	1.20
-0.48	4.43	—	—	—
0.49	—	—	—	—
1.76	—	—	—	—
1.63	—	—	—	—
Mean (SD)				
1.01	3.86	0.52	1.69	1.59
(1.16)	(1.09)	(0.84)	(0.73)	(2.05)

group receiving oleic acid only (78% (23%)). Neutrophil counts were reduced significantly in the group receiving iloprost (fig 3, table 4). Both groups receiving the oleic acid infusion had a greater fall in PaO_2 at 15 minutes than the animals receiving saline ($p < 0.001$, table 4). Mean arterial blood pressure did not change with saline but it fell by about 30% in the iloprost group and by 40% after oleic acid alone within 20 minutes of the start of the infusion; it then recovered slowly during the rest of the experiment. Heart rate was unchanged in all groups. There were no differences in lung water between the two oleic acid groups (oleic acid only 81.9% (2.3%), iloprost plus oleic acid 81.9% (0.9%)).

The plasma uric acid concentration did not differ significantly between the animals receiving oleic acid plus iloprost and those having saline but it was substantially higher in the group having oleic acid alone (table 4); this increase was significantly greater than the change after saline at 15 minutes ($p < 0.01$).

Red blood cell catalase activity fell in the iloprost group, the lowest value (58.2% (29.3%) of the initial value) being seen at 120 minutes.

Discussion

The absence of changes in transferrin accumulation in the lung after an infusion of noradrenaline or iloprost suggests that this index of permeability corrects for blood pool changes in the lungs. Noradrenaline was infused slowly to prevent endothelial cell damage from pressure effects. The unchanged heart rate indicates that little, if any, of the action of noradrenaline and iloprost is due to systemic effects. The high mean arterial blood pressure at the start of the noradrenaline infusion is likely to have led to compensatory mechanisms or tachyphylaxis as it returned towards baseline during the experiment despite the continued infusion. The low arterial pressure seen with iloprost stayed at the nadir level during the experiment, returning to baseline only after the end of the infusion. The infusion of iloprost alone to the guinea pigs in one of our groups served as an additional control for the stability of the transferrin accumulation measurements in the initial part of the study in the group receiving iloprost plus oleic acid, though the doses given to the two groups were different.

The increased pressure in the pulmonary circulation that would be expected from the noradrenaline infusion^{11,12} could have caused a protein poor hydrostatic oedema undetectable by the scintillation probes. We were unable to measure pulmonary wedge pressure to confirm the increased vascular pressure in the lung after noradrenaline infusion directly, but the trend

Table 4 Mean (SD) values for white blood cells, neutrophils, arterial oxygen tension, and plasma uric acid concentration as percentages of initial values in the five treatment groups

Time (min)	0	15	30	45	60	90	120
<i>White blood cells (% initial value)</i>							
Saline	100	103 (10)	110 (14)	—	122 (18)	116 (21)	120 (20)
Iloprost	100	122 (11)	130 (16)	—	130 (18)	126 (18)	127 (18)
Noradrenaline	100	182 (13)	188 (24)	—	186 (39)	183 (45)	195 (56)
<i>Neutrophils (% initial value)</i>							
Saline	100	103 (30)	98 (17)	—	122 (25)	129 (30)	116 (54)
Oleic acid	100	79 (28)	113 (32)	—	118 (41)	132 (28)	156 (50)
Iloprost + oleic acid	100	42 (44)	64 (29)	—	73 (45)	58 (23)	88 (34)
<i>Arterial oxygen tension (% initial value)</i>							
Saline	100	101 (13)	110 (15)	—	105 (18)	112 (19)	129 (26)
Oleic acid	100	65*** (19)	72** (24)	—	71** (26)	78* (30)	90* (33)
Iloprost + oleic acid	100	62*** (19)	91 (32)	—	89 (23)	90 (35)	83** (25)
<i>Uric acid (% initial value)</i>							
Saline	100	104 (16)	95 (25)	118 (30)	97 (23)	92 (23)	107 (34)
Oleic acid	100	151** (45)	159 (36)	190 (68)	154** (99)	161** (89)	150** (108)
Iloprost + oleic acid	100	116** (41)	138* (60)	123 (52)	109 (36)	117 (40)	104* (36)

$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ in the comparison of each treatment with the saline control.

Figure 3 Neutrophils as percentages of initial values in saline ($n = 10$), oleic acid; ($0.03 \text{ ml/kg/10 min}$); ($n = 7$), or iloprost (5 ng/min/kg) + oleic acid ($0.03 \text{ ml/kg/10 min}$) ($n = 6$) treated guinea pigs: mean values versus time. Saline versus oleic acid, saline versus iloprost + oleic acid: * $p < 0.05$, ** $p < 0.01$ (Student's t test).

towards increased lung water content supports the presence of hydrostatic pulmonary oedema. This suggests that the permeability index we used is not influenced by changes in pulmonary blood volume or increased pulmonary microvascular pressure. These results are in accordance with reports that such external radioisotope measurements are specific for pulmonary oedema with increased protein permeability.⁴ Gorin *et al*¹ showed a close correlation between lymph protein concentration measured directly and external radioflux detection in sheep after bacteraemia and Sugerman *et al*,¹⁹ using computerised gamma scintigraphy, showed that the lung:heart ratio of ^{99m}Tc labelled human serum albumin was significantly greater after oleic acid administration to dogs than the lung:heart ratio of ^{99m}Tc labelled red blood cells. These studies confirm that external detection of radiolabelled protein detects lung injury and not oleic acid induced change in pulmonary vascular volume. The findings in these animal studies have been supported by clinical studies in which patients with adult respiratory distress syndrome were compared with patients with renal or cardiogenic pulmonary oedema.^{3,8} The results of these studies support the specificity of this method as shown in our small animal model.

The reduction in oleic acid induced macromolecular permeability seen when oleic acid was preceded by iloprost infusion confirms reports of studies using other methods.^{16,20,21} The transferrin accumulation rate was linear throughout the control experiments, and the stability of the transferrin accumulation after the infusion of iloprost alone shows that the decreased transferrin accumulation after oleic acid when given with iloprost is not artefactual. The favourable effects of iloprost after oleic acid injury may have been produced

indirectly by the decreased pressure in the pulmonary circulation rather than from a direct effect on the pulmonary microvascular membrane, though prostacyclin may also be important in preserving the cellular integrity of the lungs.^{20,22} An explanation of why the Pao_2 and lung water did not return towards control values despite a decrease in transferrin accumulation could be that the permeability index is a more sensitive indicator of changes in pulmonary extravasation than traditional methods of assessment, such as wet-dry weight, and that it detects a change in extravasation earlier.⁴

The failure of iloprost to prevent the leucopenia and thrombocytopenia induced by oleic acid infusion is in keeping with the results of studies in which prostacyclin has prevented or attenuated oedema formation.²¹ The protective effects of iloprost on platelet function are probably concentration and species dependent.^{23,24} Prostacyclin also counteracts oxygen free radical activities.²⁵ We found diminished red blood cell catalase activity and uric acid production after iloprost was added to oleic acid treatment. Winterbourn *et al*²⁶ showed that red blood cells are an important component of the extracellular antioxidant defence system. The inhibition of uric acid production seen with iloprost might indicate a beneficial action of iloprost on oxygen free radical mediated injury and suggests a link between functional inhibition of neutrophils and decreased uric acid production. The neutrophil count in our study fell to a greater extent after iloprost than with oleic acid alone. Reduced circulating neutrophils are considered to indicate possible sequestration in the lung in many shock states.²⁷ Despite this, the rate of macromolecular extravasation was decreased in our study, which might indicate inactivation of the sequestered neutrophils by iloprost.

External detection of the accumulation of radiolabelled protein in the lung is a reliable means of separating protein poor oedema resulting from increased pulmonary pressure and protein rich oedema resulting from lung injury. The method of calculating the regression line slope corrected for changes in the volume of the lung blood pool. Iloprost attenuated oleic acid induced pulmonary oedema possibly through the inactivation of sequestered neutrophils. This model in the guinea pig may be of value for studying treatments that inhibit development of protein rich pulmonary oedema.

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