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

Ursula Hultkvist-Bengtsson, Lena Mårtensson

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 macro-molecular 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. Modifications of this method in large experimental animals have been reported but are not applicable to many laboratories. Clinical studies using this method have also been reported. We have developed a modification of the double isotope method for use in the guinea pig. 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, 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, increases pulmonary capillary pressure, and reduces pulmonary vascular volume by causing predominant postcapillary vasoconstriction, was used to induce hydrostatic oedema. Iloprost, a prostacyclin analogue and a potent pulmonary vasodilator, was used to increase lung blood volume. 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 (Sahlns 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 x 34 x 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, meipvacain (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-
of 180 minutes with a break for the oleic acid infusion. In the single agent experiments noradrenaline (1 μg/min/kg) or ioprost (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.

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-GENT™ 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 ioprost. 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.

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 99mTc and 113mIn 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:

\[
\frac{113mIn \text{ lung cpm}}{99mTc \text{ lung cpm}} = \frac{113mIn \text{ labelled heart cpm}}{99mTc \text{ heart cpm}}
\]

The activity ratios were plotted against time.
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 intra-vascular 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} \text{ 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 Pao2 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 Pao2 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

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Saline</th>
<th>Oleic acid</th>
<th>Noradrenaline</th>
<th>Iloprost</th>
<th>Iloprost + oleic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells (10^9/l)</td>
<td>7.1 (1)</td>
<td>5.7 (3)</td>
<td>7.6 (4)</td>
<td>8.6 (1)</td>
<td>11.0 (0)</td>
</tr>
<tr>
<td>Neutrophils (10^9/l)</td>
<td>3.2 (0)</td>
<td>2.6 (2)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Platelets (10^9/l)</td>
<td>26.8 (86)</td>
<td>315 (67)</td>
<td>273 (25)</td>
<td>310 (63)</td>
<td>321 (49)</td>
</tr>
<tr>
<td>Arterial oxygen tension (mm Hg)</td>
<td>61 (13)</td>
<td>74 (16)</td>
<td>67 (13)</td>
<td>67 (12)</td>
<td>65 (4)</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>54 (5)</td>
<td>57 (7)</td>
<td>52 (3)</td>
<td>58 (7)</td>
<td>62 (9)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>177 (27)</td>
<td>181 (26)</td>
<td>179 (16)</td>
<td>206 (29)</td>
<td>180 (12)</td>
</tr>
<tr>
<td>Plasma uric acid (μmol/l)</td>
<td>32 (18)</td>
<td>37 (10)</td>
<td>56 (23)</td>
<td>59 (32)</td>
<td>64 (25)</td>
</tr>
<tr>
<td>Red blood cell catalase (K/g Hb)</td>
<td>180 (44)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>292 (259)</td>
</tr>
</tbody>
</table>

Conversion to SI units: 1 mm Hg ≈ 0.133 kPa.

Figure 2 White blood cells (WBC), as percentages of initial values, in saline (n = 10), noradrenaline (n = 6; 1 μg/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).
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 PaO₂ 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 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 3  Regression line slope (RLS) values for transferrin accumulation (×10⁻⁴ min⁻¹) from individual animals in the five treatment groups

<table>
<thead>
<tr>
<th>Saline</th>
<th>Oleic acid</th>
<th>Noradrenaline</th>
<th>Iloprost</th>
<th>Iloprost + oleic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-20</td>
<td>5-59</td>
<td>-0-42</td>
<td>0-81</td>
<td>0-96</td>
</tr>
<tr>
<td>1-34</td>
<td>3-00</td>
<td>-0-37</td>
<td>1-49</td>
<td></td>
</tr>
<tr>
<td>2-40</td>
<td>3-57</td>
<td>1-62</td>
<td>2-73</td>
<td>-0-24</td>
</tr>
<tr>
<td>1-14</td>
<td>4-38</td>
<td>0-25</td>
<td>1-45</td>
<td>0-22</td>
</tr>
<tr>
<td>1-99</td>
<td>3-85</td>
<td>1-21</td>
<td>1-25</td>
<td>5-48</td>
</tr>
<tr>
<td>1-33</td>
<td>2-20</td>
<td>0-86</td>
<td>2-39</td>
<td>1-20</td>
</tr>
<tr>
<td>-0-48</td>
<td>4-43</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>0-49</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>1-76</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>1-63</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>3-86</td>
<td>0-52</td>
<td>1-69</td>
<td>1-59</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>White blood cells (%) initial value</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>100</td>
<td>103</td>
<td>110</td>
<td>-</td>
<td>122</td>
<td>116</td>
<td>120</td>
</tr>
<tr>
<td>Iloprost</td>
<td>100</td>
<td>122</td>
<td>130</td>
<td>-</td>
<td>130</td>
<td>126</td>
<td>127</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>100</td>
<td>182</td>
<td>188</td>
<td>-</td>
<td>186</td>
<td>183</td>
<td>195</td>
</tr>
<tr>
<td><strong>Neutrophils (%) initial value</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>100</td>
<td>103</td>
<td>98</td>
<td>-</td>
<td>122</td>
<td>129</td>
<td>116</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>100</td>
<td>79</td>
<td>113</td>
<td>-</td>
<td>118</td>
<td>132</td>
<td>156</td>
</tr>
<tr>
<td>Iloprost + oleic acid</td>
<td>100</td>
<td>42</td>
<td>64</td>
<td>-</td>
<td>73</td>
<td>58</td>
<td>88</td>
</tr>
<tr>
<td><strong>Arterial oxygen tension (%) initial value</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>100</td>
<td>101</td>
<td>110</td>
<td>-</td>
<td>105</td>
<td>112</td>
<td>129</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>100</td>
<td>65**</td>
<td>72**</td>
<td>-</td>
<td>71**</td>
<td>76**</td>
<td>90*</td>
</tr>
<tr>
<td>Iloprost + oleic acid</td>
<td>100</td>
<td>62***</td>
<td>91</td>
<td>-</td>
<td>89</td>
<td>90</td>
<td>83**</td>
</tr>
<tr>
<td><strong>Uric acid (%) initial value</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>100</td>
<td>104</td>
<td>95</td>
<td>118</td>
<td>97</td>
<td>92</td>
<td>107</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>100</td>
<td>151**</td>
<td>159</td>
<td>190</td>
<td>154**</td>
<td>161**</td>
<td>150**</td>
</tr>
<tr>
<td>Iloprost + oleic acid</td>
<td>100</td>
<td>116**</td>
<td>138*</td>
<td>123</td>
<td>109</td>
<td>117</td>
<td>104*</td>
</tr>
</tbody>
</table>

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 ml/kg/10 min); (n = 7), or iloprost (5 ng/min/kg) + oleic acid (0-03 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. Gordon et al. showed a close correlation between lymph protein concentration measured directly and external radioflux detection in sheep after bacteremia and Sugerman et al., using computerised gamma scintigraphy, showed that the lung:heart ratio of 99mTc labelled human serum albumin was significantly greater after oleic acid administration to dogs than the lung:heart ratio of 99mTc labelled red blood cells. These studies confirm that external detection of radio labelled 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. 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. 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. An explanation of why the PaO₂ and lung water did not return towards control values despite a decrease in transferrin accumulation would 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.

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. The protective effects of iloprost on platelet function are probably concentration and species dependent. Prostacyclin also counteracts oxygen free radical activities. We found diminished red blood cell catalase activity and uric acid production after iloprost was added to oleic acid treatment. Winterbourn et al. 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. 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.

We express our gratitude to Ewa Dahlsberg, Christina Johansson, and Anna-Maria Larsson for their excellent technical assistance. This study was supported by Gambio AB, the Hierta-Resitu Foundation, and the Crafoord Foundation.

4 Dauber IM, Pless WT, van Gendtelle A, Trow RS, Weil

---

**References:**


External detection of pulmonary accumulation of indium-113m labelled transferrin in the guinea pig.
U Hultkvist-Bengtsson and L Mårtensson

Thorax 1990 45: 688-693
doi: 10.1136/thx.45.9.688

Updated information and services can be found at:
http://thorax.bmj.com/content/45/9/688

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/