Dexamethasone and pentastarch produce additive attenuation of ischaemia/reperfusion lung injury

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ABSTRACT

The choice of an intravenous solution for the attenuation of ischaemia/reperfusion (I/R) lung injury is still a difficult one. Although 10% (w/v) pentastarch has been used in ICU settings, its use in I/R lung injury has not been well explored. We hypothesized that this synthetic colloid substance, which maintains colloid osmotic pressure and potentially ‘seals’ capillary leaks, in combination with an anti-inflammatory agent (i.e. dexamethasone), would ameliorate I/R lung injury. After 60 min of lung ischaemia in an isolated rat lung model, lungs were reperfused for 60 min in a closed circulating system with one of the following solutions: (1) NS (0.9% normal saline), (2) NS + Dex (dexamethasone), (3) NS + Penta (pentastarch), or (4) NS + Penta + Dex. Haemodynamic changes, lung weight gain (LWG), capillary filtration coefficient ($K_{fc}$) and lung pathology were analysed. Results showed significantly lower values of $K_{fc}$ and LWG in pentastarch- or dexamethasone-perfused groups as compared with those in the NS group. Dexamethasone as an additive to NS + Penta further decreased $K_{fc}$ and LWG. Histopathological studies showed similar decreases in injury profiles. We conclude that reperfusion with dexamethasone and pentastarch can attenuate I/R lung injury, and that dexamethasone and pentastarch have additive effects. Our data thus suggest that the combination of a colloid substance with ‘sealing effects’ and an anti-inflammatory agent may provide a better reperfusion solution for patients with I/R lung injury or for lungs stored for transplant.

INTRODUCTION

Ischaemia/reperfusion (I/R) injury is a very common clinical problem in diseases such as myocardial or cerebral vascular ischaemia, shock and organ transplantation. The type of fluid therapy that is most beneficial for attenuation of I/R lung injury is still uncertain. Specifically, the optimal composition of the intravenous infusion solution used during the treatment phase of I/R injury has not been defined. Pentastarch solution, which has extensive clinical use, has traditionally been considered to function by increasing colloid osmotic pressure. However, this synthetic macromolecule has also been shown to reduce abnormally increased microvascular permeability in an isolated rat limb I/R injury model [1]. It is postulated that these macromolecules reduce microvascular permeability by sealing the separated endothelial junctions until reperfusion allows recovery of endo-

Key words: capillary filtration coefficient, dexamethasone, haemodynamics, ischaemia/reperfusion lung injury, lung weight gain, pathology, pentastarch.

Abbreviations: ARDS, acute respiratory distress syndrome; I/R, ischaemia/reperfusion; $K_{fc}$, capillary filtration coefficient; LWG, lung weight gain; $P_{pa}$, pulmonary arterial pressure; $P_{pc}$, pulmonary capillary pressure; $P_{pv}$, pulmonary venous pressure; $R_{a}$, pulmonary arterial resistance; $R_{v}$, pulmonary venous resistance.

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thelial cell function. Data supporting a 'sealing effect' of biodegradable macromolecules without occlusion of the capillaries have been reported [2,3], e.g. pentastarch has been used to treat canine I/R myocardial injury [4]. In addition to the above-mentioned main protective effects of pentastarch, other possible effects, such as a decrease in neutrophil adherence to the endothelium, stabilization of the endothelial cell membrane [5] and a decrease in xanthine oxidase release [6], have been suggested. Although the pathogenesis of I/R lung injury is still not well known, previous studies have shown that, in addition to capillary leakage, inflammation plays an important role in producing I/R injury [7–8].

Based on these concepts, we propose that macromolecules (i.e. pentastarch) that act to seal endothelial damage and prevent capillary leakage, and a potent anti-inflammatory agent (i.e. a steroid), could be combined to develop an improved fluid therapy for prevention of impending I/R injury. To test this hypothesis, we have evaluated whether pentastarch or dexamethasone alone can reduce I/R injury, and also whether dexamethasone can enhance the effects of pentastarch to protect against I/R injury. Results from the present study demonstrate that either pentastarch or dexamethasone can partially attenuate I/R injury; however, the combination of dexamethasone and macromolecules (pentastarch) provides an additive effect that induces marked attenuation of I/R lung injury.

**METHODS**

**Preparation of isolated and perfused rat lungs**

Our *in situ* lung I/R model has been described previously [7–10]. Briefly, male Sprague–Dawley rats (250–350 g body weight) were anaesthetized intraperitoneally with sodium pentobarbital (20–25 mg). A tracheotomy was performed to permit ventilation with a Harvard rodent ventilator (Model 683; Harvard Apparatus, South Natick, MA, U.S.A.) at 55 breaths/min, at a tidal volume of 2.5 ml and a positive end-expiratory pressure of 2 cmH₂O. The inspired gas mixture contained 5% CO₂ and 95% air. After a median sternotomy was performed, heparin (1 unit/g) was injected into the right ventricle. Blood was drawn from the right ventricle and discarded. A cannula was placed into the pulmonary artery through a puncture in the right ventricle, and a tight ligature was placed around the main trunk of the pulmonary artery. A large catheter was inserted into the left atrium through the left ventricle and mitral valve, fixed by a ligature at the apex of the heart, and used to divert the pulmonary venous outflow into a reservoir. A third ligature was placed above the atrioventricular junction to prevent perfusate flowing back into the ventricles. The lungs were perfused with the chosen perfusate using a peristaltic pump (Minipulse 2; Gilson Medical Electronic, Middleton, WI, U.S.A.) at a constant flow rate of 0.03 ml·min⁻¹·g⁻¹ body weight. An initial 75 ml of the chosen perfusate, which contained residual blood cells and plasma, was discarded and not recirculated. An additional 25 ml of the chosen perfusate was recirculated in the lung. Pulmonary arterial (Pₚₐ) and pulmonary venous (Pₚᵥ) pressures were monitored continuously with pressure transducers (P23 ID; Statham, Oxnard, CA, U.S.A.) from a side-arm of the inflow and outflow cannulas, and recorded continuously on a polygraph recorder (Gould Instruments, Cleveland, OH, U.S.A.). Pᵥ was set at 2.5 mmHg by adjusting the height of the venous outflow reservoir, and zone III flow conditions (pressures: arterial > venous > alveolar) were maintained in all experiments.

The isolated perfused lung remained *in situ*, and the total weight of the rat was monitored on an electronic balance and recorded on an oscillograph after digital-to-analogue conversion. Any change in the preparation weight (body weight) was considered to be the result of a change in lung weight [7–9]. Three conditions had to be satisfied for the isolated lung preparation experiment to be continued: (1) no leakage was observed at the sites of cannula insertion, (2) no evidence of oedema was present, and (3) the lung attained an isogravimetric state, i.e. the lung was neither gaining nor losing weight.

**Perfusates**

Four different perfusates were perfused individually, followed by evaluation of I/R injury. The composition of the perfusates was as follows. (1) NS: 0.9% normal saline solution, containing 9.0 g/l NaCl (Na⁺, 154.0 mmol/l; Cl⁻, 154.0 mmol/l). (2) NS + Dex (0.04 mg/ml dexamethasone; Sigma Chemical Co., St. Louis, MO, U.S.A.). (3) NS + Penta (pentastarch): 10% HAES-sterile pentastarch was prepared by Fresenius AG, Bad Homburg, Germany. The composition of 10% HAES-sterile pentastarch is 100 g/l poly(Ö2-hydroxyethyl)-starch (hydroxyethyl starch) (molar substitution 0.40–0.55; average Mr 200000) plus 9.0 g/l NaCl (Na⁺, 154.0 mmol/l; Cl⁻, 154.0 mmol/l). The osmolarity of the perfusate was 309 mosm/l. (4) NS + Penta + Dex.

**Determination of pulmonary capillary pressure (Pᵥ)**

Pᵥ was estimated using the double-occlusion method [10,11]. Arterial inflow and venous outflow lines were occluded simultaneously, and the equilibrium Pᵥ values were measured. This equilibration pressure is the same as the isogravimetric measure of Pᵥ, and also reflects the prevailing capillary pressure when the lungs are damaged.
Calculation of pulmonary vascular resistance

Pulmonary arterial resistance ($R_a$) and venous resistance ($R_v$) were calculated using the following equations:

$$R_a = \frac{(P_{pa} - P_{pv})}{Q_a}$$

$$R_v = \frac{(P_{pv} - P_{pv})}{Q_v}$$

where $Q$ is perfusate flow.

Measurement of microvascular permeability

The pulmonary capillary filtration coefficient ($K_{fc}$) was used as an index of microvascular permeability to solvent. $K_{fc}$ was measured by a method described previously and used as an index of permeability in many published studies [10]. Briefly, after an isogravimetric state was attained in the lung, $P_{pv}$ was rapidly elevated to 6–8 cmH$_2$O for 15 min. Lung weight gain (LWG) was recorded. The recording shows a characteristic rapid weight gain (vascular filling), which is followed by a lower rate of weight gain. The rate of weight change ($\Delta W/\Delta t$) occurring in the 6–14-min interval was analysed using linear regression of the log$_{10}$-transformed rates of weight change calculated for each 1 min interval. The initial rate of weight gain was then determined by extrapolation of $\Delta W/\Delta t$ to zero time. $K_{fc}$ was then calculated by dividing $\Delta W/\Delta t$ at time 0 by the change in $P_{pv}$ that was imposed after the venous outflow pressure was increased. The $K_{fc}$ value was normalized using the baseline wet lung weight and expressed as ml·min$^{-1}$·cmH$_2$O$^{-1}$·100 g$^{-1}$ lung tissue.

Experimental protocols

The animals were divided into four groups: (1) NS, (2) NS + Dex, (3) NS + Penta and (4) NS + Penta + Dex. The isolated lungs were perfused with one of the above designated perfusates. The closed system of circulation was maintained at constant flow, volume and temperature. The experiment was initiated after haemodynamic stability had been attained for 15 min in the extracorporeal isolated lung circulation. The protocol of I/R injury challenge was as follows: the isolated lung was not ventilated or perfused for 45 min (ischaemia), followed by reinitiation of ventilation and perfusion (reperfusion) for 60 min at room temperature.

Lung histopathology

After termination of each experiment, the whole lungs were dissected and fixed immediately in 10% neutral buffered formalin. After fixation, the right middle lobes were dehydrated through a grade series of alcohol, cleared in xylene and embedded in paraffin. All sections were cut to 5 μm and stained with haematoxylin/eosin.

Statistical analysis

Values are expressed as means± S.D. Comparisons among all groups for a given variable were carried out using one-way ANOVA and Dunnett’s method of post-hoc testing. Comparison between baseline and post-reperfusion values within each group for given variables was carried out by using Student’s paired $t$ test. Statistically significant differences were accepted at $P < 0.05$.

RESULTS

LWG values

After I/R, LWG in the NS + Dex, NS + Penta and NS + Penta + Dex groups was less than that in the NS group; *$P < 0.05$ compared with NS group; †$P < 0.05$ compared with NS + Dex and NS + Penta groups; ‡$P < 0.05$ for $K_{fc}$ after I/R compared with that before I/R (baseline) in NS group.

<table>
<thead>
<tr>
<th>Group</th>
<th>$n$</th>
<th>LWG (g)</th>
<th>Before I/R (baseline)</th>
<th>After I/R</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>4</td>
<td>2.45 ± 0.84</td>
<td>0.34 ± 0.13</td>
<td>1.21 ± 0.32‡</td>
</tr>
<tr>
<td>NS + Dex</td>
<td>5</td>
<td>0.92 ± 0.16*</td>
<td>0.19 ± 0.16</td>
<td>0.30 ± 0.15*</td>
</tr>
<tr>
<td>NS + Penta</td>
<td>4</td>
<td>1.20 ± 0.29*</td>
<td>0.18 ± 0.07</td>
<td>0.32 ± 0.07*</td>
</tr>
<tr>
<td>NS + Penta + Dex</td>
<td>4</td>
<td>0.25 ± 0.08*‡</td>
<td>0.22 ± 0.10</td>
<td>0.28 ± 0.17*</td>
</tr>
</tbody>
</table>

Figure 1 LWG in the various groups during I/R

Lungs were ischaemic for the first 60 min, and then reperfused with one of four solutions for the next 60 min. In the NS group, LWG was markedly increased during reperfusion. After I/R challenge, LWG in the NS + Dex and NS + Penta groups was significantly less than that in the NS group ($P < 0.05$). Compared with the NS + Dex and NS + Penta groups, the NS + Penta + Dex group showed a further decrease in LWG ($P < 0.05$).
Table 2  Haemodynamic parameters for the various groups

Values are means ± S.D. Significance of differences: * P < 0.05 compared with baseline value.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>$P_{pa}$ (mmHg)</th>
<th>$P_{pv}$ (mmHg)</th>
<th>$P_{pc}$ (mmHg)</th>
<th>$R_a$ (cmH₂O · min⁻¹ · ml⁻¹)</th>
<th>$R_v$ (cmH₂O · min⁻¹ · ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before I/R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>4</td>
<td>11.5 ± 0.58</td>
<td>1.75 ± 0.50</td>
<td>6.04 ± 0.25</td>
<td>1.09 ± 0.11</td>
<td>0.86 ± 0.08</td>
</tr>
<tr>
<td>NS + Dex</td>
<td>5</td>
<td>12.30 ± 0.70</td>
<td>1.6 ± 0.30</td>
<td>6.31 ± 0.21</td>
<td>1.20 ± 0.15</td>
<td>0.94 ± 0.12</td>
</tr>
<tr>
<td>NS + Penta</td>
<td>4</td>
<td>15.00 ± 1.26</td>
<td>1.75 ± 0.50</td>
<td>7.58 ± 0.31</td>
<td>1.48 ± 0.19</td>
<td>1.17 ± 0.15</td>
</tr>
<tr>
<td>NS + Penta + Dex</td>
<td>4</td>
<td>16.00 ± 1.41</td>
<td>1.13 ± 0.25</td>
<td>7.67 ± 0.29</td>
<td>1.67 ± 0.15</td>
<td>1.31 ± 0.15</td>
</tr>
<tr>
<td>After I/R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>4</td>
<td>19.00 ± 3.16*</td>
<td>1.75 ± 0.50</td>
<td>9.34 ± 1.48*</td>
<td>1.93 ± 0.35*</td>
<td>1.51 ± 0.27*</td>
</tr>
<tr>
<td>NS + Dex</td>
<td>5</td>
<td>15.00 ± 1.30</td>
<td>1.75 ± 0.50</td>
<td>7.58 ± 0.63</td>
<td>1.45 ± 0.18</td>
<td>1.17 ± 0.15</td>
</tr>
<tr>
<td>NS + Penta</td>
<td>4</td>
<td>16.80 ± 1.50</td>
<td>2.0 ± 0.60</td>
<td>8.51 ± 0.66</td>
<td>1.66 ± 0.17</td>
<td>1.30 ± 0.13</td>
</tr>
<tr>
<td>NS + Penta + Dex</td>
<td>4</td>
<td>16.30 ± 1.71</td>
<td>1.75 ± 0.50</td>
<td>8.15 ± 0.72</td>
<td>1.63 ± 0.21</td>
<td>1.28 ± 0.19</td>
</tr>
</tbody>
</table>

Figure 2  Light micrographs of lung tissue treated with various fluid regimens, showing differing severity of I/R lung injury

Lung tissue was fixed in formalin and stained with haematoxylin/eosin. (A) Lung tissue from the control (NS) group, demonstrating marked inflammatory cell infiltration in the perivascular and alveolar regions that reflects severe I/R injury. (B) The NS + Dex group showed less leucocyte infiltration than the NS group. (C) The NS + Penta group showed less leucocyte infiltration than the NS group, but there was still a significant inflammatory cell infiltrate. (D) Tissue from the NS + Penta + Dex group revealed an almost normal appearance, except for mild perivascular oedema. Magnification × 170.

control group. Furthermore, among all groups, the NS + Penta + Dex group had the greatest decrease in LWG (Table 1; Figure 1). These data indicate that dexamethasone and pentastarch each attenuate the pulmonary oedema elicited by I/R lung injury, and that dexamethasone and pentastarch produced additive attenuation of pulmonary oedema in I/R lung injury.

$K_v$ values

The value of $K_v$ in the NS group after I/R lung injury
was significantly greater than that at baseline. In comparison with the NS group, the NS + Dex, NS + Penta and NS + Penta + Dex groups showed a significant decrease in microvascular permeability at 60 min after reperfusion, as shown by lower values of $K_{pa}$ ($P < 0.05$) (Table 1).

**Haemodynamics**

Before I/R, no significant differences in haemodynamics were found among the four groups. However, after I/R challenge, the NS group had significantly higher values of $P_{pa}$, $P_{pa}$, $R_a$ and $R_v$ than at baseline (Table 2).

**Histological findings**

Lungs from the NS control group demonstrated marked perivascular oedema and profound interstitial and intra-alveolar leucocyte infiltration that reflected severe I/R injury (Figure 2A). The NS + Dex and NS + Penta groups showed less leucocyte infiltration than the NS group (Figures 2B and 2C); however, there was still clearly identifiable inflammatory cell infiltration in the NS + Penta group. The data indicate that pentastarch might have some anti-inflammatory effect. The lungs from the NS + Penta + Dex group revealed an almost normal appearance, except for mild perivascular oedema (Figure 2D).

**DISCUSSION**

The pathogenesis of I/R lung injury is still unclear; however, one possible mechanism that has been proposed is that hypoxia induces tissue macrophages to release pro-inflammatory cytokines [7,12–17]. Cytokines mediate polymorphonuclear neutrophil adherence to endothelial cells, which release their own oxygen radicals, and are likely to contribute to a complex phenomenon that results in vascular injury [18–22] and infiltration of polymorphonuclear neutrophils into both the interstitium and the alveoli. Additional inflammatory cells are recruited into the interstitial spaces and alveoli following this initiating sequence. Although it is clear that the neutrophil–endothelial interactions, regulated by both humoral and local mediators, are necessary for I/R injury [23], it is also known that oxygen-derived free radicals, proteases, cytokines, eicosanoids, complement activation products, platelet-activating factor, adhesion molecules and nitric oxide are involved as signalling and effector molecules related to lung endothelial damage [24].

Although the physiological and cell molecular biological events occurring in I/R lung injury are complex, in the present study we observed two major presentations of I/R lung injury: permeability pulmonary oedema (as reflected by increases in LWG and $K_{pa}$) and acute inflammation (demonstrated by the presence of inflammatory cell infiltration). Therefore the aims of the use of infusion solutions to treat I/R lung injury are not only to inhibit inflammation, but also to decrease permeability oedema. Theoretically, a substance such as pentastarch could ameliorate pulmonary oedema both by providing a colloid solution that maintains osmotic pressure (to favour the retention of protein in the intravascular space) and potentially by sealing the capillary leak due to the large molecular size of the colloid substance. However, for prevention and treatment of the inflammatory process, one would also need to use an anti-inflammatory agent such as dexamethasone.

In the control NS group, I/R lung injury induced: (1) haemodynamic changes, characterized by increases in $P_{pa}$, $P_{pa}$, $R_a$ and $R_v$ (2) an increase in microvascular permeability, reflected by an increase in $K_{pa}$ (3) pulmonary oedema, reflected by elevated LWG, and (4) inflammation, consisting of inflammatory cell infiltration. These findings were consistent with previous studies on I/R lung injury in isolated lung models [7–10]. The values of LWG and $K_{pa}$ in the NS + Dex and NS + Penta groups were significantly lower than those in the NS control group. These data indicate that the NS + Dex and NS + Penta groups were partially protected against I/R injury. In addition, a further marked decrease in LWG was observed in the NS + Penta + Dex group, along with lung histology that showed near normal morphology. These results show that the NS + Penta + Dex perfusate induced the most marked attenuation of I/R lung injury.

Our study presents the first evidence that pentastarch infusion gives partial protection against I/R lung injury. These results are similar to those from previous studies in other tissues, such as myocardium [4], brain [25,26] and spinal cord [27], and other conditions, such as limb ischaemia [1], sepsis [28] and thermal injury [29]. In those studies it was suggested that pentastarch molecules of an appropriate size could ‘plug’ the injured sites and obstruct the capillary leak [3,28]. The elegant anatomical images produced by Webb et al. [30] support this mechanism of a direct occlusive effect, rather than purely an osmotic effect on the injured sites. In addition, pentastarch may minimize the development of endothelial damage during the evolution of I/R by two additional mechanisms [28]. First, hyperosmolar solutions have been shown to decrease vascular resistance. This effect may explain our findings of reduced endothelial swelling, secondary to improved blood flow and prevention of irreversible cell damage [28,31]. Secondly, colloid solutions may alter cell integrity by such diverse effects as $O_2$ free-radical scavenging [6] and stabilization of fragile cell membranes [28]. For example, in a study by Collis et al. [5], hydroxyethyl starches inhibited the stimulated release of von Willebrand Factor, suggesting a possible beneficial role in the inhibition of endothelial activation, thus preventing neutrophil adhesion. Since our results showed that the lungs of animals...
treated with the NS + Pentastarch solution still had significant leucocyte infiltration, it appears that the anti-inflammatory effects of pentastarch were limited in our model.

Our results also showed that dexamethasone alone in the reperfusion fluid attenuated I/R lung injury. Some previous similar studies have supported the beneficial role of steroids in I/R lung injury [7,8,32,33]. However, the study of Boros et al. [34] showed that steroids were ineffective in diminishing I/R injury. The reason for these different results with steroids in I/R injury is unknown. In addition, we present the first evidence that dexamethasone, as an additive, promotes the attenuation of I/R injury by 10% pentastarch solution. This effect is likely to be due to the known anti-inflammatory actions of steroids; it is known that steroids favourably alter granulocyte function by reducing lysosomal enzyme release and superoxide anion production by neutrophils [10]. In addition, steroids inhibit complement-induced neutrophil aggregation and block granulocyte arachidonic acid metabolism, possibly by inhibiting phospholipase A₂ activity [35]. Steroids also inhibit the biosynthesis of tumour necrosis factor-α, both by diminishing the quantity of mRNA and by preventing its translation [7,36]. In animal experiments, steroids have an attenuating effect on acute lung injury due to potent anti-inflammatory. Corticosteroids have been used for the treatment of patients with acute respiratory distress syndrome (ARDS), particularly after early initial studies [37] suggested a possible benefit. However, subsequent investigations [38,39] demonstrated that high-dose methylprednisolone was not useful in preventing ARDS or treating it in its early stages. The main reasons for these discrepancies were different causes of ARDS in patients that were enrolled, and the different timing of intervention with steroid treatment. In the future it will be necessary to carefully select patients to ensure the same cause of ARDS and comparable timing of treatment, in order to study the different aetiologies of ARDS in the early phase. However, in recent clinical trials in late-stage ARDS (fibroproliferative phase), beneficial effects of steroid treatment on mobility and mortality were reported, suggesting that steroids can inhibit fibrosis and remodelling of lung parenchyma [40,41]. Because steroids suppress immunity, which could lead to the rapid spread and invasion of micro-organisms, use of steroids to treat ARDS induced by sepsis might be a relative contra-indication. Theoretically, except for the case of sepsis, use of anti-inflammatory steroids to treat acute lung injury is reasonable.

In conclusion, 10% (w/v) pentastarch or 0.04 mg/ml dexamethasone in the lung perfusate has been shown to partially attenuate I/R lung injury. In addition, perfusion of dexamethasone and pentastarch together produces additive effects and marked attenuation of I/R lung injury. These data suggest that infusion solutions for the treatment of acute I/R injury that contain colloid substances with ‘sealing effects’ and anti-inflammatory agents should be useful. Clinically, we propose that 10% pentastarch + dexamethasone solution might be more beneficial in preventing acute lung injury than 10% pentastarch alone.

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