Coagulopathy and plasma transfusion in critically ill patients
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Chapter 15

Methylprednisolone fails to attenuate lung injury in a mouse model of transfusion-related acute lung injury

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Abstract

Background: Transfusion-related acute lung injury (TRALI) is the leading cause of transfusion-related morbidity and mortality. Anecdotally, TRALI patients have been treated with corticosteroids. However, evidence for its therapeutic rationale in TRALI is lacking. We determined the effects of corticosteroids on lung injury in a ‘two hit’ mouse model of antibody mediated TRALI.

Methods: BALB/c mice were primed with lipopolysaccharide, after which TRALI was induced by injecting MHC-I antibody against H2Kd. Mice infused with phosphate-buffered saline served as controls. Simultaneously, one group of TRALI mice was infused with methylprednisolone (MPS; 2 mg/kg). Mice were supported by mechanical ventilation for 2 hours, after which bronchoalveolar lavage fluid (BALF) and lung homogenate were obtained. Statistics were obtained by one-way analysis of variance or Kruskall Wallis.

Results: Injection of MHC-I antibodies resulted in TRALI, indicated by pulmonary edema and increased BALF levels of protein and pro-inflammatory mediators macrophage inflammatory protein-2, keratinocyte-derived chemokine, and interleukin (IL)-6. Administration of MPS did not affect the amount of edema nor pulmonary protein and chemokine levels. MPS reduced systemic inflammatory reaction as well as IL-6 levels in the BALF.

Conclusion: In a ‘two-hit’ model of antibody-mediated TRALI, MPS attenuated the IL-6 host response, but failed to prevent the development of lung injury.
Introduction

Transfusion-related acute lung injury (TRALI) is the leading cause of transfusion-related mortality and morbidity [1]. In critically ill and surgical patients, relatively high incidences of TRALI have been reported [2-4], associated with prolonged mechanical ventilation, increased hospital mortality and decreased long-term survival [3-5]. Indeed, the clinical picture of TRALI can be devastating, with fulminant edema and ensuing hypoxia [5]. Therapy is supportive including mechanical ventilation and hemodynamic support. Case reports show that patients are often treated with high-dose corticosteroids [5-7].

The rationale for treatment with steroids may be that acute respiratory distress syndrome (ARDS) and TRALI show clinical and histopathological similarities, with an inflammatory response leading to the disruption of the lung alveolar-capillary permeability barrier [8]. In severe ARDS, corticosteroids were shown to reduce mortality [9,10]. However, there are no data that support the use of steroids in TRALI. In the present study, we investigated the effect of methylprednisolone (MPS) on pulmonary inflammation in a murine “two-hit” model of antibody-mediated TRALI. This model corresponds well with human TRALI [11], as an inflammatory condition of the patient at the time of the transfusion increases the susceptibility for a TRALI reaction.

Materials and methods

Experiments were performed with male BALB/c mice (Charles River, Someren, the Netherlands), aged 10-12 weeks and weighing 22-27 g, randomly assigned to 3 groups (n=16 per group). The study was approved by the Animal Care and Use Committee of the Academic Medical Center at the University of Amsterdam, the Netherlands (protocols 102190-1 and 102190-2). Animal procedures were carried out in compliance with Institutional Standards for Use of Laboratory Animals.
Experimental study protocol

We performed a stepwise dose finding experiment in order to assess optimal dose of major histocompatibility complex (MHC)-I antibodies capable of inducing TRALI in a ‘two hit’ TRALI model in mice primed with lipopolysaccharide (LPS, from *Escherichia Coli* 0111:B4) which was administered intraperitoneally at a dose of 0.1 mg/kg. Twenty-four hours after LPS, mice were anesthetized by intraperitoneal injection with a mix containing ketamine (EurovetAnimal Health BV, Bladel, the Netherlands), medetomidine (Pfizer Animal Health BV, Capelle a/d Ijssel, the Netherlands) and atropine (Pharmachemie, Haarlem, the Netherlands), as described previously [12]. A tracheostomy was inserted and animals were mechanically ventilated in a pressure-controlled mode, with an inspiratory pressure of 12 cm H$_2$O, PEEP of 2 cm H$_2$O (resulting in V$_T$ of approx. 7.5 mL/kg), respiratory rate 100 breaths/min and FiO$_2$ of 0.5 (Servo 900 C, Siemens, Sweden). After exposure of the jugular vein, using a 30-gauge sterile needle attached to polyethylene tubing, venous blood was aspirated from the jugular vein to verify intravascular placement of the needle. TRALI was induced by injecting MHC-I antibody against H2K$^d$ (IgG2a,κ). In previous experiments, we found that matched isotype antibody (IgG$_{2a}$, CRL-1908, American Type Culture Collection) did not differ from vehicle (phosphate buffered saline [PBS]) [13]. Therefore, control mice received PBS. A dose of 2 mg/kg was found to induce lung injury without increased mortality (data not shown), which is in line with previous reports on this model [11]. Immediately prior to infusion of antibodies, MPS (Solumedrol®, Pfizer, Capelle a/d Ijssel, The Netherlands) in a dose of 2 mg/kg was infused [14,15]. Previous experiments showed no differences between a volume matched injection of NaCl 0.9% instead of treatment, and no treatment of mice experiencing TRALI. Therefore, in order to limit the number of animals, we have omitted the saline group. After 2 hours of mechanical ventilation, mice were bled from the carotid artery. Blood gas analysis was done in a blood gas analyzer (Rapidlab 865, Bayer, Mijdrecht, the Netherlands). Bronchoalveolar lavage fluid (BALF) was obtained from the right lung and cell counts were determined using a hemacytometer (Beckman Coulter, Fullerton, CA, USA). Differential counts were done on cytospin preparations stained with a modified Giemsa stain, Diff-Quick (Dade Behring AG, Düdingen, Switzerland). Supernatant was stored at -80°C for total protein level and cytokine measurement.
The left lung was weighed and dried for three days in an oven at 65°C. The ratio of wet weight to dry weight represents tissue edema.

In a second set of experiments, right lungs were used for preparation of homogenate after dilution in saline and Greenberger lysis buffer using a tissue homogenizer (Biospec products, Bartlesville, OK, USA). Supernatant was stored at -80°C for cytokine measurements. Left lungs were fixed in 4% formalin and embedded in paraffin for histopathology. Four-micrometer sections were stained with hematoxylin-eosin and analyzed by a pathologist who was blinded for group identity. To score lung injury, we used a modified VILI histology scoring system as previously described [16]. In short, four pathologic parameters were scored on a scale of 0–4: 1) alveolar congestion, 2) haemorrhage, 3) leukocyte infiltration, and 4) thickness of alveolar wall/hyaline membranes. A score of 0 represents normal lungs; 1, mild, less than 25% lung involvement; 2, moderate, 25% to 50% lung involvement; 3, severe, 50% to 75% lung involvement; 4, very severe, more than 75% lung involvement. The total histology score was expressed as the sum of the score for all parameters.

**Assays**

Total protein levels (Bradford Protein Assay Kit, OZ Bioscience, Marseille, France) were measured in BALF. Keratinocyte-derived chemokine (KC), macrophage inflammatory protein-2 (MIP-2) and interleukin-6 (IL-6) were measured in BALF, homogenate and plasma using enzyme-linked immunosorbent assay (ELISA), according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN, USA).

**Statistics**

Data are expressed as mean ± standard deviation (SD) or median [interquartile range] when appropriate. Comparisons between groups were performed using one-way analysis of variance followed by t-test or Kruskall-Wallis followed by Mann Whitney U-test depending on data distribution. A p value of less than 0.05 was considered statistically significant. Statistical analyses were performed with Prism version 5.0 (GraphPad Software, San Diego, CA, USA).
Results

Data preliminary studies

Preliminary experiments did not demonstrate differences between mice infused with isotype antibody or vehicle (PBS). Pulmonary edema expressed as wet/dry ratio was low in both groups (4.2 (±0.3) versus 4.5 (±0.3), p=0.07). Furthermore, total protein levels in BALF did not differ (106 ug/ml [84-162] vs. 81 ug/ml [61-151], p=0.16), nor did KC levels in BALF (54 pg/ml (±16) vs. 56 pg/ml (±12), p=0.76). Lung injury scores were low in mice infused with isotype and those infused with PBS (1.3 (±1.1) vs. 1.9 (±0.8), p=0.21). To determine whether volume of MPS infusion had an effect on outcome, we compared infusion of MPS with a volume matched injection of 0.9% NaCl. No differences were found in wet/dry ratio (4.7 (±0.3) in 0.9% NaCl vs. 4.8 (±0.2) in MPS, p=0.25), total protein levels in BALF (117 ug/ml (±65) in 0.9% NaCl vs. 177 ug/ml (±71) in MPS, p=0.11) and pulmonary levels of KC (65 pg/ml [58-90] in 0.9% NaCl vs. 111 pg/ml [70-113] in MPS, p=0.07).

Induction of TRALI with MHC-I antibody

After the induction of TRALI, six of the 32 animals deceased prematurely, of whom four in the control group and two in the group treated with MPS. Mortality was related to instrumentation and did not differ significantly between groups. These animals were excluded from analysis. Injection of MHC-I antibodies resulted in TRALI, indicated by edema as reflected by an increase in wet-to-dry ratio of the lungs compared to controls, with increased BALF protein levels due to leakage of protein in the pulmonary compartment (figure 1). Lung injury was further characterized by an increase in both lung and BALF levels of chemokines MIP-2 and KC and the pro-inflammatory cytokine IL-6 (figure 2). TRALI did not result in increased pulmonary neutrophil influx within 2 hours (0.0 [0.0-1.0] in controls vs. 4.5 [0.0-9.3] in TRALI, p=0.08). Lung injury scores were significantly higher in TRALI mice compared to controls (4.6 (±0.7) vs. 1.9 (±0.8), p<0.001). TRALI also induced a systemic inflammatory reaction illustrated by increased plasma levels of both KC and IL-6 (figure 3).
Methylprednisolone in a murine model of TRALI

Figure 1: Wet-to-dry ratio of the lungs and BALF protein levels in mice injected with antibodies that induce TRALI, treated with MPS.

![Box plots showing wet-to-dry ratio and BALF protein levels](image)

Data expressed as mean, whiskers indicate minimum and maximum.

TRALI = transfusion related acute lung injury
PBS = phosphate buffered saline
MPS = methylprednisolone
BALF = broncho-alveolar fluid.

The effect of MPS

Administration of MPS did not decrease wet-to-dry ratio or BALF protein levels (figure 1). BALF and lung homogenate levels of chemokines MIP-2 and KC were also not affected by administration of steroids, while IL-6 homogenate and BALF levels were reduced (p=0.002 and p=0.03) (figure 2). Lung injury scores did not differ between treated (3.6 (±1.9) and untreated mice (4.9 (±0.7), p=0.12). Although steroids only reduced IL-6 in the lung, steroids reduced plasma levels of both KC and IL-6 (figure 3), validating the anti-inflammatory capacity of the selected dose of 2 mg/kg MPS in this model.
Figure 2: BALF and lung homogenate levels of MIP-2, KC and IL-6 in mice injected with antibodies that induce TRALI, treated with MPS. Data expressed as median, whiskers indicate minimum and maximum.

TRALI = transfusion related acute lung injury
PBS = phosphate buffered saline
MPS = methylprednisolone
BALF = broncho-alveolar fluid.
Discussion

Steroids did not improve markers of lung injury in a two-hit TRALI model, as pulmonary edema persisted, due to leakage of protein into the alveolar compartment. MPS did attenuate the IL-6 host response in the lung and both IL-6 and KC systemically.

In patients with early acute ARDS, MPS reduces lung injury, duration of mechanical ventilation and mortality, which, at least in part, was attributed to reduced plasma levels of IL-6 [17]. The latter is in line with our findings in a TRALI model, which show a clear reduction of pulmonary as well as systemic IL-6 levels after MPS treatment. However, in our study, despite the reduction of the IL-6 host response, inflammatory damage persisted. A possible explanation could be related to timing. TRALI is a hyperacute syndrome, developing within hours after transfusion, whereas ARDS takes a more prolonged course. In line with the clinical syndrome, we used a hyperacute model in which mice were euthanized after 2 hours, when inflammation has shown to be maximal [18]. In ARDS, beneficial effects of steroids were only shown after infusion for 14 days [17].
Another possible explanation of the lack of effect of MPS on lung injury might be the fact that inflammatory damage in TRALI is mediated via pathways which are not affected by the administration of steroids. In this model of antibody mediated TRALI, complement activation and macrophages were shown to be crucial contributors to the development of TRALI [19]. Of note, MPS can increase C1q production by macrophages [20], hereby enhancing complement activation with subsequent inflammatory injury.

The dose of MPS used in this study equals the dose which was shown to be beneficial in late ARDS [10] and higher than the dose that was beneficial in early ARDS [9]. Also, the chosen dose has shown to attenuate pulmonary inflammation in mice experiencing LPS induced acute lung injury [14,15]. As MPS attenuated the systemic inflammatory response, we think that under dosing of MPS is an unlikely explanation for the absence of beneficial effects on lung injury. However, the absence of pharmacokinetic data is a limitation to our study.

Our study has also other limitations. We used an acute model and cannot exclude that prolonged administration of steroids may be beneficial. Also, our model of antibody-mediated TRALI does not take into account other factors that are also capable of inducing TRALI [21]. Possibly, the response to MPS may be different in non-immune mediated TRALI. Finally, this supported TRALI model does not allow the study whether steroids affect mortality, which would be the most relevant outcome measure.

Our findings do not demonstrate a beneficial effect of corticosteroids in murine TRALI. A change in practice in human TRALI, based upon these findings, would be premature. Further research on timing and duration of corticosteroids in different models of TRALI is warranted.
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References


