Immunobiology 222 (2017) 1074–1080

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Research Paper

A double blind randomized experimental study on the use of IgM-enriched polyclonal immunoglobulins in an animal model of pneumonia developing shock

Rosanna Vaschetto[a,1], Nausicaa Clemente[b,1], Aline Pagni[c], Teresa Esposito[d], Federico Longhini[c], Francesca Mercalli[e], Elena Boggio[b], Renzo Boldorini[e], Annalisa Chiocchetti[b], Umberto Dianzani[b,*], Paolo Navalesi[c,d,f]

[a] SCDU Anestesia e Rianimazione, Azienda Ospedaliero Universitaria "Maggiore della Carità", Novara, Italy
[b] IRCAD and Università del Piemonte Orientale "Amedeo Avogadro", Dipartimento di Scienze della Salute, Novara, Italy
[c] SC Anestesia e Rianimazione, Ospedale Sant'Andrea (ASL VC), Vercelli, Italy
[d] Dipartimento di Medicina Traslazionale, Università del Piemonte Orientale "Amedeo Avogadro", Novara, Italy
[e] SCDU Anatomia Patologica, Azienda Ospedaliero Universitaria "Maggiore della Carità", Novara, Italy
[f] CRRF Mons. L. Novarese, Moncrivello, VC, Italy

ARTICLE INFO

Keywords:
Sepsis
Pneumonia
Immunoglobulins
Interleukin 10
Tumor necrosis factor

ABSTRACT

Background: Patients with severe pneumonia often develop septic shock. IgM-enriched immunoglobulins have been proposed as a potential adjuvant therapy for septic shock. While *in vitro* data are available on the possible mechanisms of action of IgM-enriched immunoglobulins, the results of the *in vivo* experimental studies are non-univocal and, overall, unconvincing. We designed this double blinded randomized controlled study to test whether IgM-enriched immunoglobulins administered as rescue treatment in a pneumonia model developing shock, could either limit lung damage and/or contain systemic inflammatory response.

Methods: Thirty-eight Sprague Dawley rats were ventilated with injurious ventilation for 30 min to prime the lung. The rats were subsequently randomized to received intratracheal instillation of either lipopolysaccharide (LPS) (12 mg/kg) or placebo followed by 3.5 h of protective mechanical ventilation. IgM-enriched immunoglobulins at 25 mg/h (0.5 mL/h) or saline were intravenously administered in the last hour of mechanical ventilation. During the experiment, gas exchange and hemodynamic measurements were recorded. Thereafter, the animals were sacrificed, and blood and organs were stored for cytokines measurements.

Results: Despite similar lung and hemodynamic findings, the administration of IgM-enriched immunoglobulins compared to placebo significantly modulates the inflammatory response by increasing IL-10 levels in the bloodstream and by decreasing TNF-α in bronchoalveolar lavage (BAL) fluid. Furthermore, in vitro data suggest that IgM-enriched immunoglobulins induce monocytes production of IL-10 after LPS stimulation.

Conclusions: In an *in vivo* model of pneumonia developing shock, IgM-enriched immunoglobulins administered as rescue treatment enhance the anti-inflammatory response by increasing blood levels of IL-10 and reducing TNF-α in BAL fluid.

1. Introduction

Severe bacterial pneumonia is a major challenge in the Intensive Care Unit (ICU) and represents the leading cause of mortality (De et al., 2012). Several patients with pneumonia develop septic shock, which is a further independent risk factor of death in these patients (Valles et al., 2003). The fundamentals of the treatment of septic shock is based on effective antimicrobial therapy (Ferrer et al., 2014; Kumar et al., 2006)

Abbreviations: ABG, arterial blood gas; BAL, bronchoalveolar lavage; CTR, control group; FiO_2, fraction of inspiratory oxygen; IFN-γ, interferon-γ; IgG, Immunoglobulin G; IgM, Immunoglobulin M; IL, interleukin; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; MAP, mean arterial pressure; OPN, osteopontin; $PaCO_2$, arterial carbon dioxide tension; PaO_2, arterial partial pressure of oxygen; PCR, polymerase chain reaction; PEEP, positive end-expiratory pressure; PNT, pentaglobiln; P_{PEAK}, peak pressure; RR, respiratory rate; SEM, standard error of the mean; SpO_2, peripheral oxygen saturation; TNF-α, Tumor necrosis factor-α

* Corresponding author at: IRCAD and Università del Piemonte Orientale "Amedeo Avogadro", Dipartimento di Scienze della Salute, Novara, Italy.
E-mail address: umberto.dianzani@med.uniupo.it (U. Dianzani).
[1] Equally contributed.

http://dx.doi.org/10.1016/j.imbio.2017.09.002
Received 4 April 2017; Accepted 7 September 2017
Available online 08 September 2017
0171-2985/

and optimization of homeostasis, *i.e.,* fluid resuscitation (Dellinger et al., 2013; Myburgh and Mythen, 2013), and adequate gas exchange (Acute Respiratory Distress Syndrome Network et al., 2000; Dellinger et al., 2013). Many adjuvant therapies have been proposed in the last decades, aimed at modulating the immune system activation. Although these attempts often resulted in negative or conflicting results when targeting and blocking one specific mediator of the inflammatory cascade (Abraham et al., 1998, 2001; Cohen and Carlet, 1996; Ranieri et al., 2012), promising preliminary results have been obtained by unspecific neutralization of endotoxins (Cruz et al., 2009) and adsorption of multiple mediators (Hu et al., 2012).

Immunoglobulins have been proposed as a potential adjuvant therapy for severe sepsis and septic shock (Alejandria et al., 2002). Patients with septic shock have low IgG and IgM levels (Bermejo-Martin et al., 2014; Venet et al., 2011), which are inversely correlated with 28-days risk of death (Tamayo et al., 2012). The spectrum of action for immunoglobulins should be quite broad as neutralize endotoxins by scavenging lipopolysaccharide (LPS), opsonize phatogens, and modulating proinflammatory mediators (McCuskey et al., 1996; Rieben et al., 1999; Shankar-Hari et al., 2012). Among the different immunoglobulins preparations, IgM-enriched solution, might have some advantages (Hoffman et al., 2008; Rieben et al., 1999).

Despite some small trials indicate a potential survival benefit in patients treated with immunoglobulins (Rodriguez et al., 2005; Weisman et al., 1992), meta-analyses provide inconclusive results (Alejandria et al., 2013; Laupland et al., 2007; Soares et al., 2014). Their use is therefore, still controversial and not currently recommended by international guidelines for the treatment of sepsis (Dellinger et al., 2013; Rhodes et al., 2017). While *in vitro* data are available (Lamari et al., 1999; Rieben et al., 1999; Rossmann et al., 2015; Trautmann et al., 1998), the results of the *in vivo* experimental studies are non-univocal and, overall, unconvincing (Barratt-Due et al., 2013; Hoffman et al., 2008; Lachmann et al., 2004; Rossmann et al., 2015; Shmygalev et al., 2015). In fact, in some of these studies the IgM-enriched immunoglobulins are administered near-simultaneously (Stehr et al., 2008) or even prior to the noxa priming (Barratt-Due et al., 2013; Hoffman et al., 2008; Lachmann et al., 2004; Rossmann et al., 2015; Shmygalev et al., 2015). Because both scenarios are far from mimicking the clinical conditions where the drug is obviously administered subsequent to the noxa, the mechanisms of action of the IgM-enriched immunoglobulins remain unclear.

Administering IgM-enriched immunoglobulins after the lung has been challenged could either limit the lung damage and/or contain the systemic inflammatory response. To address this issue, we designed this double blinded randomized controlled study where IgM-enriched immunoglobulins were given as rescue treatment in an experimental model of pneumonia with shock, obtained in mechanically ventilated rats by intra-tracheal nebulization of LPS associated with 30 min of injurious ventilation.

2. Methods

2.1. Animal preparation

The study was approved by the institutional animal care committee at the University of Piemonte Orientale “A. Avogadro”, Novara, Italy (protocol n°2481 of the 26th February 2014) and, for the part involving cells, by the Ethical Committee of the hospital called Comitato Etico Interaziendale A.U.O. “Maggiore della Carità” ASL BI, ASL NO, ASL VCO” (Protocol 1036/CE, study n. CE 168/16). The study was performed according to the principles outlined in the Declaration of Helsinki and in the ARRIVE guidelines. Thirty-eight male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN, USA) were housed in a controlled 12-h light/dark cycle environment with food and water ad libitum. Animals weighing 290 ± 15 g (8–9 weeks old) were anesthetized with intraperitoneal injection of a mixture of Zoletil (tiletamine and zolazepam) 60 mg/kg (Virbac s.r.l., Milan, Italy) and Xylazine 20 mg/kg (Bayer S.p.A., Milan, Italy). Anesthesia was maintained with a second injection after 2 h of the same drugs at half dosage; muscle relaxation was achieved by continuous intravenous administration of cisatracurium (Mylan Inc., Milano, Italia) at 3.6 mg/kg/h. Rats were placed on a heating pad to maintain core temperature at 37 °C. A tracheostomy was performed for intratracheal cannulation (14 gauge). The right carotid artery was catheterized for blood sampling and continuous arterial blood pressure measurements.

2.2. Experimental protocol

The rats were initially ventilated in volume controlled mode to target a peak pressure (P_{PEAK}) of 22 cmH_2O with a positive end-expiratory pressure (PEEP) of 5 cmH_2O (Merlin Small Animal Ventilator; Vetronic Services, Newton Abbot, United Kingdom), a respiratory rate (RR) between 25 and 50 breath/min to maintain normocapnia, inspiratory to expiratory time of 1:2, and fraction of inspiratory oxygen (FiO_2) of 21%. After a baseline arterial blood gas (ABG) measurement (Radiometer ABL 700; Diamond Diagnostics, Holliston, MA) to confirm similar gas exchange conditions in all animals (T0), a mild injurious ventilation was set for 30 min to prime the lung i.e., increasing P_{PEAK} at 25 cmH_2O. Thereafter, ventilatory setting was restored and rats were blindly randomized into three groups and ventilated for 4 h in total: 1) LPS group in which LPS (055:B5; Sigma-Aldrich, St. Louis, MO) at a dose of 12 mg/kg in 0.5 mL normal saline was administered at T30 (Time 30 min) by using an intratracheal aerosolizer (PennCentury Inc., Philadelphia, PA). At T150, when clinical signs of septic shock were evident, continuous placebo infusion i.e., normal saline, was administered intravenously (iv) at 0.5 mL/h till T240; 2) pentaglobin group (LPS + PNT) in which LPS was administered as previously described, and at T150 a continuous infusion iv of IgM-enriched polyclonal immunoglobulins (Pentaglobin, Biotest, Dreieich, Germany) at 25 mg/h (0.5 mL/h) was infused till T240; 3) control group (CTR) in which placebo was administered both intratracheally and iv with the same procedures as the previous groups. Before nebulizing LPS or placebo, FiO_2 was increased at 92% (Oxygen Concentrator LFY-I-5A, Zhejiang Longfei Industry Co., Ltd, Mainland, Cina) for 30 min. Thereafter FiO_2 was maintained at 21%, and increased to 92% only when mean arterial pressure (MAP) dropped below 40 mmHg.

Randomization followed a computerized previously generated random sequence held by an investigator not involved in the animal experiment, who was in charged to prepare LPS, drug, and placebo accordingly.

2.3. Measurements

MAP, heart rate, and peripheral oxygen saturation (SpO_2) were continuously monitored. ABGs were analyzed at baseline (T0) and hourly thereafter. About 100 µL of whole blood left from ABG was centrifuged, aliquoted and stored for cytokines analysis. Upon completion of mechanical ventilation, whole blood was collected for measurements of cytokines, and the animals were sacrificed. Lungs were harvested for histological examination. Plasma was aliquoted and stored at −80 °C until assayed.

2.4. Bronchoalveolar lavage (BAL)

The left upper lobe was excised for histological examination, as previously explained (Vaschetto et al., 2008). Briefly, the right lower lobe was snap frozen for cytokine measurements. The left lower and the right upper lobes were lavaged by intratracheal instillation of 2 mL cold phosphate-buffered saline (Sigma-Aldrich). After 5 s, the BAL was obtained. This procedure was repeated twice. After centrifugation, the supernatant was aliquoted and frozen at −80 °C until further analysis.