Journal of Neuroimmunology 309 (2017) 58–67

Contents lists available at ScienceDirect

Journal of Neuroimmunology

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

Anti-inflammatory and immunomodulatory potential of human immunoglobulin applied intrathecally in Lewis rat experimental autoimmune neuritis

Kalliopi Pitarokoili*, Felix Kohle, Jeremias Motte, Oluwaseun Fatoba, Xiomara Pedreiturria, Ralf Gold, Min-Suk Yoon

Department of Neurology, St. Josef Hospital, Ruhr-University of Bochum, Germany

ARTICLE INFO

Keywords:
Autoimmune neuropathies
Immunoglobulins
Experimental autoimmune neuritis
Autoimmunity
Inflammation
Immunotherapy

ABSTRACT

Intravenous human immunoglobulins dominate in the treatment of autoimmune neuropathies. We introduce intrathecal application as a new option for experimental autoimmune neuritis in Lewis rats. After immunisation with neuritogenic P2 peptide, we show a therapeutic and preventive effect of intrathecal human immunoglobulins (5–40 mg/kg) on clinical and electrophysiological neuritis signs. Histology corroborated a lower degree of inflammation, demyelination, ICAM-1-dependent blood-nerve-barrier permeability and complement activation in the sciatic nerve. After preventive application, immunoglobulins induced a Th2 cytokine shift in the peripheral nerves already before clinical neuritis signs. Intrathecal immunoglobulin application could be a novel immunomodulatory option for autoimmune neuropathies.

1. Introduction

The first use of subcutaneous human immunoglobulins dates back to 1952 for patients with agammaglobulinemia (Bruton, 1952; Buckley, 1998). Intravenous human immunoglobulins (IVIg) were shown effective for idiopathic thrombocytopenic purpura in 1981 (Imbach et al., 1981). Since then, their application has been extended to many autoimmune disorders including diseases of the central and peripheral nervous system. Their immunomodulatory properties combined with an attractive safety profile are the main reasons for their establishment as a first-line treatment for autoimmune diseases of the peripheral nervous system (PNS). Patients with the acute form (Guillain-Barré Syndrom, GBS) or the chronic form of peripheral autoimmune neuritis (chronic inflammatory demyelinating polyradiculoneuropathy, CIDP) improve rapidly during the first 24–48 h after intravenous application of IVIg and remain stable during the following weeks, as immunoglobulin G (IgG) has a long half-life of two to three weeks (Lünemann et al., 2015). Human IVIg formulations available in market consist of purified IgG products from pooled human plasma of up to 60.000 donors. The content of unmodified IgG is approximately 95% with only trace amounts of immunoglobulin A or immunoglobulin M. Patients with GBS are treated with 0.4 g/kg body weight daily for 5 days whereas patients with CIDP receive initially 2 g/kg body weight in 2–4 days followed by a maintenance treatment of 1 g/kg body weight every 3–4 weeks intravenously (Hughes et al., 2008, Léger et al., 2013). Taking into account the price per gram of IVIg (approximately $70), it is obvious that the overall cost of this treatment for CIDP patients is extremely high (Blackhouse et al., 2010). The optimal dosing schema has to be adjusted individually as clinical experience shows that the optimal combination of dosage and frequency of application varies between subjects and during the course of disease (Yoon et al., 2011; Willison et al., 2016). Subcutaneous route of administration has been tested in neuritis patients in terms of safety, tolerability and patients preference, as a dose-saving option, however, the dosages required remain relatively high (0.2 g/kg–0.4 g/kg twice a week) (Van Schaik et al., 2016).

The immunomodulatory mechanisms of immunoglobulin in autoimmune PNS diseases have been widely investigated in human disease. Because of their polyvalent nature, a wide spectrum of immunomodulatory effects mostly in the inflamed PNS either through the $F(ab')_2$ or through the Fc segment has been shown. $F(ab')_2$ segment can block different cellular receptors and neutralize cytokines, complement,

Abbreviations: AUC, Area under curve; CFA, complete Freund's adjuvant; CIDP, chronic inflammatory demyelinating polyneuropathy; cMAP, compound muscle potentials; DAPI, 4′,6-Diamidin-2-phenylindol; EAN, experimental autoimmune neuritis; GBS, Guillain-Barré syndrome; ICAM1, intercellular adhesion molecule 1; IgG, immunoglobulin G; i.p., intraperitoneally; IVIg, intravenous immunoglobulins; MNCV, motor nerve conduction velocity; PBS, phosphate buffer saline; p.i., post immunisation; PNS, peripheral nervous system

* Corresponding author at: Dept. of Neurology, Ruhr University, St. Josef-Hospital, Gudrunstr. 56, 44791 Bochum, Germany.
E-mail address: kalliopi.pitarokoili@ruhr-uni-bochum.de (K. Pitarokoili).

http://dx.doi.org/10.1016/j.jneuroim.2017.05.008
Received 14 February 2017; Received in revised form 14 May 2017; Accepted 15 May 2017

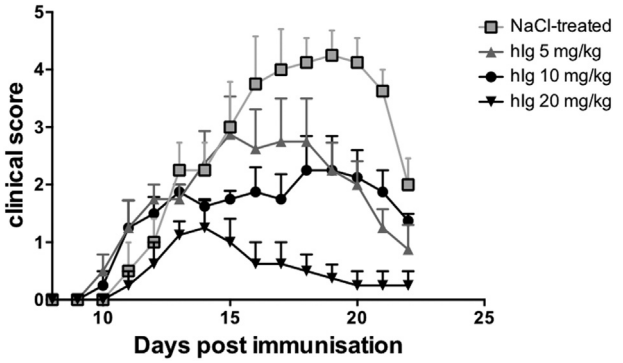

Fig. 1. Representative clinical course of EAN rats (n = 4). EAN was induced in Lewis rats by immunisation on day 0 with P2 peptide 53–78 plus CFA. Rats received human immunoglobulins (hIgs) intrathecally at doses of 5 mg/kg, 10 mg/kg and 20 mg/kg on days 11,13 and 15 post immunisation. Control rats received NaCl 0.9% only. The experiment was repeated three times, each time with n = 4/group. Mean values and SEM are depicted. Statistical analysis and p-value calculation were performed calculating the area under the curve. Comparisson of AUC was done using one-way ANOVA combined with Tukey's multiple comparisons test. NaCl treated vs 20 mg/kg ***p < 0.0001.

adhesion-molecules and autoantibodies (Viard et al., 1998; Le Pottier et al., 2007; Schwab and Nimmerjahn, 2013). Fc Segment can modulate activating (high-affinity FcγRIA, FcγRIIA/C, FcγRIIIA/B, FcγRIV) and inhibitory (FcγRIIB) FcγR expression on immune cells (Samuelsson et al., 2001; Hansen and Balthasar, 2002; Anthony et al., 2011). These mechanisms have been widely investigated in the prototype model of animal autoimmune PNS disease, the experimental autoimmune neuritis (EAN), revealing a complex and not yet fully understood immunomodulatory effect (Enders et al., 1997; Gabriel et al., 1997; Hadden et al., 2002; Lin et al., 2007a, 2007b; Eftimov et al., 2013; Klehmet et al., 2014; Niknami et al., 2013; Kajii et al., 2014). In view of this complexity, it has to be pointed out that the influence of each these pathways from IVIg, depends not only on the particular autoimmune disease but also on the route of administration.

In this study, we describe the immunomodulatory effects of human immunoglobulin (hIg) applied intrathecally in the Lewis rat model of EAN (Waksman and Adams, 1955). This route of administration has gained a preference for multiple diseases with central (spinal) nervous system involvement such as baclofen application against spasticity or chemotherapy in leptomeningeal neoplastic involvement (Otero-Romero et al., 2016; Mack et al., 2016). Intrathecal opioid injection can modulate central/spinal sensitisation in chronic regional pain syndromes by bypassing the blood-brain barrier and providing direct accessibility to central and peripheral neural structures (Pope et al., 2016). Furthermore, an increasing number of publications have reported the intrathecal use of monoclonal antibodies in multiple sclerosis (Studer et al., 2014; Bonnan et al., 2014; Tomita et al., 2015; Doorduijn et al., 2016). We present the first report of a new effective and dose-saving route of administration of immunoglobulins for dysimmune neuropathies.

2. Materials and methods

2.1. Antigens

The neuritogenic P2 peptide, corresponding the amino acids 53–78 of rat myelin P2 protein, was synthesized by Dr. Rudolf Volkmer from Charité Universitätsmedizin (Berlin, Germany).

2.2. Human immunoglobulin preparation

A human immunoglobulin preparation 10% Privigen® (CSL Behring) was used for all experiments. 1 ml of this preparation contains 100 mg of human immunoglobulin produced from the plasma of human donors (purity of at least 98% IgG). The distribution of the IgG subclasses is (approx. values): IgG1 67.8%, IgG2 28.7%, IgG3 2.3%, IgG4 1.2%. The maximum IgA content is 25 µg/ml. It contains approximately 250 mmol/l (range: 210 to 290) of L-proline.

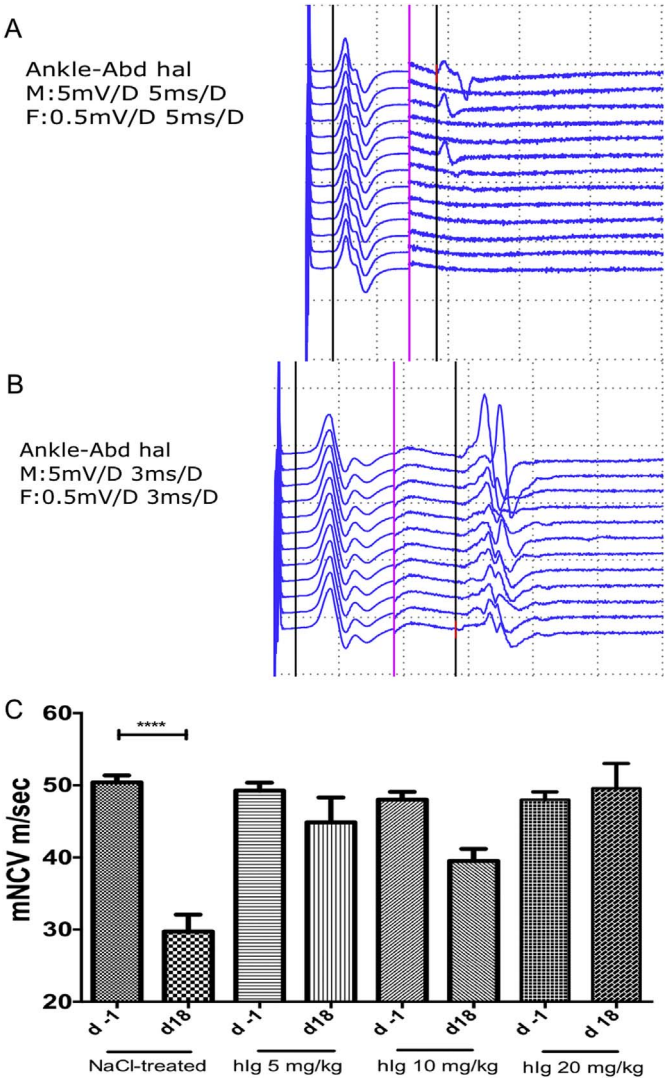

Fig. 2. Representative traces of F waves at disease maximum (day 18 p.i.) from NaCl-treated rats (A) and 20 mg/kg intrathecal Igs-treated rats (B). NaCl treated rats presented with a reduced persistence of F-wave response whereas Ig-treated rats showed a normal response indicating the effect of effect of Igs in reducing proximal demyelination. C. Motor nerve conduction velocity of the sciatic nerve one day before immunisation (d-1) and at the peak of disease (day 18 p.i.) (n = 4/group): After proximal and distal stimulation of the sciatic nerve the conduction velocity was calculated. A statistical significant reduction of the MNCV appeared only for the NaCl-treated group (n = 4) but no difference in the mNCV was seen for all immunoglobulin-treated groups, indicating a protective role of immunoglobulins against distal demyelination. Mean values and SEM are depicted, p-values ****p < 0.0001. The experiments were repeated three times with n = 4/group.

2.3. Disease induction and clinical score assessment

A total of 48 female Lewis rats were randomised for the therapeutic concept and a total of 59 rats were randomised for the preventive concept as described in the following section.The rats were 6–8 weeks old, they were purchased from Charles River Co. (Sulzfeld, Germany) and weighed 160–180 g when used for the following experiments. They were anesthetized by 1.5%–2.0% halothane in oxygen. They were immunised by subcutaneous injection of 250 µg $P2_{53–78}$ peptide in phosphate buffered saline (PBS) into the root of the tail, emulsified in