RESEARCH REPORT

Beneficial effect of a multimerized immunoglobulin Fc in an animal model of inflammatory neuropathy (experimental autoimmune neuritis)

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Abstract Intravenous immunoglobulin (IVIg) is one of the first-line therapies for inflammatory neuropathies. Clinical use of IVIg for these disorders is limited by expense and availability. Here, we investigated a synthetic product alternative to IVIg. The aim of this study was to test the therapeutic efficacy of a novel recombinant polyvalent murine IgG2a Fc compound (stradomer™) in experimental autoimmune neuritis (EAN). Seventy-four Lewis rats were immunized with myelin, randomized into three groups, and were treated with albumin, IVIg, or stradomer at 1% of IVIg dose. Rats were assessed clinically, electrophysiologically, and histologically. The clinical disease severity was evaluated by clinical grading and weight changes. The electrophysiological studies recorded motor conduction velocity (MCV), amplitudes, and latencies of the evoked compound muscle action potential (CMAP) and spinal somatosensory evoked potential. The treatment efficacy of the IVIg and stradomer groups was compared to the albumin (control) group. We demonstrate that stradomer has a similar therapeutic efficacy to human IVIg in EAN. Rats receiving stradomer or IVIg showed significantly lower clinical scores and less prominent weight loss compared with controls. A statistically significant improvement in both MCV and the amplitudes of distal and proximal evoked CMAP was observed in the stradomer and IVIg groups. Finally, treatment with both IVIg and stradomer resulted in statistically less inflammation and demyelinating changes in the sciatic nerve as evidenced by lower histological grade. These results reveal the potential of using fully recombinant multimerized immunoglobulin Fc instead of IVIg for treating inflammatory neuropathies.

Key words: experimental autoimmune neuritis, Fcγ receptor, intravenous immunoglobulin, stradomer™

Introduction Intravenous immunoglobulin (IVIg) is a highly effective agent for treating various autoimmune and systemic inflammatory diseases (Kotlan et al., 2009; Tournadre et al., 2010) and is now increasingly used as a successful first-line initial and maintenance treatment for neurological conditions such as Guillain-Barré syndrome (GBS), chronic inflammatory demyelinating polyneuropathy (CIDP), and multifocal motor neuropathy (MMN) (Nobile-Orazio et al., 2010). Despite such wide use, its costly preparation requires pools of plasma from 3 to 100,000 blood donors (Crow et al., 2006). A cost-effectiveness analysis from the National Blood Authority of Australia (with a population...
of only 22 million) in 2009–2010 indicated that 1,124,604 g of IVIg was issued for these neuropathies alone, representing a cost of nearly $69 million nationally (National Blood Authority Australia, 2010). Further, a recent study estimated that the direct cost of IVIg treatment for GBS is US $10,305 per person weighing 70 kg (Winters et al., 2011). The high cost of IVIg becomes even more significant because most patients with CIDP and MMN need high maintenance doses of IVIg at 2–6 weekly intervals on a continuing basis (Joint Task Force of the EFNS and the PNS, 2010). In addition to high cost, IVIg has been associated with unwanted side effects, including headache, chills, migraine, dizziness, fever, nausea/vomiting, fatigue, and dermatitis (Bertorini et al., 1996; Czernik et al., 2012). Moreover, renal failure, thromboembolic events, and anaphylaxis after IVIg treatment, although relatively rare, warrant consideration (Bertorini et al., 1996; Czernik et al., 2012). Thus, developing an alternative for IVIg therapy in inflammatory neuropathies is desirable.

Development of an IVIg mimetic has been impeded by a limited understanding of its mechanisms of action. A wealth of animal and human studies now suggests that the anti-inflammatory effects of IVIg reside within the Fc domain. Furthermore, in a previous study in experimental autoimmune neuritis (EAN), the animal model of inflammatory neuropathy, our group showed that the efficacy of IVIg resided in the constant Fc component of IVIg not the variable Fab region (Lin et al., 2007). Despite the knowledge that Fc fragments mediate many of the anti-inflammatory effects of IVIg, the exact mechanisms of Fc-mediated immune tolerance are controversial, including, but not limited to, Fc blockade of canonical Fcγ receptors (FcγRs) (Dhodapkar et al., 2007; Jacob and Rajabally, 2009), upregulation of the FcγRIIB inhibitory receptor (Dalakas, 2004), competitive inhibition of complement activation (Dalakas, 2004; Shahnizaila and Yuki, 2011), and a reduction in pathogenic autoantibodies through occupation of the neonatal Fc receptors (FcRn) (Sewell and Jolles, 2002; Clynes, 2007).

From a biologic perspective, it is well known that the majority of FcγRs are low affinity, meaning that they bind Fc bearing immune aggregates more efficiently than the homodimeric Fc fragments that comprise normal IgG (Karsten and Köhl, 2012). This observation is biologically relevant to the function of IVIg, as several studies now indicate that aggregated IgG fragments are required to suppress inflammation in idiopathic thrombocytopenic purpura (ITP) and inflammatory arthritis animal models (Morgan and Tempelis, 1978; Teeling et al., 2001; Bazin et al., 2004; Siragam et al., 2005; Deng and Balthasar, 2007). To capitalize on this knowledge for clinical translation, we employed fully recombinant multimeric Fc molecules, stradomers™, which bind with high affinity to canonical FcγRs and are effective in treating collagen-induced arthritis and in preventing murine ITP (Jain et al., 2012).

In this study, we sought to compare the therapeutic efficacy of a novel fully recombinant form of a multimerized murine IgG2a Fc, stradomer, in rat EAN with that of IVIg. Our findings indicate that both IVIg and stradomer, administered at a 2 log order lower dose than IVIg, are effective in the treatment of EAN. These data suggest that stradomer mediates similar therapeutic effects as IVIg in EAN, and may be a useful IVIg mimetic for the treatment of human inflammatory neuropathies.

Materials and Methods

For the purpose of this study, two experiments, namely experiment A and B, were carried out. The two studies were performed to test the efficacy of stradomer in two different circumstances. In experiment A, we generated a moderate disease, whereas in experiment B, severe disease was induced. The condition and procedures of both experiments were similar unless otherwise stated in the relevant methods section.

Reagents

Human IVIg (OCTAGAM®) and albumin (20% NSA) were kindly provided by Octapharma, Sydney, Australia and CSL Bioplasma, Hong Kong, respectively. Fully recombinant forms of multimerized murine IgG2a Fc were supplied by Gliknik Inc. (Baltimore, MD, USA). We tested two similar stradomers in the two experiments both comprised of mouse IgG2a Fc. IgG2a is the mouse homologue of human IgG1 that has been multimerized with a human IgG2 hinge multimerization domain. Each multimer is a highly ordered multimer of the homodimer. Thus, the second multimer band contains two Fc homodimers covalently and stably bound through disulfide bonds, the third multimer band contains three Fc homodimers covalently bound, etc.

Stradomer protein was manufactured in a shake flask system using transient transfection of an HEK cell line and purified on a GE AktaXpress system using GE mAb Select Protein A affinity columns (Jain et al., 2012).

Animals

Female inbred Lewis rats weighing 150–200 g (8–10 weeks old) were housed in a non-specific pathogen-free animal facility (Blackburn Building, University of Sydney, Australia). The animals were allowed to adapt to their housing conditions for 5 days,
and experiments were conducted subsequently. All animal protocols were performed in compliance and with approval from the Animal Care and Ethics Committee of the University of Sydney (Ethics approval number: K14/8-2009/2/5081).

Preparation of myelin antigen

Myelin was isolated from fresh bovine cauda equina according to the method described by Norton and Poduslo (1973).

Anaesthesia

Immunization, intravenous injections, and electrophysiological procedures were performed under halothane/oxygen mixture.

Induction of EAN and clinical score assessment

EAN is a well-accepted animal model of the human inflammatory demyelinating neuropathies (Maurer et al., 2002). In experiment A, EAN was induced in 43 adult female Lewis rats by injecting each hind footpad with 50 μl of bovine peripheral nerve myelin emulsified with 1:1 saline: incomplete Freund’s adjuvant (Sigma, Sydney, Australia) and Mycobacterium tuberculosis (strain H37Ra, BD Bioscience, Sydney, Australia) at a concentration of 40 mg/ml myelin and 5 mg/ml Mycobacterium. In experiment B, EAN was induced in 31 adult female Lewis rats, but the dosage of myelin increased to 50 mg/ml. All animals were weighed and scored for evidence of clinical disease before inoculation and then daily for 30 days. Clinical disease severity was scored from 0 to 5 using the following scale: 0, normal; 1, limp tail; 2, mild hind limb weakness, abnormal posture, or waddling gait; 3, moderate hind limb weakness and difficulty in walking; 4, paraplegia and useless hind limbs; and 5, moribund (Spies et al., 1995).

Treatment of EAN rats

Immunized rats were randomly placed into three groups. In experiment A, group 1 (n = 15) received injections of 100 mg/100 g body weight of albumin once daily for 2 days. Group 2 (n = 14) received injections of 100 mg/100 g body weight of IVlg once daily for 2 days. Group 3 (n = 14) received injections of 1 mg/100 g body weight of stradomer once daily for 2 days. In experiment B, group 1 (n = 10) received injections of 100 mg/100 g body weight of albumin once daily for 2 days. Group 2 (n = 10) received injections of 100 mg/100 g body weight of IVlg once daily for 2 days. Group 3 (n = 11) received injections of 1 mg/100 g body weight of stradomer once daily for 2 days. The injection for each rat was given intravenously commencing from the onset of neurological deficit usually 9–10 days after immunization. This dose of IVlg was equivalent to that used in patients (0.4 g/kg given daily over 5 days or 1 g/kg given daily over 2 days).

Electrophysiological studies

Electrophysiological studies were conducted using a Neuromax (XLTEK, Ontario, Canada) neurophysiology machine prior to footpad inoculation and on days 10, 16, 22, and 30 in experiment A and prior to footpad inoculation and then on days 8, 10, 12, 14, 16, 18, 22, 26, and 30 in experiment B. To assess sciatic nerve motor conduction, the sciatic nerve was stimulated through paired needle electrodes at the ankle (distal) and the sciatic notch (hip; proximal). The nerve was stimulated with supramaximal rectangular pulses of 0.1-ms duration and the amplitudes and latencies of the evoked compound muscle action potential (CMAP) recorded from needle electrodes inserted subcutaneously into the dorsal foot muscles. Motor conduction velocity (MCV) was calculated by measuring the distance between the hip and ankle stimulating electrodes and dividing it by the difference in latency of the response (Harvey and Pollard, 1992; Spies et al., 1995). In experiment B, sensory nerve conduction was assessed using spinal somatosensory evoked potentials (S wave) (Wietholter and Hulser, 1985). Supramaximal rectangular pulses of 0.1-ms duration were used to stimulate sciatic nerve at the ankle and the spinal evoked responses were recorded through needle electrodes placed between the spinous processes of T13 and L1. The S wave latency was used as a measure of sensory conduction through the dorsal roots because nerve roots are a site of maximal pathology in EAN. Two averaged traces, each containing at least 20 responses, were recorded for each nerve (Harvey and Pollard, 1992; Spies et al., 1995).

Histological analysis

In experiment A, five animals from each group were randomly selected and sacrificed at days 16–18 and another five at day 30. Proximal sections of sciatic nerves were then collected. Nerves were fixed in 2.5% glutaraldehyde overnight, post-fixed in Dalton’s chrome osmium, and embedded in Spurr’s resin. Transverse sections of 0.5-μm thickness were cut on a microtome, stained with toluidine blue, and examined by light microscopy. The percentage of normal fibres and those showing demyelination and axonal degeneration was studied for each nerve in 20 perivascular areas of each group at days 16–18 (mid-study). The same parameters and a measure of axonal regeneration (cluster formation) were evaluated at day 30 (end of study). In addition, a semi-quantitative assessment of inflammation was made by grading the extent of inflammation and demyelination in
perivascular areas using the following scale: grade 0, normal perivascular area; grade 1, mild cellular infiltrate adjacent to a vessel; grade 2, cellular infiltrate plus demyelinating fibres adjacent to a vessel; and grade 3, cellular infiltrate plus demyelination around a vessel and at more distant sites (Heininger et al., 1988). All histological studies and grading were performed blindly by an independent researcher.

Statistical methods

The statistical software SPSS® 20 was used for analysis. Data were analyzed by Student’s t-test, one-way analysis of variance (ANOVA) using Tukey post hoc test, or Mann-Whitney U-test as appropriate. Results are expressed descriptively as the mean ± SD. A two-tailed p value <0.05 was considered significant.

Results

The animals in the albumin (control) group developed more severe disease compared with the IVIg and stradomer groups in both experiments. This was indicated by both higher clinical score (Figs. 1A and 1C) and more marked weight loss (Figs. 1B and 1D).

Clinical score

In experiment A, higher clinical scores were observed from day 10 until day 30 in the albumin group compared with the IVIg and stradomer groups. Differences between albumin and IVIg groups were statistically significant from day 13. The significance was observed from day 19 when clinical scores of the albumin group were compared to that of the stradomer group. Recovery from the illness in the IVIg and stradomer groups was faster than that in the albumin group. The average clinical scores of IVIg and stradomer groups dropped to 1.3 (±0.5) and 1.5 (±1.2), respectively, on day 30 while remaining at 2.3 (±0.5) in the albumin group at the same day (Fig. 1A). Similar trends were observed in experiment B; however, the differences between albumin and both the IVIg and stradomer groups were statistically significant from day 12 (Fig. 1C).

Weight changes

The results shown in Figs. 1B and 1D indicate that IVIg and stradomer treatments reduced the weight loss that occurred in EAN rats. The extent of mean weight loss was the greatest in the albumin groups.
followed by the stradomer groups and then the IVIg groups (Figs. 1B and 1D). In experiment A, the maximal mean weight loss was 49.5 (±10) g on day 15 in the albumin group, 33.5 (±9) g on day 14 in the IVIg group, and 33.8 (±8) g on day 15 in the stradomer group. When the IVIg group was compared to the albumin group, significant differences in weight change were evident from days 14 to 19 and from days 23 to 30. Significant differences in weight change were seen from days 14 to 19 and from days 25 to 30 when comparing stradomer and albumin groups (Fig. 1B). Comparable results were observed in experiment B, but statistical differences between albumin and both the IVIg and stradomer groups were seen from days 14 and 17, respectively (Fig. 1D).

Electrophysiology

Motor conduction velocity

Overall, the MCV was significantly higher in the treatment groups than in the albumin group (Figs. 2A and 2B). In experiment A, the mean MCV gradually decreased in all albumin and treatment groups from day 10 and then recovered from day 22 in the IVIg and stradomer groups. There was a slight recovery recorded for mean MCV in the albumin group at the end of the experiment. A statistically significant difference between the IVIg and albumin groups was evident from day 22 and continued until day 30. A statistical difference was also observed between the stradomer and albumin treatment groups at day 30 (Fig. 2A). Similarly, in experiment B the mean MCV decreased in all groups (on day 10 in the albumin group and day 12 in the IVIg and stradomer groups) and then slowly recovered on day 26 in the albumin group, day 18 in the IVIg group, and day 22 in the stradomer group. Statistically significant differences were observable from day 16 until day 30 between the albumin and IVIg groups, and from days 22 until day 30 between the albumin and stradomer groups (Fig. 2B).

CMAP amplitude

After stimulation at the ankle (distal) (Figs. 3A and 3C) and the hip (proximal) (Figs. 3B and 3D), the CMAP amplitudes recorded from the dorsal foot muscles were better preserved in the IVIg and stradomer groups when compared with the albumin groups in both experiments.

In experiment A, the mean amplitudes of distal CMAP dropped at day 10 and then continued to decrease until the maximal reduction was reached on day 22 in the albumin group (79% reduction) and on day 16 in the IVIg group (62% reduction) and stradomer groups (74% reduction) (Fig. 3A). It then increased until day 30. Overall CMAP recovery was greater in the treated groups compared with the albumin group.

In experiment B, the mean amplitudes of distal CMAP dropped to the lowest point on day 22 in the albumin group (77% reduction) and on day 16 in the IVIg group (50% reduction) and stradomer groups (66% reduction) (Fig. 3C). It then increased until day 30, again to a greater extent in the treated groups.

As seen in Figs. 3B and 3D, we observed a reduction in mean amplitudes of proximal CMAP in all groups. In experiment A, the maximal reduction of mean proximal CMAP amplitude occurred on day 16; in the albumin group 74%, in the IVIg group 63%, and in the stradomer group 66% reduction was found (Fig. 3B). In experiment B, the maximal reduction of the mean proximal CMAP amplitude occurred on day 18 in the albumin group (66% reduction), on day 14 in the IVIg group (38% reduction), and on day 16 in the stradomer group (60% reduction) (Fig. 3D).

In experiment A, when the IVIg group was compared to albumin, significant differences were observed from day 16 in both distal and proximal CMAP amplitudes. The difference was significant at day 30 for distal CMAP amplitudes and from days 22
Figure 3. The amplitudes of dorsal foot muscle compound muscle action potential (CMAP) after stimulation at ankle (distal) (+SD in the intravenous immunoglobulin [IVIg] and stradomer™ groups and −SD in the albumin group) after immunization with (A) 40 mg/ml and (C) 50 mg/ml myelin in animals treated with albumin, IVIg, and stradomer. The amplitudes of CMAP after stimulation at hip (proximal) (+SD in the IVIg and stradomer groups and −SD in the albumin group) after immunization with (B) 40 mg/ml and (D) 50 mg/ml myelin in animals treated with albumin, IVIg, and stradomer. (E) The mean latency to peak (+SD in the albumin and stradomer groups and −SD in the IVIg group) and (F) amplitude of S wave (+SD in the IVIg and stradomer groups and −SD in the albumin group) after immunization with 50 mg/ml myelin in animals treated with albumin, IVIg, and stradomer. * indicates significant difference between the albumin and IVIg groups. # indicates significant difference between the albumin and stradomer groups (p < 0.05).

Spinal somatosensory evoked potential (S wave)

Latency to peak and amplitudes of S waves were measured in experiment B. Latency to peak of the S wave was more prolonged in the albumin group compared with the IVIg and stradomer groups. The mean S wave latencies increased in all albumin and

to 30 for proximal CMAP amplitudes when stradomer and albumin groups were compared. In the stradomer group, less reduced amplitudes of distal and proximal CMAP were observed from day 16; however, the difference failed to reach statistical significance at the mentioned day (Figs. 3A and 3B). In experiment B, significant differences between the IVIg and albumin groups were observed from day 14 in both distal and proximal CMAP amplitudes. The difference was significant from day 26 for distal CMAP amplitudes and at days 18, 26, and 30 for proximal CMAP amplitudes when the stradomer and albumin groups were compared (Figs. 3C and 3D).
Figure 4. Toluidine blue-stained transverse section of sciatic nerve after experimental autoimmune neuritis (EAN) induction with 40 mg/ml myelin, magnification x40. More widespread perivascular demyelination, axonal degeneration, and cellular infiltration in the sciatic nerve of (A) the albumin group compared with the (B) intravenous immunoglobulin (IVIg) and (C) stradomer™ groups 16–18 days after EAN induction. More demyelination in (D) the albumin group compared with (E) the IVIg and (F) stradomer groups 30 days after EAN induction.

Histology

Histological studies were carried out in experiment A. Various degrees of demyelination and axonal degeneration were evident in sciatic nerves of animals in all three groups at days 16–18 (mid-study). However, more extensive demyelination, axonal degeneration, and cellular infiltration were found in the albumin group (Fig. 4A) compared with the IVIg (Fig. 4B) and stradomer groups (Fig. 4C). Histological studies at the end of the study (day 30) showed less demyelination in stradomer (Fig. 4F) and IVIg (Fig. 4E) groups than the albumin group (Fig. 4D).

Semi-quantification of histological changes

A semi-quantitative assessment of histological changes was carried out based on results from experiment A. At days 16–18 (mid-study), more normal fibres were evident in the IVIg- and stradomer-treated groups compared with the albumin group. Eighty-six and 87% of fibres in the prevascular area of the IVIg and stradomer groups were normal, respectively, whereas only 66% of all fibres in the albumin group remained normal (Fig. 5A). The extent of demyelination was significantly greater in nerves from the albumin group (8.81%) in comparison with the IVIg (4.1%) and stradomer (3.7%) groups (Fig. 5B). As shown in Fig. 5C, both the IVIg- and stradomer-treated groups had almost 3.3 times less fibres undergoing axonal degeneration compared with the albumin group. When the extent of perivascular inflammation and demyelination was graded, nerves from the albumin
treatment groups at day 12 and then decreased after reaching the maximal latency on day 16 in the IVIg and compound groups and day 22 in the albumin group. Statistically significant differences were observed from day 14 until day 30 between the albumin and IVIg groups, and at days 22 and 30 between the albumin and stradomer groups (Fig. 3E).

The corresponding amplitudes of S waves were less preserved in the albumin group compared with the IVIg and stradomer groups. The mean amplitudes of the S waves declined from day 14 in all albumin and treatment groups until the maximal reduction on day 22 in the albumin group (55% reduction), day 16 in the IVIg group (29% reduction), and day 18 in the stradomer group (45% reduction) and then slowly increased during the recovery period. Statistically significant differences were evident from day 14 until day 30 between the IVIg and albumin groups and from day 22 until day 30 between the stradomer and albumin groups (Fig. 3F).
group had more extensive changes than those from the IVIg and stradomer groups, evidenced by a higher histological score. The percentage of the perivascular area with a given grade of demyelination and inflammation showed that nerves from the IVIg and stradomer groups had a lower percentage of grade 3 (Fig. 5D).

At the end of the study (day 30), the percentage of completely demyelinated fibres in the IVIg and stradomer groups was less than that of control group (Fig. 5E). In the treated groups, more cluster formation was observed compared with the control group (Fig. 5F). The difference in extent of cluster formation reached a significant level in the stradomer group. When the extent of perivascular inflammation and demyelination was calculated, the percentage of grade 0 in both treated groups was greater than that in the control group (Fig. 5G).

Discussion

In this study, the therapeutic efficacy of a novel fully recombinant multimerized murine IgG2a Fc compound was investigated and compared with that of IVIg in rat EAN. IVIg preparations have more than 60% IgG1 subtype and this subclass is considered to be the major effect of anti-inflammatory activity (Aloulou et al., 2012). The compound consists of mouse IgG2a, which is a homologue of human IgG1. It is the murine equivalent of the human stradomer with the murine IgG2a Fc substituted for the human IgG1 Fc.

IVIg and stradomer administered at the onset of disease were effective in reducing further progression of the disease and shortening disease duration as demonstrated by clinical, electrophysiological, and histological findings. We used two doses of myelin antigen to generate severe and moderate disease, in order to assess the efficacy of the stradomer in those different circumstances. We reasoned that the efficacy of the treatment would be more evident in severely affected animals. Our results showed that the differences in clinical scores, weight change, electrophysiology, and histology between the stradomer-treated and albumin groups in severe disease were more profound. However, as expected, more animals were lost particularly in the albumin (control) group. The moderate disease was generated to reduce animal loss.

The therapeutic effect of IVIg and stradomer, at a 2 log order lower dose, was similar and there was no significant difference in their effects on clinical score and weight loss. Both treatments were similarly effective compared with the albumin group in the treatment of EAN. At the peak of disease severity, rats treated with IVIg had the lowest clinical score amongst the three groups in both experiments. Less severe disease and a shorter clinical course were seen in both
IVlg and stradomer groups; recovery was also faster in these groups compared with the albumin group. The significant differences between the stradomer and albumin groups were evident later than that between the IVlg and albumin groups in experiment A. However, the recovery rate in both stradomer groups was similar to that in the IVlg groups. Similarly, the extent of mean weight loss was the greatest in the albumin group compared with the IVlg and stradomer groups. Significant weight gain in the stradomer groups started later than that of IVlg groups but reached the same ultimate level, suggesting that the treatment with the stradomer and IVlg had similar effectiveness in disease recovery in EAN.

Furthermore, in both experiments, a fall in MCV, which is characteristic of demyelinating neuropathies, was observed in all animals. In the treated animals the maximum drop had occurred at days 16–18, and thereafter recovery was seen. In the albumin group, however, MCV continued to fall and slightly recovered close to the end of the study. Although significant differences between the albumin and IVlg groups were evident earlier than that of between albumin- and stradomer-treated animals, the recovery rate of MCV was fastest in the latter animal groups. Results from electrophysiological studies of the CMAP amplitudes showed that both IVlg and stradomer treatments were effective in restoring S wave and proximal and distal CMAP amplitudes. As shown in Fig. 3, IVlg was superior to stradomer in maintaining the amplitudes of S wave and both distal and proximal CMAP. Again, significant differences between the albumin and IVlg groups were apparent earlier than those between albumin- and stradomer-treated animals in both experiments, but the recovery rate of amplitudes was faster in stradomer-treated animals, especially in experiment B.

Histological findings in the acute phase of disease indicated that the percentage of normal fibres in the prevascular areas in the IVlg and stradomer groups was significantly more than in the albumin group. In line with this finding, the extent of perivascular inflammation and demyelination in both IVlg and stradomer groups was less than the albumin group. In the recovery phase, more cluster formation (axonal regeneration) and less perivascular inflammation and demyelination were observed in both treated groups compared with the control group. The differences in demyelination and cluster formation were more prominent when the stradomer group was compared with the albumin and the IVlg groups, suggesting that stradomer treatment was most effective in accelerating regeneration and reducing demyelination (Fig. 5). Finally, although the differences between treated (IVlg and stradomer) and albumin groups in clinical, electrophysiological, and histological studies were not profound, they are highly analogous to the situation when GBS patients are treated with IVlg. Unlike the situation in the chronic disorder CIDP, when dramatic recovery may be seen following IVlg use, in GBS it is only when large numbers of patients are entered into clinical trials, the benefit of IVlg is seen by statistical differences.

The current and our previous studies (Lin et al., 2007) showed that IVlg is an effective therapy in EAN. However, two previous studies reported different results. One study found that IVlg therapy failed to be effective in EAN (Enders et al., 1997) and another reported that although intravenous human lg had minimal beneficial effect in EAN, intraperitoneal human Ig at the onset of disease accelerated recovery from EAN (Gabriel et al., 1997). Methodological differences between our study and these two studies may contribute to the differences found. First, we regularly assessed animal's clinical, electrophysiological, and histological changes throughout the 30-day period rather than at a terminal examination or a shorter period of observation (e.g., 20 days) in the other two studies. The beneficial responses after IVlg therapy were usually more prominent after day 20 in our study. Second, rats in our study received injections of 100 mg/100 g body weight of IVlg once daily for 2 days, whereas rats in the other two studies received 40 mg/100 g body weight for 5 days. Moreover, very significant benefits were found in a previous study in which IVlg and its Fc component were compared to the Fab portion and albumin (Lin et al., 2007).

The results of this study indicated that IVlg and recombinant multimers of IgG Fc had similar therapeutic characteristics even though there were some differences between them when compared with albumin. These differences in efficacy may be owing to differences in mechanism of action or dose differences between IVlg and stradomer. Multimerized murine IgG Fc that presents polyvalent Fc to FcγRs, which was used in this study, consists of fully recombinant stradomer binding strongly to FcγRs. The interaction of the Fc fragment with FcγR is necessary for IgG-mediated effector functions. FcγRs are divided into different subclasses, such as FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16) (Lisi et al., 2011). Of these, FcγRI, FcγRIIA, and FcγRIII are activating receptors that are primary mediators for proinflammatory activity in autoimmune disorders; FcγRIIB is the only inhibitory receptor, which is believed to have anti-inflammatory properties (Tackenberg et al., 2010). Immune complexes trigger anti-inflammatory responses by binding to activating and inhibitory FcγRs that are present on most innate immune effector cells such as basophils, mast
cells, neutrophils, monocytes, and macrophages. In this process, both activating and inhibitory signalling pathways are triggered by immune complexes depending on their affinity for the activating and inhibitory FcγRs. Further, only FcγRIIB is present on B cells and transduces an inhibitory signal, when interacting with the B cell receptor, preventing B cells from entering the germinal centre and transforming into plasma cells (Nimmerjahn and Ravetch, 2008; Tackenberg et al., 2010).

Results from our previous study showed that from the multiple mechanisms proposed for the action of IVIg (Ephrem et al., 2005; Siberil et al., 2007; Tackenberg et al., 2009; Piepers et al., 2010), FcγR-mediated mechanisms play a more significant role than that of anti-idiotypic antibody in rat EAN (Lin et al., 2007). We demonstrated that the Fc fraction of IVIg rather than the Fab portion was the main factor in preventing further disease progression, reducing disease length, better maintaining electrophysiological parameters, and preventing extensive inflammation and demyelination in nerve roots of the rat EAN model (Lin et al., 2007). Similarly, IVIg was shown to be more effective compared with Fab fragments in suppressing the clinical signs of rat EAN, suggesting that Fc portion may play an important role (Miyagi et al., 1997). In line with these findings, it is documented that FcγRIIB is necessary for the anti-inflammatory and protective effects of IVIg in various animal models and humans (Nimmerjahn and Lunemann, 2011). IVIg upregulates FcγRIIB and modulates the FcγRI/II/III ratio on macrophages, which determines the final immune response (Samuelsson et al., 2001). In murine models, FcγRIIB expression was shown to be upregulated by IVIg (Kaneko et al., 2006; Nimmerjahn and Ravetch, 2007) and was documented to be vital for the action of IVIg in a murine model of ITP (Samuelsson et al., 2001; Nimmerjahn and Ravetch, 2006). We propose that stradomer acts similarly through these FcγR mechanisms.

The administered dose of stradomer might be responsible for the detected differences between IVIg and stradomer. In this study, we used stradomer only at 1% of IVIg dose because it has been suggested that in IVIg, a small amount of aggregated IgG (roughly 1%) rather than IgG monomers constitutes the active component of IVIg mediating its functions through Fc receptors (Teeling et al., 2001; Ramakrishna et al., 2011). As several studies demonstrated that the aggregated Fc fragment of IVIg is responsible for its immunomodulatory function, the dose of stradomer may play a significant role in its therapeutic function (Ling et al., 1990). For instance, it has been reported that the efficacy of IVIg treatment of patients with ITP was associated with the presence of IgG aggregates not with monomeric IgG levels. More importantly, the stepwise increase in IgG aggregates significantly improved the efficacy of IVIg treatment during therapy (Augener et al., 1985). Therefore, increasing the dose of stradomer may promote its therapeutic effect and consequently reduce its difference with IVIg.

The findings of this study raise the possibility that stradomer has the potential to be used as an alternative for IVIg in the treatment of inflammatory neuropathies. Moreover, stradomer could be safer than IVIg as a smaller dose is required and, more importantly, it could be supplied without limit thus saving a precious resource. Further studies are required to answer questions about safety and dose response.

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References


