Modulatory Effects on Axonal Function After Intravenous Immunoglobulin Therapy in Chronic Inflammatory Demyelinating Polyneuropathy

Cindy Shin-Yi Lin, PhD; Arun V. Krishnan, PhD; Susanna B. Park, PhD; Matthew C. Kiernan, DSc

Objective: To investigate the immediate and longitudinal mechanisms of action of intravenous immunoglobulin (IVIg) on axonal function in chronic inflammatory demyelinating polyneuropathy (CIDP).

Design: Prospective single-center study.

Setting: Hospitals and outpatient clinics.

Participants: Clinical and functional assessment, nerve conduction studies, and 526 motor excitability studies were undertaken in 27 patients, matched before and immediately after infusion and followed up longitudinally.

Main Outcome Measures: Axonal excitability variables were measured before and immediately after infusion and compared with matched studies and findings in healthy controls.

Results: Immediately after infusion, patients demonstrated decreased threshold, with significant reduction in strength-duration time constant \((P = .003)\), reduction in accommodation to depolarization \((P = .04)\), and reduced threshold change during hyperpolarization \((P = .003)\), accompanied by significant decreases in superexcitability \((P = .03)\) and subexcitability \((P = .02)\). In contrast, changes were absent in disease controls, confirming a specific IVIg action in CIDP patients. Longitudinally, changes correlated with clinical improvement (mean [SE] increase in the Medical Research Council sum score, 2.7 [0.7]; \(P = .005\)). Increased compound muscle action potential amplitude was associated with reduction in terminal latency (correlation coefficient, \(-0.65; P = .02\)). In addition, these changes translated into improvement in functional assessment with the adjusted Inflammatory Neuropathy Cause and Treatment score, which demonstrated a significant correlation with nerve excitability variables longitudinally \((P = .01)\).

Conclusions: Findings from the present series establish a modulatory effect of IVIg on axonal function in CIDP patients, suggesting that IVIg stabilizes axonal membrane potential and promotes axonal recovery.

Arch Neurol. 2011;68(7):862-869

CHRONIC INFLAMMATORY demyelinating polyneuropathy (CIDP) is characterized by progressive weakness accompanied by sensory dysfunction. With an estimated prevalence of 2 to 7 per 100 000 population, CIDP leads to progressive and severe disability in most patients. A combined spectrum of clinical presentations, electrodiagnostic changes, and cerebrospinal fluid analysis are typically used to confirm a diagnosis.

Intravenous immunoglobulin (IVIg) therapy is an effective treatment for CIDP despite the high demand and cost of therapy, producing clinically meaningful improvement. However, IVIg is not effective in all CIDP patients, and a response to IVIg is not always apparent. Furthermore, the precise mechanism of action by which IVIg exerts its immunomodulatory effects and the pathophysiological processes that underlie the clinical improvement after IVIg treatment have not been elucidated.

CME available online at www.jamaarchivescme.com and questions on page 840

For editorial comment see page 844

Although demyelination and subsequent axonal loss account for many of the symptoms and clinical features of CIDP, the rapidity of clinical improvement in some patients after IVIg treatment raises the possibility that changes in axonal ion channel function may underlie beneficial treatment effects. Consequently, we undertook the present study to investigate the potential mechanism of effect of IVIg on...
axonal ion channel function in CIDP patients, immediately and longitudinally across treatment cycles, using novel nerve excitability techniques.9

METHODS

STUDY DESIGN

Patients receiving maintenance IVIg treatment were prospectively and consecutively recruited from a specialized neuropathy clinic. The diagnosis of CIDP was determined using a combination of clinical and electrophysiological features, fulfilling the current European Federation of Neurological Societies and Peripheral Nerve Society recommended guidelines.10 Before inclusion in the study, patients underwent induction therapy consisting of a 5-day course of IVIg infusions (0.4 g/kg/d), followed by monthly maintenance therapy (0.4 g/kg), that was continued according to clinical response. At the time of the study, all patients were clinically responsive to IVIg treatment and were receiving IVIg as their sole maintenance therapy. Patients were not prescribed corticosteroids or other concurrent immunomodulatory therapy.

To control for potential confounding factors related to IVIg treatment, including dilutional effects, a disease control group of patients (5 patients, aged 37-59 years) was selected representing a range of disorders without peripheral nerve involvement, including multiple myeloma, polymyositis, dermatomyositis, immunoglobulin deficiency, and graft-vs-host disease. Patients were excluded if they had a history of other conditions known to cause neuropathy. A cohort of healthy individuals (n=58) were age matched for comparison as a control group. All participants gave informed consent to the procedures, which were approved by the Human Research Ethics Committee of the University of New South Wales and the South Eastern Sydney Area Health Service Human Research Ethics Committee. The studies were performed in accordance with the Declaration of Helsinki.

CLINICAL NERVE EXCITABILITY MONITORING

Excitability studies were undertaken according to previously described protocols.9,11 Stimulus current was applied at the wrist to the median nerve and the anode located 10 cm proximal. Stimulation patterns were generated using computerized software (QTRAC, version 8.2; Institute of Neurology, London, England) and converted to current by an isolated linear bipolar constant-current stimulator (DSS; Digitimer, Ltd, Welwyn Garden City, England). Compound muscle action potentials (CMAPs) were recorded using surface electrodes overlying the abductor pollicis brevis, with the active electrode at the motor point and the reference electrode 4 cm distal.

Multiple nerve excitability variables were recorded using the following protocol and measures9,11:

- **Threshold (current)** refers to the stimulus current required to excite a single unit or to evoke a compound potential that was a defined fraction of the maximum;
- **Rheobase** is the threshold for stimuli of infinitely long duration;
- **Strength-duration time constant (SDTC)** is an apparent membrane time constant inferred from the relationship between threshold current and stimulus duration, indirectly reflecting nodal persistent sodium ion (Na+) conductances;
- **Refractoriness** is measured at short interstimulus intervals (2.5 ms), reflecting inactivation of transient Na+ channels;
- **Superexcitability** is measured as the maximum threshold decrease from a 5- to 7-millisecond interstimulus interval, demonstrating increased excitability due to the depolarizing after-potential13; and
- **Subexcitability** is assessed as the maximum threshold increase at an interstimulus interval of greater than 10 milliseconds, during which time axonal excitability is reduced, reflecting the kinetics of voltage-dependent slow potassium ion (K+) channels.13,15

**Accommodation** was defined as the tendency of the membrane potential to return toward the resting level despite a sustained depolarizing or hyperpolarizing stimulus, using the following protocol:

- **Threshold electrotonus (TE)** is the only method with which to examine the behavior of internodal conductances in human axons.16,17 In TE, the threshold changes produced by prolonged subthreshold depolarizing or hyperpolarizing currents are measured. The threshold changes generally parallel the electrotonic potentials responsible for them and therefore reflect membrane potential. With the use of subthreshold polarizing currents, all patients were clinically responsive to IVIg treatment and were receiving IVIg as their sole maintenance therapy. Patients were not prescribed corticosteroids or other concurrent immunomodulatory therapy.

To control for potential confounding factors related to IVIg treatment, including dilutional effects, a disease control group of patients (5 patients, aged 37-59 years) was selected representing a range of disorders without peripheral nerve involvement, including multiple myeloma, polymyositis, dermatomyositis, immunoglobulin deficiency, and graft-vs-host disease. Patients were excluded if they had a history of other conditions known to cause neuropathy. A cohort of healthy individuals (n=58) were age matched for comparison as a control group. All participants gave informed consent to the procedures, which were approved by the Human Research Ethics Committee of the University of New South Wales and the South Eastern Sydney Area Health Service Human Research Ethics Committee. The studies were performed in accordance with the Declaration of Helsinki.

CLINICAL FUNCTIONAL GRADING SCALES

The Medical Research Council (MRC) sum scores19 for grading muscle power were assessed bilaterally for the following muscles: shoulder abduction, elbow flexion, wrist extensors, first dorsal interosseous, hip flexion, knee extension, and ankle dorsiflexion. Each muscle was graded with a maximum score of 5 for a total maximal score of 70 (normal strength). Assessment of functional disability was undertaken via the adjusted Inflammatory Neuropathy Cause and Treatment (INCAT) disability score, with scores of 0 to 5 for arm and leg disability summed to give a maximal total of 10 (severe disability).8,20

STATISTICAL ANALYSIS

Paired t tests or Wilcoxon signed rank tests, depending on normality (assessed using the Lilliefors test), were used to compare preinfusion and postinfusion recordings and longitudinal data for each patient. Correlations were performed using Pearson correlation coefficients. All data were expressed as mean (SE), and P < .05 was regarded as statistically significant.

RESULTS

From a combined total of 617 new referrals to a specialized neuromuscular clinical service, 22 CIDP patients were...
The clinical and neurophysiological profiles of the patient cohort are summarized in the Table. The clinical and neurophysiological profiles of the patient cohort are summarized in the Table.

AFTER A SINGLE IVIg INFUSION

Motor axonal function after a single IVIg infusion

To compare the changes before and after a single infusion, motor excitability recordings were paired for each patient within a single IVIg infusion. A detailed analysis of a representative CIDP patient is depicted in Figure 1. After the IVIg infusion, there were significant differences in multiple excitability profiles between pretreatment recordings and those obtained 1 week and 2 weeks after treatment. In contrast to the preinfusion recordings, studies 1 week after the infusion demonstrated a marked reduction in SDTC (Figure 1A), accompanied by a fanned-in appearance in TE waveforms (Figure 1B), with greater inward rectification in the hyperpolarizing direction and decreased superexcitability and subexcitability in the recovery cycle (Figure 1C). However, 2 weeks after the IVIg infusion, these variables reverted toward the preinfusion values (Figure 1A-C). This pattern of change occurred repeatedly over time, as demonstrated in Figure 1D, with immediate paired cycles and longitudinal changes in SDTC in this representative patient plotted during a course of 22 months.

This pattern of change in excitability recordings was confirmed by group data (Figure 2). Significant changes developed in multiple excitability variables in the absence of any marked change in motor amplitudes. In contrast to preinfusion recordings, postinfusion recordings demonstrated a reduction in the threshold current required to stimulate the nerve (preinfusion, 4.9 [1.2] mA; postinfusion, 4.0 [1.2] mA; \( P = .15 \); Figure 2A). As a marker of persistent Na+ conductances, SDTC demonstrated a significant reduction immediately after IVIg treatment from 0.47 (0.02) to 0.41 (0.02) milliseconds (\( P = .003 \); Figure 2B). These changes were accompanied by lesser S2 accommodation (preinfusion, 22.7% [1.1%]; postinfusion, 19.7% [1.3%]; \( P = .04 \); Figure 2C) and reduced threshold change in the hyperpolarizing direction (TEh [90-100 ms] preinfusion, −124.2% [6.5%]; postinfusion, −104.3% [4.3%]; \( P = .003 \); Figure 2D). Prominent changes were also noted in recovery cycle measures, with reduction in superexcitability (preinfusion, −24.0% [1.6%]; postinfusion, −22.1% [1.4%]; \( P = .03 \);
Figure 2E) and subexcitability (preinfusion, 13.3% [0.9%]; postinfusion, 10.0% [1.0%]; P = .02; Figure 2F). Taken in total, the excitability changes suggested that axons in CIDP patients were relatively hyperpolarized before IVIg treatment and that IVIg treatment exerted a stabilizing effect on resting membrane potential.

An additional series of studies was undertaken in a cohort of disease controls who were receiving IVIg for indications other than peripheral neuropathy and in whom peripheral nerve function was normal. In contrast to CIDP patients, disease controls did not demonstrate any immediate modulation in their excitability profiles immediately after a single infusion, suggesting that the changes noted in CIDP patients were disease specific (data not shown).

LONGITUDINAL ASSESSMENT OF AXONAL FUNCTION WITH IVIg TREATMENT

Longitudinal assessment of motor nerve excitability variables was undertaken during a total of 36 months (mean duration, 15.2 [2.0] months per patient). In motor axons, longitudinal paired data (n = 12) demonstrated trends qualitatively similar to those noted in the immediate postinfusion studies. Specifically, there was a significant increase in SDTC (0.08 [0.03] ms; P = .04; Figure 3A) and a reduction in the stimulus to elicit 50% of the maximal response (1.66 [1.15] mA; P = .004; Figure 3B), accompanied by a significant reduction of the rheobasic current (1.78 [1.14] mA; P < .001), indicating that axons became less resistant and more excitable and confirming that IVIg reduced the threshold current required for axonal stimulation after long periods of treatment.

A representative CIDP patient has been depicted to illustrate the effects of IVIg modulation of SDTC longitudinally (Figure 1D). Immediately after IVIg, there was reduction in SDTC (90-100 ms; 2.0% [0.9%]; TEh [90-100 ms], 13.5% [5.7%]; P = .04; Figure 3C and D) along with a significant reduction in superexcitability (2.9% [1.0%]; P = .01; Figure 3E). These changes corresponded to the longitudinal improvement in the MRC sum score (2.7 [0.7]; P = .005; Figure 3F). Specifically, patients who had a greater than 2-increment increase in sum MRC scores with long-term IVIg treatment demonstrated significant correlation with axonal excitability variables, including accommodation half-time (R = 0.66;
In addition, the adjusted INCAT score was significantly correlated with TEd (90-100 ms), an excitability variable that is exquisitely sensitive to changes in resting membrane potential (R = 0.68; P = .01).

LONGITUDINAL CHANGES IN CIDP VS HEALTHY CONTROLS

The longitudinal changes observed in CIDP patients were taken to reflect the normalization of excitability variables in response to long-term IVIg treatment, particularly when compared with the findings in 58 healthy age-matched controls (Figure 4). Specifically, when compared with normative values, initial preinfusion recordings demonstrated a significant reduction in SDTC (preinfusion, 0.42 [0.02] ms; healthy controls, 0.53 [0.02] ms; P = .005; Figure 4A) with less accommodation to depolarizing currents (TEd [90-100 ms] preinfusion, 48.4% [1.2%]; healthy controls, 44.8% [0.54%]; P = .007; Figure 4C) and hyperpolarizing currents (TEh [90-100 ms] preinfusion, −128.8% [5.1%]; healthy controls, −120.5% [2.5%]; P = .17; Figure 4D) in accordance with previous findings. However, in the final recordings taken longitudinally across an average duration of 15.2 (2.0) months per patient, these excitability variables approached values for healthy controls, indicating these variables were within the normative range, confirming the benefit of long-term IVIg treatment.

The present series of studies assessed the immediate and long-term effects of IVIg therapy on axonal function in vivo in patients diagnosed as having CIDP. The changes observed across multiple nerve excitability variables suggested that IVIg exerted a modulatory effect on axonal function that was evident not only immediately after a single infusion but also as a sustained effect on axonal function in patients who responded to IVIg treatment over time. These studies have established the following immediate changes in nerve excitability variables: a shift of stimulus-response curves toward normal, a shortening of SDTC, a

©2011 American Medical Association. All rights reserved.

Figure 2. Changes in motor excitability with intravenous immunoglobulin (IVIg) treatment in patients with chronic inflammatory demyelinating polyneuropathy (CIDP). Motor excitability variables are reported as mean (SE) before and after a single IVIg infusion in CIDP patients. The threshold to elicit a 50% maximum response decreased after the infusion (A), and reductions were observed in the strength-duration time constant (SDTC) (B), S2 accommodation (C), and hyperpolarizing threshold electrotonus (TEh) (90-100 ms) (D). The recovery cycle demonstrated a significant decrease in superexcitability (E) and in subexcitability (F). NS indicates not significant (P = .15). *P < .01. †P < .05.

Figure 3. Longitudinal changes with intravenous immunoglobulin (IVIg) treatment in patients with chronic inflammatory demyelinating polyneuropathy. Longitudinal changes in motor axons with IVIg treatment are depicted in strength-duration time constant (SDTC) (A), stimulus for 50% maximum response (B), depolarizing threshold electrotonus (TEd) (90-100 ms) (C), hyperpolarizing TE (TEh) (90-100 ms) (D), superexcitability (E), and Medical Research Council (MRC) sum score (F). *P < .05. †P < .01.
MECHANISMS UNDERLYING CHANGES IN AXONAL PROPERTIES

Before interpretation of findings from the present study, we acknowledge that the precise mechanism of action of IVIg in patients with CIDP remains unclear. Proposed mechanisms include effects mediated by cellular and humoral immune systems, with IVIg working to suppress inflammation, inhibit autoantibody production, curb complement and cytokine function, and modulate Fc receptor function to reduce antibody-dependent cytotoxicity. It is likely that a combination of mechanisms act in concert to exert the clinical effects of IVIg. As such, the effects documented on axonal function and specific conductances may have occurred secondary to such processes and also potentially secondary to structural changes within the axon and myelin sheath. Regardless, the improvement in axonal function corresponded to clinical change and, as such, would seem intrinsic to a mechanism of effect of IVIg operating at a molecular level within the axon itself.

The present study demonstrated a consistent pattern of excitability modulation after repeated administrations of IVIg treatment in CIDP patients. Overall, these findings suggest that axons in CIDP patients existed in a relatively less excitable state before infusion and that IVIg treatment had a normalizing effect on membrane potential, as established by significant reduction in axonal threshold and improvement in accommodative properties. In addition, reduction in SDTC occurred after IVIg treatment. These changes corresponded to the time course of clinical improvement as experienced by CIDP patients after maintenance IVIg treatment. Reduction in the single variable of SDTC has been previously identified after a 5-day induction course of IVIg in CIDP patients. Although changes in SDTC may reflect modulation of Na+ currents, the mechanisms underlying cli-
cal improvement after IVIg treatment are complex, perhaps reflecting a variety of secondary and compensatory factors.

What then may underlie the baseline changes of axonal hyperpolarization in CIDP patients? Resting membrane potential in human axons is determined by a number of factors, including activation of persistent Na\(^+\) conductances\(^{17}\) and the activity of the energy-dependent axonal Na\(^+/K\(^+\) pump.\(^{26,27}\) Although recent studies have provided evidence of Na\(^+\) channel redistribution in CIDP axons,\(^{28}\) the changes in excitability variables noted in the present study are not purely those that have been described with alteration in Na\(^+\) conductances in humans.\(^{29}\)

In some CIDP patients, the rapid course of clinical improvement after IVIg treatment would be more suggestive of a change in axonal function (eg, related to ion channel properties) rather than structural change. The changes observed in axonal excitability immediately after IVIg infusion in CIDP patients in the present study may qualitatively reflect a pseudonormalization of functional abnormalities.\(^{23,24}\) As such, one can speculate that these immediate changes reflect stabilization of axonal function in CIDP patients with IVIg treatment that ultimately may help to promote structural recovery.

Alteration in Na\(^+\)/K\(^+\) pump activity remains another potential explanation for the excitability changes noted in pre-infusion recordings. The Na\(^+\)/K\(^+\) pump is electrogenic, with 3 Na\(^+\) ions being pumped out for every 2 K\(^+\) ions pumped into the axon, leading to a net deficit of positive charge on the axonal membrane.\(^{30}\) Heightened activity of the Na\(^+\)/K\(^+\) pump may induce membrane hyperpolarization due to increased extrusion of positive charge from the axon. Morphological changes in the axon may also provide the basis for heightened pump activity, producing hyperpolarization in CIDP.\(^{31}\) Specifically, remyelination leads to shortening of internodes, with an attendant increase in the number of Na\(^+\) channels. This in turn may lead to a greater Na\(^+\) influx during impulse conduction, thereby providing a strong stimulus for increased pump activity. Of clinical importance, increased pump activity occurs during prolonged natural activity, and previous studies\(^{32}\) in CIDP have demonstrated that membrane hyperpolarization after a period of natural activity may be sufficient to induce activity-dependent conduction failure. Of further relevance, a potential clinical correlation of activity-dependent conduction failure is the symptom of fatigue, a prominent feature of CIDP.\(^{33}\)

**LONGITUDINAL EFFECTS OF IVIg**

Of further clinical relevance, the improvements in excitability were sustained in longitudinal recordings, with normalized SDTC, reduced threshold, and increased accommodation suggesting that improvements in biophysical properties may in part underlie the clinical improvement that occurs with long-term IVIg therapy.\(^{8}\) These longitudinal findings in CIDP patients treated with immunoglobulin were the opposite of the longitudinal changes in excitability observed during Wallerian degeneration.\(^{31}\) With Wallerian degeneration, a progressive decrease in CMAP was associated with increased threshold, enhanced superexcitability, increased rheobase, increased SDTC, and decreased voltage-dependent Na\(^+\) and K\(^+\) conductances. Findings during the latent phase of Wallerian degeneration were interpreted to suggest gradual disruption in ion channel function that preceded conduction failure and axonal degeneration.

The converse of these changes as identified in the present series of CIDP patients suggests that the longitudinal changes in excitability were not related to ongoing demyelination. Indeed, a potential explanation is that these changes may be related to promotion of remyelination by IVIg, thereby preventing secondary axonal degeneration. Although this hypothesis requires further investigation, improvements in peak CMAP amplitude were associated with a decrease in latency longitudinally across treatment in the present CIDP patient cohort, which would support such a hypothesis. In addition, it remains plausible that some of the longitudinal changes noted in excitability variables were related to morphological alterations, as has been previously proposed.\(^{31}\)

Ideally, selecting the best immunotherapy for an individual patient should be based on the proven efficacy of a chosen regimen in modulating the pathophysiological mechanism underlying the neuropathy, the anticipated duration of disease, patient age, and metabolic derangement. Coexisting processes of inflammation, demyelination, degeneration, and regeneration only serve to contribute to the complexity of the long-term efficacy of IVIg immunotherapy. Findings from the present study may imply that the immediate role of immunotherapy appears to stabilize membrane potential, to thereby prevent further axonal degeneration and promote remyelination and recovery. The modulation established in nerve excitability profiles across the present series of studies may thereby be taken to reflect axonal plasticity in CIDP patients. More important, the longitudinal findings confirm that axonal accommodative properties were improved after long-term IVIg treatment. As such, multiple nerve excitability testing may serve as a useful biomarker to identify disease response to immunotherapy and to guide more specific and tailored treatment strategies for individual CIDP patients.

**Accepted for Publication:** December 6, 2010.

**Correspondence:** Cindy Shin-Yi Lin, PhD, Faculty of Medicine, School of Medical Science, Wallace Wurth Building, University of New South Wales, Sydney, New South Wales, Australia 2051 (c.lin@unsw.edu.au).

**Author Contributions:** The authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. **Study concept and design:** Lin and Kiernan. **Acquisition of data:** Lin, Krishnan, and Park. **Analysis and interpretation of data:** Lin, Krishnan, Park, and Kiernan. **Drafting of the manuscript:** Lin and Kiernan. **Critical revision of the manuscript for important intellectual content:** Lin, Krishnan, Park, and Kiernan. **Statistical analysis:** Lin and Kiernan. **Obtained funding:** Lin and Kiernan. **Administrative, technical, and material support:** Lin and Kiernan. **Study supervision:** Lin. **Financial Disclosure:** None reported.

**Funding/Support:** This study was supported by an Early Career Research Grant from the University of New South Wales.
Wales (Dr Lin), by National Health and Medical Research Council of Australia Career Development Award 568680 (Dr Krishnan), and by an Australian Postgraduate Award (Dr Park).

REFERENCES