MECHANISMS OF INTRAVENOUS IMMUNOGLOBULIN IN THE TREATMENT OF EXPERIMENTAL AUTOIMMUNE NEURITIS

Hsin Hsin Lin

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Department of Medicine
The University of Sydney

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SUMMARY

The aims of this study were to test the efficacy of immunoglobulin and its Fab and Fc fragment in the treatment of experimental autoimmune neuritis (EAN) in Lewis rats, to investigate which portion of immunoglobulin is operative in the effect of IVIg, and to clarify the possible mechanisms by which immunoglobulin exerts its action in the treatment of EAN in the rat.

EAN was induced by immunization with whole bovine peripheral nerve myelin. The immunized rats were randomized into groups, assessed clinically, electrophysiologically, and histologically, and intravenously injected with normal saline, albumin, human IVIg preparation, purified Fab or Fc fragments. The clinical disease severity was evaluated by the daily clinical grading and weight change. The electrophysiological studies included the spinal somatosensory evoked potential (S wave) and the compound muscle action potential (CMAP). The histopathological findings were analysed semiquantitatively. The treatment efficacy was compared between the normal saline and albumin groups, albumin and IVIg groups, albumin and Fab groups, albumin and Fc groups, Fab and Fc groups, Fab and IVIg groups, and Fc and IVIg groups. Methods of myelin isolation, antibody purification, and Western blot techniques were also applied.

The results revealed that treatment with Fc fragment and IVIg administered at the onset of signs of disease effectively prevented further progression of disease, shortened disease duration, and facilitated recovery from illness as shown in clinical, electrophysiological and histological parameters.

In the study in which the efficacy of the normal saline and albumin was compared, no significant difference was noted between these two groups. By day 30, 1 out of 9 rats
(11%) in the normal saline group and 2 out of 9 (22%) in the albumin group completely recovered from the clinical disease. In the study in which the efficacy of the albumin and IVIg was compared, more rats completely recovered from the clinical disease in the IVIg group (29% in the albumin group and 71% in the IVIg group) by day 30. The animals receiving IVIg treatment exhibited significantly lower clinical scores, less prolongation of S wave latencies, better maintained S wave amplitudes, less reduction of distal motor conduction velocities (MCVs), better maintained distal and proximal amplitudes of CMAPs, and lower histological grades. In the study in which the efficacy of the albumin, Fab fragment, Fc fragment, and IVIg was compared, more rats completely recovered from the clinical disease in the Fc and IVIg groups (0% in the albumin group, 13% in the Fab group, 50% in the Fc group, and 67% in the IVIg group) by day 30. The animals receiving Fc fragment and IVIg treatment exhibited significantly lower clinical scores, less prominent weight loss, less prolongation of S wave latencies, better maintained S wave amplitudes, less reduction of distal MCVs, better maintained distal and proximal CMAP amplitudes, and lower histological grades.
DECLARATION

I hereby declare that this submission is my own work and to the best of my knowledge it contains no material previously published or written by other person, nor material which to a substantial extent has been accepted for the award of any other degree or diploma at the University of Sydney or any other educational institution. Any contribution made to the research by others, with whom I have worked at the University of Sydney, is explicitly acknowledged in the thesis.

I also declare that the intellectual content of this thesis is the product of my own work, except to the extent that assistance from others in the project’s design and conception or in style, presentation and linguistic expression is acknowledged.

All the experiment described in this thesis was performed in the Neurology laboratory of the Department of Medicine at the University of Sydney between April 2003 to December 2005.
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I would like to dedicate this work to my husband and daughter, Chien Hui and Yu Ling.
SCIENTIFIC COMMUNICATIONS ARISING
FROM THIS THESIS

Papers

HH Lin, JM Spies, JD Pollard
Effective treatment of experimental autoimmune neuritis with human immunoglobulin
(submitted)

HH Lin, MX Wang, JM Spies, JD Pollard
Effective treatment of experimental autoimmune neuritis with Fc fragment of human
immunoglobulin (submitted)

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HH Lin, JM Spies, JD Pollard
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Journal of Neuroimmunology 2004, 154: 145

HH Lin, MX Wang, JM Spies, JD Pollard
Effective treatment of experimental autoimmune neuritis with Fc fragment of human

HH Lin, MX Wang, JM Spies, JD Pollard
Effective treatment of experimental autoimmune neuritis with Fc fragment of human
immunoglobulin – Journal of the Neurological Sciences 2005, 238 (Suppl. 1): S190
Best Poster Award

Effective treatment of experimental autoimmune neuritis with human immunoglobulin

*IVIG in Neurological disease – 1st Asia Pacific Symposium, Singapore, November 2004*

Oral presentations

Effective treatment of experimental autoimmune neuritis with human immunoglobulin

*11th Asian & Oceanic Congress of Neurology, Singapore, November 2004*

Poster presentations

Effective treatment of experimental autoimmune neuritis with human immunoglobulin

*7th International Congress of Neuroimmunology, Venice, Italy, September 2004*

Effective treatment of experimental autoimmune neuritis with human immunoglobulin

*IVIG in Neurological disease – 1st Asia Pacific Symposium, Singapore, November 2004*

Effective treatment of experimental autoimmune neuritis with Fc fragment of human immunoglobulin

*2005 Meeting of the Peripheral Nerve Society, Tuscany, Italy, July 2005*

Effective treatment of experimental autoimmune neuritis with Fc fragment of human immunoglobulin

*18th World Congress of Neurology, Sydney, Australia, 2005 November*
Abbreviations

ADCC  antibody-dependent cellular cytotoxicity
AIDP  acute inflammatory demyelinating polyradiculoneuropathy
Alb   albumin
AM    adhesion molecule
AMAN  acute motor axonal neuropathy
AMSAN acute motor sensory axonal neuropathy
ANOVA analysis of variance
AP    alkaline phosphatase
APC   antigen-presenting cell
AT-EAN adoptive transfer experimental autoimmune neuritis
BCR   B-cell receptor
BNB   blood-nerve barrier
C     complement
C domain constant domain of IgG molecule
C. jejuni Campylobacter jejuni
CMAP  compound muscle action potential
CMV   Cytomegalovirus
CNS   central nervous system
CR    complement receptor
CSF   cerebrospinal fluid
C terminal carboxyl terminal of IgG molecule
CV    conduction velocity
EAN   experimental autoimmune neuritis
ELISA enzyme-linked immunosorbent assay
EM    electro-microscopy
FcγR  Fc gamma receptor
FcR   Fc receptor
<table>
<thead>
<tr>
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<th>Definition</th>
</tr>
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<tr>
<td>Gal-C</td>
<td>galactocerebroside</td>
</tr>
<tr>
<td>GalNAc-GD1a</td>
<td>ganglioside N-acetylgalactosaminyl GD1a</td>
</tr>
<tr>
<td>GBS</td>
<td>Guillain-Barré syndrome</td>
</tr>
<tr>
<td>GD1a</td>
<td>disialoganglioside-GD1a</td>
</tr>
<tr>
<td>GD1b</td>
<td>disialoganglioside-GD1b</td>
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<tr>
<td>GM1</td>
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<td>GQ1b</td>
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<tr>
<td>GT1a</td>
<td>trisialoganglioside-GT1a</td>
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<tr>
<td>H/A ratio</td>
<td>proximal/distal CMAP amplitude ratio</td>
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<tr>
<td>H chain</td>
<td>heavy chain of IgG molecule</td>
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<tr>
<td>H. influenzae</td>
<td>Haemophilus influenzae</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
</tr>
<tr>
<td>HLA</td>
<td>human leukocyte antigen</td>
</tr>
<tr>
<td>HNK</td>
<td>human natural killer</td>
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<tr>
<td>IC</td>
<td>immune complex</td>
</tr>
<tr>
<td>ICAM</td>
<td>intercellular adhesion molecule</td>
</tr>
<tr>
<td>IFN</td>
<td>interferon</td>
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<tr>
<td>Ig</td>
<td>immunoglobulin</td>
</tr>
<tr>
<td>IgA</td>
<td>immunoglobulin A</td>
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<td>IgG</td>
<td>immunoglobulin G</td>
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<tr>
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<td>immunoglobulin M</td>
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<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>ITAM</td>
<td>immune-receptor tyrosine-based activation motif</td>
</tr>
<tr>
<td>ITIM</td>
<td>immune-receptor tyrosine-based inhibitory motif</td>
</tr>
<tr>
<td>ITP</td>
<td>idiopathic thrombocytopenic purpura</td>
</tr>
<tr>
<td>i.v.</td>
<td>intravenous injection</td>
</tr>
<tr>
<td>IVIg</td>
<td>intravenous immunoglobulin</td>
</tr>
<tr>
<td>kDa</td>
<td>kilo dalton</td>
</tr>
<tr>
<td>L chain</td>
<td>light chain of IgG molecule</td>
</tr>
<tr>
<td>LFA</td>
<td>lymphocyte function associated antigen</td>
</tr>
<tr>
<td>LM</td>
<td>light microscopy</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
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<tr>
<td>LM1</td>
<td>sialosylneolactotetraosylceramide</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>mAb</td>
<td>monoclonal antibody</td>
</tr>
<tr>
<td>MAC</td>
<td>membranolytic attack complex</td>
</tr>
<tr>
<td>MAG</td>
<td>myelin-associated glycoprotein</td>
</tr>
<tr>
<td>MBP</td>
<td>myelin basic protein</td>
</tr>
<tr>
<td>MCV</td>
<td>motor conduction velocity</td>
</tr>
<tr>
<td>MFS</td>
<td>Miller Fisher syndrome</td>
</tr>
<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
</tr>
<tr>
<td>MIP</td>
<td>macrophage inflammatory protein</td>
</tr>
<tr>
<td>MMP</td>
<td>matrix metalloproteinases</td>
</tr>
<tr>
<td>MS</td>
<td>multiple sclerosis</td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
<tr>
<td>NK</td>
<td>natural killer</td>
</tr>
<tr>
<td>NMJ</td>
<td>neuromuscular junction</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>N/S</td>
<td>non significance</td>
</tr>
<tr>
<td>N terminal</td>
<td>amino terminal of IgG molecule</td>
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<tr>
<td>P0</td>
<td>peripheral myelin protein zero</td>
</tr>
<tr>
<td>P2</td>
<td>peripheral myelin protein 2</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>PE</td>
<td>plasma exchange</td>
</tr>
<tr>
<td>PNM</td>
<td>peripheral nerve myelin</td>
</tr>
<tr>
<td>PNS</td>
<td>peripheral nervous system</td>
</tr>
<tr>
<td>SC</td>
<td>Schwann cell</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulfate</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>SDS-polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>SSEP</td>
<td>spinal somatosensory evoked potential</td>
</tr>
<tr>
<td>S wave</td>
<td>spinal somatosensory evoked response</td>
</tr>
<tr>
<td>TBS</td>
<td>Tris buffered saline</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
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<tr>
<td>TCR</td>
<td>T-cell receptor</td>
</tr>
<tr>
<td>TGF</td>
<td>transforming growth factor</td>
</tr>
<tr>
<td>Th cell</td>
<td>T helper cell</td>
</tr>
<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
</tr>
<tr>
<td>TTBS</td>
<td>Tris buffered saline with Tween 20</td>
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<tr>
<td>VCAM</td>
<td>vascular cell adhesion molecule</td>
</tr>
<tr>
<td>V domain</td>
<td>variable domain of IgG molecule</td>
</tr>
<tr>
<td>VLA</td>
<td>very late antigen</td>
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