MECHANISMS OF INTRAVENOUS IMMUNOGLOBULIN IN THE TREATMENT OF EXPERIMENTAL AUTOIMMUNE NEURITIS

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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)

Published abstracts

HH Lin, JM Spies, JD Pollard

Effective treatment of experimental autoimmune neuritis with human immunoglobulin – 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 immunoglobulin – Journal of the Peripheral Nervous System 2005, 10 (Suppl): S53

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

Gal-C	galactocerebroside
GalNAc-GD1a	ganglioside N-acetylgalactosaminyl GD1a
GBS	Guillain-Barré syndrome
GD1a	disialoganglioside-GD1a
GD1b	disialoganglioside-GD1b
GM1	monosialoganglioside-GM1
GQ1b	tetrasialoganglioside-GQ1b
GT1a	trisialoganglioside-GT1a
H/A ratio	proximal/distal CMAP amplitude ratio
H chain	heavy chain of IgG molecule
H. influenzae	Haemophilus influenzae
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HNK	human natural killer
IC	immune complex
ICAM	intercellular adhesion molecule
IFN	interferon
Ig	immunoglobulin
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IL	interleukin
ITAM	immune-receptor tyrosine-based activation motif
ITIM	immune-receptor tyrosine-based inhibitory motif
ITP	idiopathic thrombocytopenic purpura
i.v.	intravenous injection
IVIg	intravenous immunoglobulin
kDa	kilo dalton
L chain	light chain of IgG molecule
LFA	lymphocyte function associated antigen
LM	light microscopy

LM1	sialosylneolactotetraosylceramide
LPS	lipopolysaccharide
mAb	monoclonal antibody
MAC	membranolytic attack complex
MAG	myelin-associated glycoprotein
MBP	myelin basic protein
MCV	motor conduction velocity
MFS	Miller Fisher syndrome
MHC	major histocompatibility complex
MIP	macrophage inflammatory protein
MMP	matrix metalloproteinases
MS	multiple sclerosis
MW	molecular weight
NK	natural killer
NMJ	neuromuscular junction
NO	nitric oxide
N/S	non significance
N terminal	amino terminal of IgG molecule
P0	peripheral myelin protein zero
P2	peripheral myelin protein 2
PBS	phosphate buffered saline
PE	plasma exchange
PNM	peripheral nerve myelin
PNS	peripheral nervous system
SC	Schwann cell
SD	standard deviation
SDS	sodium dodecyl sulfate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SSEP	spinal somatosensory evoked potential
S wave	spinal somatosensory evoked response
TBS	Tris buffered saline

TCR	T-cell receptor
TGF	transforming growth factor
Th cell	T helper cell
TNF	tumor necrosis factor
TTBS	Tris buffered saline with Tween 20
VCAM	vascular cell adhesion molecule
V domain	variable domain of IgG molecule
VLA	very late antigen

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