UNIVERSITY OF TECHNOLOGY SYDNEY

Systemic Administration of a Connexin43 Mimetic Peptide: A Treatment Option for Spinal Cord Injury

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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Certificate of Original Authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as part of the collaborative doctoral degree and/or fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Abbreviation

3Rs:	Replacement, Reduction and Refinement
ACEC:	Animal Care and Ethics Committee
AMPA:	a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
ANOVA:	Analysis of Variance
AS-ODN:	Antisense oligodeoxynucleotide
ATP:	Adenosine triphosphate
BBB:	Basso, Beattie and Bresnahan
BSB:	Blood spinal cord barrier
CNS:	Central nervous system
FITC:	Fluorescein isothiocyanate
FL:	Front limb
GABA:	γ-aminobutyric acid
GFAP:	Glial fibrillary acidic protein
H&E:	Haematoxylin and Eosin
HL:	Hind limb
HREC:	Human Research Ethics Committee
IHC:	Immunohistochemistry
MASCIS:	Multicentre Animal Spinal Cord Injury Study
MP:	Methylprednisolone
mRNA:	Messenger ribonucleic acid
MS:	Mass spectrometry
NASCIS:	National Acute Spinal Cord Injury Studies
NeuN:	Neuronal nuclei
NGS:	Normal goat serum
NMDA:	N-methyl-D-aspartate
OSP:	Oligodendrocyte Specific Protein
P5:	Peptide5
PBG:	Phosphate buffer with 5% normal goat serum
PFA:	Paraformaldehyde
ROS:	Reactive oxygen species
SCI:	Spinal cord injury

SDS:	Sodium dodecyl sulphate
SEM:	Standard error of the mean
SP:	Scrambled peptide
TBS:	Tris-buffered saline
UNSW:	University of New South Wales
UTS:	University of Technology Sydney

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Abstract

Spinal cord injury (SCI) is a major cause of morbidity, and leads to significant physical, emotional and financial burdens for individuals and their families. There is no known cure to date and current treatment options are limited. A cascade of secondary tissue damage starts within minutes to hours of an initial traumatic SCI, worsening the cellular and functional recovery. The time course of secondary damage does, however, offer a potential window of opportunity for intervention. It may be possible to administer new therapeutics during this early period, to prevent the extensive spread of damage to adjacent healthy tissue.

Over the past two decades, regulation of Connexin43 hemichannels has provided a novel avenue of research into limiting the secondary damage of SCI. Peptide5, a Connexin43 mimetic peptide, is a promising candidate that shows efficacy in blocking Connexin43 hemichannels, thus preventing the propagation of neurotoxic molecules from the injured cells to the nearby intact tissue in a number of neural injury models. Peptide5, in particular, has been shown to reduce secondary tissue damage and to improve functional recovery when delivered directly to the site of SCI. However, direct application of peptides to a spinal cord lesion is likely to be impractical in a clinical setting, and might result in delays to the initiation of treatment. Therefore, the current research project aims to demonstrate that the systemic administration of Peptide5 is a clinically relevant treatment option for traumatic SCI by (1) optimising the systemic administration protocol of Peptide5; (2) investigating the cellular and function improvements in SCI rats from the Peptide5 systemic administration; and (3) examining the safety profile of Peptide5 systemic administration protocol.

Mass spectrometry and electrophoresis were used to determine the *in vitro* degradation curves in normal rat and human sera. A rat model of contusive SCI was then employed to optimise the dosing regimen and investigate the efficacy of Peptide5 in limiting secondary tissue damage and improving functional recovery. The lesion size was assessed on Haematoxylin and Eosin stained histological sections, and fluorescent immunohistochemistry was utilised to examine the Connexin43 protein and hemichannels, as well as cellular responses. The behavioural improvements were evaluated from motor (open field and error ladder tests) and sensory (mechanical pain hypersensitivity) functions. Fluorescent-labelled Peptide5 was delivered to the SCI and normal rats systemically in order to determine the *in vivo* distribution.

It was found that the *in vitro* half-life of Peptide5 was 2 hours in normal rat serum and that the most effective dosing regimen was three injections of Peptide5 at 0 (10 mg/kg), 2 (5 mg/kg) and 4 (2.5 mg/kg) hours post-injury. Compared with controls, Peptide5 treated rats showed significant improvements in hind limb locomotor function and mechanical pain hypersensitivity. Peptide5 treatment led to a reduction in Connexin43 protein level, hemichannel opening, lesion size, astrogliosis, macrophage and/or microglial activation, and neuronal cell death. There was no evidence to suggest that Peptide5 had any adverse systemic effects in normal and SCI animals, or influenced other connexins in the spinal tissue.

The current research project demonstrated that systemic administration of Peptide5 is a feasible, neuroprotective and safe treatment option for ameliorating secondary damage and improving functional recovery in a contusive SCI model of rats. These findings provide strong support for the clinical use of Peptide5 in the near future.