Development of NNZ-2566 as a Drug Candidate for Traumatic Brain Injury: The Neuren Story

Margaret A. Brimble* and Paul W. R. Harris

Department of Chemistry, Maurice Wilkins Centre for Molecular Biodiscovery and The Institute for Innovation in Biotechnology, University of Auckland, Auckland 1010
(e-mail: *m.brimble@auckland.ac.nz*)

About Margaret Brimble

Margaret Brimble MNZM, FRSNZ, FRSC, FNZIC, FRACI holds the Chair of Organic and Medicinal Chemistry at the University of Auckland. She graduated with an MSc (Hons) in Chemistry in 1982 from that university and gained her PhD in organic chemistry as a Commonwealth scholar under the direction of Prof. Ray Baker at the University of Southampton in 1986. Margaret then took up a lectureship at Massey University where she began her independent bioactive natural products work. She was a visiting professor at the UC-Berkeley in 1992, moved to Sydney University in 1994, and returned to NZ as Professor of Organic Chemistry at the University of Auckland in 1999. She was instrumental in establishing the first new interdisciplinary degree in medicinal chemistry in NZ and is now Chairperson of Organic and Medicinal Chemistry and was Director of Medicinal Chemistry, Neuren Pharmaceuticals Ltd.

Margaret was appointed Titular Member of IUPAC Organic and Biomolecular Chemistry Division (Division III), is Co-Chair Organising Committee – Zing European Gordon Conference on Synthesis and Biosynthesis of Natural Products to be held in Egypt in February 2012 and was co-Chair of the IUPAC 14th International Conference on Organic Synthesis held in Christchurch in 2002. She is a Trustee and Chairperson of RSNZ's Rutherford Foundation, past-President of the International Society of Heterocyclic Chemistry and a past member of the Marsden Fund Council (Chairperson of the PCB panel). Her editorial board responsibilities include Organic and Biomolecular Chemistry and Natural Product Reports, Synthesis and Synlett, Scientific Reports, the Wiley-Blackwell Postgraduate Chemistry Series, the Journal of Heterocyclic Chemistry, Marine Drugs, Chemistry Insights, Perspectives in Medicinal Chemistry.

Margaret has received numerous national and international accolades, not least those of her MNZM and the L'Oreal-UNESCO Laureate and the RSC Natural Products awards that have been recorded in these pages. Most recently she opened the 2011 Marie Curie *Women in Science* lecture series in Wellington in February.

Neuren Pharmaceuticals, Neuroprotection and NNZ-2566

Neuren Pharmaceuticals Ltd.[1] is a NZ based biopharmaceutical company whose principal business activities are the discovery, development and commercialization of pharmaceuticals for the treatment of brain injury and neurodegeneration. The company's focus is the preservation, treatment and monitoring of neuronal function in neurodegenerative disease in chronic conditions such as Parkinson's and Alzheimer's disease and following acute ischemic and traumatic brain injury. Herein, we outline the medicinal chemistry effort that led to the discovery of NNZ-2566, currently in phase II human clinical trials as a potential therapeutic agent to treat traumatic brain injury (TBI) with $US18 million funding from the US Army Medical Research and Material Command.

The ability to reduce the damage and consequences of brain injury is referred to as neuroprotection and NNZ-2566 is the most promising neuroprotective drug currently in development for TBI. This is a considerable achievement as there is currently no neuroprotective drug approved for TBI on the market. The fact that the US Army is prepared to fund the trial to the extent of over $US18 million speaks for itself and validates NNZ-2566 as a valuable drug candidate establishing Neuren Pharmaceuticals Ltd. as a major player at the forefront of clinical research into TBI. Few biotech companies find themselves in such a position with the real possibility of dramatically improving shareholder value and making a major contribution to public health. NNZ-2566 is Neuren's lead clinical stage asset. The discovery and development of NNZ-2566 was undertaken through a contractual arrangement with Neuren Pharmaceuticals Ltd. administered by Auckland Uniservices Ltd. All of the medicinal chemistry was conducted in the Brimble laboratory in Auckland University's Chemistry Department.

Drugs for Traumatic Brain Injury (TBI): An Unmet Medical Need with Significant Market Opportunity

In the US, traumatic brain injury (TBI) is the primary cause of death and disability in persons under 45 years old, occurring more frequently than breast cancer, HIV-AIDS, multiple sclerosis, and spinal cord injury combined.[2,3] In the US alone, approximately 1 million TBI patients are either treated and released from the emergency department or admitted to the hospital each year. Of the approximately 300,000 hospital admissions, nearly half have mild or moderate TBI. This trauma affects up to 90 New Zealanders every day and can result in lifelong disability. The cost of medical care for these individuals exceeds $NZ1 billion dollars p.a. and places considerable financial burden on the limited resources of our small country. Moreover, the societal and personal impact is equally severe.

Overall, the leading causes of TBI are falls and motor vehicle accidents; however, penetrating ballistic-like brain injury (PBBI) represents one of the most severe categories and is the leading cause of TBI-related death in the US in both civilian and military populations.[4,5] With no drugs approved for this indication, TBI represents a large unmet medical need as well as a significant market opportunity. Neuren Pharmaceuticals Ltd. has estimated total sales of NNZ-2566 in the first 10 years following approval to exceed $US2 billion in the US alone, with peak gross revenues of $US341 million. Assuming drug registration success, this would result in a net present value exceeding $US250 million.

In addition to the damage caused by the initial brain injury, secondary injury takes place in the minutes and days following the injury (Fig. 1). These processes, which include alterations in cerebral blood flow and the pressure within the skull, contribute substantially to the damage from the initial injury. NNZ-2566 provides a unique opportunity for neuronal rescue therapy designed to ameliorate the effects of secondary injury caused by brain trauma.

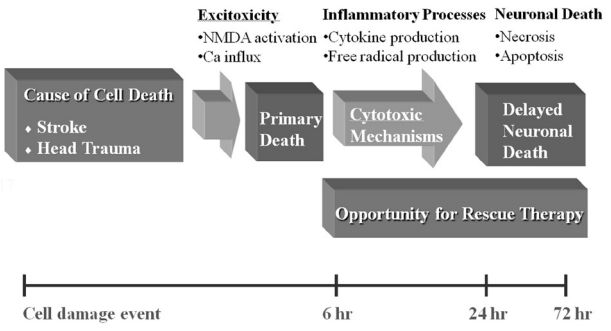


Fig. 1. The brain injury cascade.

Besides the direct cellular and molecular effects of TBI, up to one-third or more of patients experience non-clinical or non-convulsive seizures in the acute, post-injury period. Non-convulsive seizures (NCSs) are associated with increased brain injury and long-lasting cognitive and neurological deficits. Generalized convulsive seizures are readily recognizable, but NCSs occur without motor manifestation and, therefore, are often undiagnosed. Even though NCSs are difficult to detect and treat, prompt medical intervention should be provided to prevent synergistic brain damage and worsening of the prognosis.[6] The unanswered medical need to control and treat NCSs effectively in brain-injured patients underscores the importance to develop novel therapeutic drugs with both neuroprotective and antiepileptic properties.

Independent animal studies conducted by the Walter Reed Army Institute of Research (WRAIR) indicated that NNZ-2566 significantly reduced the number and duration of non-convulsive seizures following brain injury.[7] The compound possesses a unique therapeutic potential as a safe prophylactic agent that synergistically provides neuroprotection and reduces injury-induced seizures. The WRAIR suggest that neuroprotective effects of NNZ-2566 may, in part, be functionally attributed to the compound's ability to modulate expression of multiple neuroinflammatory mediators in the injured brain.[8]

Presently, there are no drugs approved for TBI. This and the serious and life-threatening nature of the condition supported the approval of NNZ-2566 for Fast Track designation by the US FDA.

The Initial Lead: Discovery of GPE

Studies have shown that NNZ-2566 prevents secondary damage to brain cells in patients with TBI by interfering with the inflammatory and apoptotic phenomena that are up-regulated following an acute brain injury, thereby reducing the number of brain cells impacted upon by the initial injury. NNZ-2566 is a synthetic analogue of a naturally occurring molecule produced by the brain in response to injury. The natural molecule is a small part of the endogenous insulin-like growth factor-1 (IGF-1) protein that can be truncated in the brain by an acid protease to form the des(1-3)IGF-1 fragment and the N-terminal tripeptide, glycine–proline–glutamate (GPE) (Fig. 2).[9]

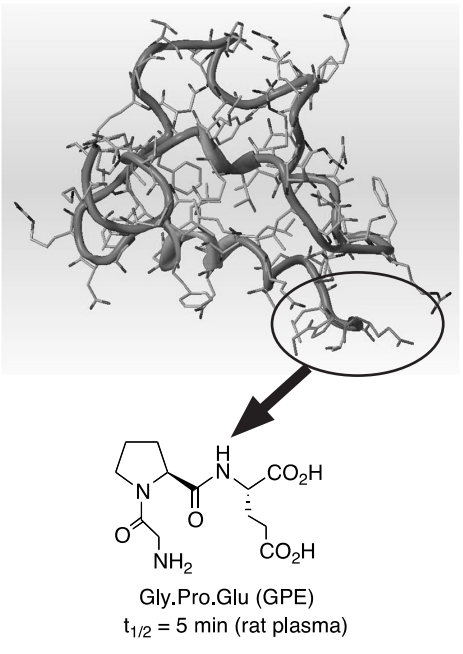


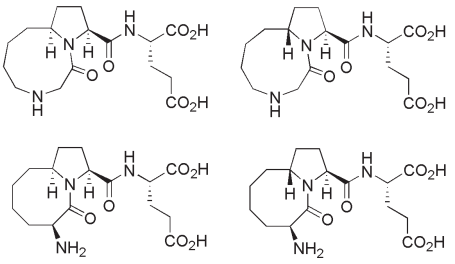
Fig. 2. Cleavage of tripeptide Gyl.Pro.Glu. (GPE) from the N-terminus of insulin-like growth factor 1 (IGF-1).

IGF-1 is neuroprotective and improves long-term function after brain injury. However, its clinical application to neurological disorders is limited by its large molecular size, poor central uptake and mitogenic potential. The endogenous tripeptide GPE also exhibits neuroprotective properties in animal models for ischemic injury. GPE thus provided a novel lead molecule for the development of new drugs to treat neurological disorders. However, it is not enzymatically stable and has a plasma half-life of less than 5 min. in rats;[10] hence, intravenous infusion of GPE becomes necessary for stable and potent neuroprotection. Our goal, therefore, was to find a synthetic analogue of GPE that was a more potent neuroprotection agent, had a longer half-life, and was able to penetrate the blood-brain barrier.

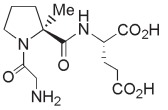
The Development of NNZ-2566

For the invention of NNZ-2566, the challenge was that the initial neuropeptide lead GPE did not target a single, highly specific component in a well-known biological pathway. We had to design and develop synthetic analogues of the naturally occurring neuropeptide to modulate expression of multiple inflammatory mediators in the injured brain. Over 120 such compounds were synthesized.[11] The process to optimize GPE into therapeutic leads involved investigation into areas such as improving proteolytic stability, improving bioavailability (transport across the gastrointestinal tract and blood-brain barrier), and formulation development. Synthetic approaches to achieve these parameters included increasing the lipophilicity of the analogues,[12] removal of amino acid characteristics such as the α- and γ-carboxylic acid groups in the glutamic acid residue,[13-15] using D amino acids, introducing bulky α,α-dialkyl amino acids and *N*-methyl amino acids that are not recognized by proteases. Further structural changes included introducing appropriate functionality to alter the *cis-trans* conformation of the proline *N*-carbonyl to the side chain amido carbonyl attached to C2,[16] modification of the peptide linkages, and reduction in the degrees of freedom of the peptidomimetic by linking the side chains using Grubbs' ring-closing metathesis.[17] Examples of compounds from this latter stratagem are summarized in Chart 1. Formulation development included the selection of suitable protease inhibitors and permeation enhancers for optimal oral delivery.

Chart 1. Examples of synthesized macrocyclic analogues of GPE.



To overcome the instability of tripeptide GPE in plasma, one of the GPE analogues resulted from α-methylation of the proline ring to give GPE G-2MePE (now known as NNZ-2566). Its half-life[18] in the blood and in the brain was significantly prolonged compared with that of GPE, and oral bioavailability improved considerably compared to the parent peptide. *In vivo*, NNZ-2566 reduced injury size in rats subjected to acute focal stroke.[18] An intravenous infusion of NNZ-2566 of 4 h duration (3–10 mg/kg/h), initiated 3 h after endothelin-induced middle-cerebral artery constriction (MCAO), significantly reduced infarct area as assessed on day five. Neuroprotective efficacy in the MCAO model was also observed following oral administration of the drug (30–60 mg/kg) (Fig. 3).



$t_{1/2}$ (rat brain) = 74 min

NNZ2566

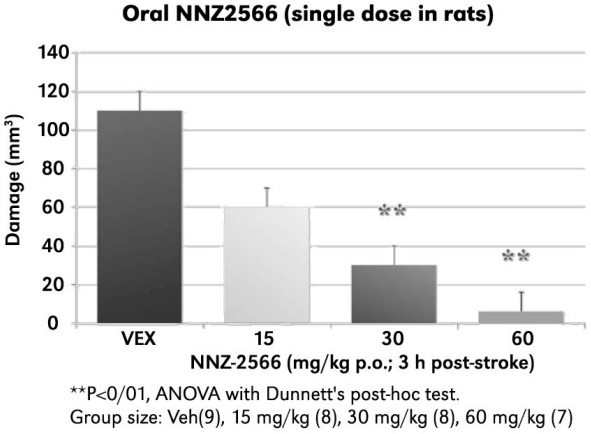


Fig. 3. MCAO-induced ischemic brain damage: effect of oral NNZ2566 – see ref. 18.

In vitro, NNZ-2566 significantly attenuates apoptotic cell death in primary striatal cultures, suggesting that attenuation of apoptosis is one mechanism of action underlying its neuroprotective effects. Independent experiments carried out by the WRAIR demonstrated that in animal models NNZ-2566 reduced the level of expression of genes associated with inflammation, necrosis and apoptosis – key elements of the brain injury cascade – and reduction in the functional deficits induced by these phenomena.[7,8] These data further supported the development of NNZ-2566 as a neuroprotective agent for acute brain injury, and it was shown to have a good safety profile in preclinical and phase I studies. It is currently in phase II clinical trials for the treatment of cognitive deficits following traumatic brain injury.

After the discovery of NNZ-2566 we provided on-going analytical and preparative support for the scale-up and current Good Manufacturing Practice (cGMP) production of the newly developed active pharmaceutical ingredient (API). We also went on to develop two new classes of compounds for Neuren Pharmaceuticals Ltd. called diketopiperazines (DKPs) and macrocyclics that have been rationally designed as candidates for both acute and chronic neurological conditions. Our second drug candidate NNZ-2591 is also under serious consideration by Neuren Pharmaceuticals Ltd. for entry to human clinical trials for TBI.

The Future of Peptide Chemistry at The University of Auckland

The discovery of NNZ-2566 as a pre-eminent drug candidate for TBI not only has obvious potential health benefits in NZ but also for the world population. The global market for novel therapies in areas of significant unmet medical need such as TBI is large and the downstream economic benefits to NZ from the development of the world's first drug for TBI also would be significant.

Importantly for our medicinal chemistry team, the work carried out for Neuren Pharmaceuticals Ltd. was a catalyst for the development of a larger peptide and peptidomimetic synthesis laboratory for NZ biopharma that is now located in the Institute for Innovation in Biotechnology (IIB) in our university. The purpose-built peptide synthesis laboratory is also equipped to undertake the synthesis of peptides under cGMP. In partnership with the Malaghan Institute of Medical Research, and with funding from the Health Research Council, this state-of-the-art peptide chemistry laboratory is set to produce long peptides as components for melanoma vaccines to be used in human clinical trials.

Acknowledgements

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