Laminin: a targeted therapy for the CNS

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Understanding molecular mechanisms of neuronal viability & neurite outgrowth. At present, the treatment of CNS trauma and disease remains largely one of medicine's unachieved goals. For example, current treatments of spinal injuries and stroke are often purely palliative and fail to provide the means to facilitate functional recovery or offer the patient a return to normal life [1,2]. Current treatment of Parkinson's disease still only aims at replacing the dopamine missing in higher brain centers, rather than preventing the death of the mid-brain dopaminergic neurons [3], and novel treatments for Alzheimer's disease and amyotrophic lateral sclerosis (ALS) lead only to marginal, if any, improvements [4–6]. Even though genetic linkage studies and basic neuroscience research have identified numerous genes and mechanisms involved in CNS trauma and the progression of Alzheimer's disease, ALS and Parkinson's disease [7–13], none of the currently used or tested medications promote efficient survival of neurons and their connections; they simply serve to alleviate the symptoms caused by the lack of function in certain groups of brain cells.

Stem cells: the magic bullet

In recent years, stem cell research has offered hope as the first biological alternative to designed drugs in the treatment of CNS trauma and neurological disorders [14,15]. More specifically, stem cell therapy has been proposed as the future for the treatment of spinal cord injuries (SCIs), and Parkinson's and Alzheimer's disease. Either olfactory epithelial cells derived from the patient's own nasal epithelium or stem cells from the patient's own blood have been proposed and used to treat paralysis and ALS. However, the effectiveness of such treatments remains marginal and somewhat controversial [16]

and, indeed, it sometimes seems as though stem cells are being promoted as a 'magic bullet', a potential cure for almost anything. However, there seems to be a lack of reasoning as to its real usability and an underestimation of potential problems. Indeed, rather than being skeptical, many seem to be carried along by a wave of stem cell optimism that is based on far too little hard evidence. This brings to mind the fable of the Emperor's New Clothes...

In reality, as long as nobody knows how stem cells work, how to make them work and whether or not they are safe, they will remain just a hope. What is needed is a pure, clear-cut and simple approach that draws upon, and develops, scientifically identified beneficial factors for their use in the treatment of neurological disease and trauma. Indeed, the value of basic science, such as KDI tripeptide protein (Lys-Asp-Ile) research, lies precisely in gathering information regarding CNS development and function that can then be projected into the clinical world in a beneficial manner within a more realistic timescale.

Naturally occurring small molecules may aid the treatment of neurological disease & trauma

Naturally occurring small molecules, such as biologically active domains of laminin, can overcome problems associated with stem cells and may provide a short cut for effective treatment of neurological disease and trauma. During the past 20 years, an extracellular matrix glycoprotein, initially named laminin and first studied by cancer biologists [17], has been identified as an active mediator of neuronal development, response to trauma and the regenerative potential of the CNS [7,8,18]. Recent research from my laboratory has shown that a short biologically active KDI tripeptide derived from γ 1-laminin [19,20], which is one of the 15 currently known isoforms of laminin [21], promotes both functional recovery and survival of CNS neurons [19,20]. This KDI tripeptide supports functional recovery of adult rat SCIs to such an extent that animals with severed lumbar spinal cords, initially unable to walk, recovered to a

level where they could at least bear weight on their hind limbs, or in the best cases even walk after 3 months of local application of the KDI tripeptide at the severed site [22].

Laminin is it, KDI does it!

In 2001, we identified the KDI tripeptide from its 10 amino acid-long precursor [19] and found that it bears both the neurite outgrowth-promoting and neuronal survival-supporting properties of γ 1-laminin [20].

Studies on the human embryonic CNS indicated that γ 1 laminin was expressed in spatial and temporal correlation with pioneer axon growth, and that the KDI domain promoted directional axon growth of the human embryonic spinal cord neurons in a 3D culture system [23]. The KDI tripeptide also promoted survival and neurite outgrowth of human embryonic neurons and neutralized both glial scar- and soluble myelin-derived inhibitors of axon growth [24]. These results indicate that the KDI domain of γ 1-laminin is a naturally occurring factor that regulates normal human spinal cord development and supports both survival and neurite outgrowth of human spinal cord neurons against both glial- and myelin-derived inhibitory signals [23,24]. Thus, the KDI tripeptide may be the first targeted medication for CNS trauma and neurodegenerative disorders by promoting both survival and axon growth of the injured CNS neurons.

Efficacy of KDI

In addition to its laminin-like function in promoting neurite outgrowth and survival of central neurons, the KDI tripeptide was found to protect the CNS against glutamate-induced neurotoxicity [25], which is the most common mechanism of neuronal death in all forms of CNS trauma and disease [26–28].

This protection is dose-dependent and based on the ability of the KDI tripeptide to inhibit the function of all major ionotropic glutamate receptors [29]. This ability, together with laminin's own neurotrophic function, allows the KDI tripeptide to save the CNS from total destruction and tissue lysis and thus also to preserve CNS function.

Natural, small, safe & easy to apply

Unlike drugs designed by the pharmaceutical industry, the KDI tripeptide is a naturally occurring biological alternative that could herald a new generation of future medications. KDI is a

small, biologically active molecule, which not only produces laminin-like and growth factor-type effects on neurons, but can additionally inhibit the function of ionotropic glutamate receptors. Its efficacy is probably based on this multitude of supplementary effects. The advantage of the KDI tripeptide over designed drugs is that it occurs naturally in our CNS and, therefore, increasing its concentration in the event of injury or neurological disease is unlikely to produce harmful side effects.

'The logic behind the KDI application is to keep the existing neurons alive and protect their existing neuronal projections'

For example, the use of sole glutamate receptor inhibitors in the treatment of neurodegenerative disorders has been ruled out due to the fact that when high concentrations of these inhibitors, such as MK-801, are applied they result in catalepsy, even in rats, whereas the injection of a very high concentration of the KDI tripeptide into the CNS of rats does not produce any harmful effects and they remain fully alert, well and show perfectly normal behavior [Liesi P, Unpublished Data]. Thus, apart from possessing the beneficial effects of glutamate receptor inhibitors against neuronal death, the KDI tripeptide does not have the usual negative side effects of such inhibitors but produces highly tolerable beneficial effects, presumably owing to its laminin-like, growth-promoting functions.

The advantage of the KDI tripeptide over the stem cell concept is that application of a small synthetic tripeptide is a far more simple and workable option than isolating stem cells, growing them, transporting them back into an individual and expecting them to replace the missing neurons or to support the growth of injured neuronal pathways. Significantly, one of the reasons why stem cells, particular the olfactory epithelial stem cells, may work could be the fact that they would produce KDI tripeptide, which acts as a neuronal survival and neurite outgrowth-promoting factor. Instead of transplanting stem cells to make new neurons that should eventually build new neuronal pathways, the logic behind the KDI application is to keep the existing neurons alive and protect their existing neuronal projections. Only in this way do we have a chance of maintaining viable neurons with functional projections.

KDI offers the first treatment to increase survival of dopaminergic neurons that die in Parkinson's disease

Most recently, the great potential of the KDI tripeptide in promoting the survival of CNS neurons has been verified using a well-established animal model of Parkinson's disease [30]. In this animal model, the KDI tripeptide was the first ever compound to be used that protected the dopaminergic neurons of the rat substantia nigra (SN) against death induced by high doses of a dopamine analog, 6-hydroxydopamine (OHDA) [30]. A single, unilateral injection of 6-OHDA into the rat SN destroys almost all dopaminergic neurons, resulting in complete depletion of dopamine in the striatum, which leads to a Parkinson's-like disease condition in rats [31]. However, a single injection of the KDI tripeptide prior to 6-OHDA protected the dopaminergic neurons from death and neutralized the neurotoxic effect of 6-OHDA to such an extent that over 30% of the dopaminergic neurons of the injected SN remained viable [30]. If this much neuronal protection were to occur in humans with Parkinson's disease, they would be able to live their lives asymptotically, as it is known that clinical symptoms of Parkinson's disease are not manifested until less than 30% of neurons are functional [32,33].

KDI in the near future: working towards clinical testing

Currently, laboratory research on KDI focuses on further testing its potential as a treatment in animal models of neurological diseases, such as ALS and Parkinson's disease, and in CNS trauma situations, such as stroke. An important line of research involves confirming its effective concentrations, establishing the best methods of application in different trauma and neurological diseases and isolating the neuronal receptor for KDI. These lines of research will help to design more targeted treatments for forthcoming human applications.

The future for KDI also involves a systematic effort in obtaining approval for human testing as soon as possible. The required steps include obtaining the appropriate permits and collaborating with those clinicians who are in a position to apply KDI into the cerebrospinal fluid of human volunteers with either ALS or acute SCI. However, progress towards human clinical tests depends on appropriate financial support to enable completion of the laboratory work required for the permit application and for the production of sufficient human-grade, sterility-controlled KDI tripeptide to administer to human volunteers.

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