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Research Article

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Intrathecal injection and *in vivo* gene delivery: A probable therapy to reduce the secondary damage caused by acute spinal cord injury

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ABSTRACT

Spinal cord injury is a kind of serious disease mostly caused by injury. It will lead to function deficit of limbs and even do harm to life some time. Now, little measures are effective except early stage administration of great dosage of methylprednisolone and decompression surgery. But in vein usage of the drug may cause many side effects and operation can only remove the mass that compress the dura or spinal cord. We need some more effective way. Through gene delivery technique, continuous expression of functional protein, such as neuro-protective factor at local site may be possible. We postulate intrathecal injection of certain carrier with target gene, which can reduce secondary damage and prevent spinal cord from further attack. It may be become a novel therapy for acute spinal cord injury in the near future.

Key words: Spinal cord injury; Intrathecal injection; Secondary damage

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INTRODUCTION

The result of spinal cord injury may be decided by instant condition of the trauma. For poor reproductive ability of central nerve cell compared to peripheral nerves, and the inhospitable milieu of injured spinal cord, which does not support survival of neural cells, we still have little method to treat the disease. Although spinal cord injury (SCI) causes loss of neurons and glial cells at the lesion site, functional deficits result primarily from loss of descending and ascending axons in the spinal cord by direct trauma and progressive damage to initially intact axons by complex secondary injury mechanisms[1,2]. Extensive studies have been made in animal models to understand the pathophysiological mechanisms of tissue damage and the consequent disability [3,4]. The cascade of secondary degenerative events constitutes a range of therapeutic targets which, if successfully treated, could significantly ameliorate functional loss after traumatic Spinal Cord Injury (SCI). In early hours after injury, the level of neurotrophic factor was down-regulated. In cell level, activiation of neurotrophic factor and inhibition of apoptostic gene may have theraprutic effect[5]. Neuroprotective repair strategies aimed at reducing secondary injury to descending axonal pathways surrounding the lesion cavity may significantly improve functional recovery. Such factor like glial cell line-derived neurotrophic factor (GDNF) [6-10], transforming growth factor-β1(TGF-β1)[11-15], and etc[16] has potential neuroprotective and neurotrophic effects on several neural cell types in the central nervous systems (CNS). And according to former researches, continuously secreting such neurotrophic factor maybe one of the reasons of peripheral nerve regeneration. Therefore, applying neurotrophic factor maybe one of the possible strategies of gene therapy for spinal cord injury (SCI)[17]. Although the administration of exogenous neurotrophic proteins[18] or other medicine[19,20] has therapeutic potential, the limitations imposed by short serum half life period, large molecular weight, high cost and the blood brain barrier (BBB) could restrict the clinical utility of this approach. During the past few years, significant progress has been made in the development of techniques for transfecting genes into spinal cord[21,22] and exploring their potential to

treat SCI[23].

Hypothesis

A powerful approach to determining the functional role of specific genes in neural processes is to over- express their translation products in vivo. To date, this has mainly been achieved by using transgenic animals. But there are several drawbacks to transgenic systems, which obscuring the functional role of the over expressed gene product [24]. Gene transfer allows both systemic and localized expression. Its potential in the central nervous system (CNS) has been appreciated for some time, not only as an investigational tool in experimental animals but a possible novel therapeutic principle [24-27] as well. Though ex vivo cell culture and gene transfer is effective in experimental research, in vivo application is undoubtedly has its special clinic advantage. Under such technique of intrathecal injection of certain carrier with target gene, can we postulate local, long term, continuous expression of neuroprotective factor is possible which will alleviate the symptoms and limb function of those SCI patients.

RESULTS AND DISCUSSION

One of the factors affect regeneration of central nerve is the microenvironment of spinal cord [4]. After primary injury, local damage and inflammation initializes the "waterfall" secondary change, which causes cell degeneration, co liquation and death [1]. At that time, lack of neurotropic factor may inhibit regeneration of neuron and glia cell. The mechanism is that such factor like TGF-beta, GDNF can regulate inflammation reaction and inhibit the cell infarct. So local use of these factor will improve limbs function after spinal cord injury.

To reconstruct its microenvironment, some people tend to infuse above cell factor in vein. Besides its high cost, great dosage will also cause some side effects. So we turn to gene therapy, which can make local expression of functional protein possible. Accompanied with in vein administration of methylprednisolone, the therapy can not only reduce the secondary damage but accelerate cell regeneration as well.

Commonly employed approaches for CNS gene transfer have utilized modified viral vectors as carriers of genetic material, and have yielded promising results in functional studies both using herpes simplex [24,25] and adeno-associated [26] vectors. Immune responses to viral structures, and the potential for transactivation of onco-genes, may, however, limit the utility of viral mediated gene transfer [27]. Developing a safe and efficient non-viral method for delivery and expression of target genes in the adult rat spinal cord would therefore greatly enhance the appeal of gene transfer as an investigational tool in functional studies. Some physical methods may efficient but is rarely used in vivo[28]. Complex-binding of plasmid DNA to cationic lipids is rapidly becoming a standard method for mediation of cellular entry and expression of plasmid DNA [29-33], and has shown some promise for in vivo use outside of the CNS [34-42]. Within the CNS, few attempts have been published, and these have yielded mixed results [43-51]. We hypotheses, an expression construct encoding Lac Z cDNA [52] could be delivered into the spinal cord of adult rats by cation transfection agent. This led to cellular uptake of the complexes, and presence of transgene mRNA for enough long period (more than 2 weeks). By optimizing delivery and expression strategies, detectable amounts of functional protein can be expressed following lipid mediated gene transfer in the CNS [53], thus the protein can work at local damaged site where they may play their positive role in greatest extent. The expression vector(Fig.1) and primer(Fig.2) can be seen as follows:

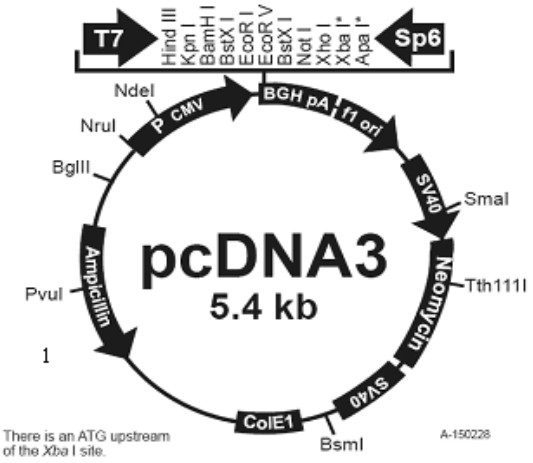

Fig.1 Atlas of eukaryotic expression vector: pcDNA3

GENE	3'PRIME	5'PRIME	SIZE
GDNF	GGA TTT TAT TCA AGC CAC CAT	TCA GAT ACA TCC ACA CCG TTT AG	400
β-actin	AAC GAG CGG TTC CGA TGC CCT GAG	TGT CGC CTT CAC CGT TCC AGT T	600

Fig.2 Primer design of PCR

In actual application, most patients with canal entrapment may need decompression surgery. By intrathecal injection technique, can we put certain carrier with related factor into injured site directly during operation which will not add extra trauma onto patient. Local expression of target gene will just work at that site. While in vein use of such factors can not penetrate the blood brain barrier (BBB) for its great molecular quality. Besides, its short half life period prevents it from taking effect for a long time.

CONCLUSION

Intrathecal injection of certain carrier with target gene can reduce secondary damage and prevent spinal cord from further attack. It may be become a novel complementary therapy for acute spinal cord injury in the near future besides surgical operation.

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