Iranian Journal of Pharmaceutical Sciences
Winter 2008: 4(1): 11-16
www.ijps.ir

Original Article

# Effects of Biodegradable Polymers on the Rat's Damaged Spinal Cord Neural Membranes

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**Abstract**

The overall goal of this study was to identify the appropriate biomaterials able to facilitate the regeneration in rat's injured adult spinal cord. Acute damage to axons is manifested as a breach in their membranes, ionexchange distortion across the compromised region, local depolarization and even conduction block. It would be of particular importance to interrupt the progress of events happening after acute injury to the spinal cord. Repair strategies using transplants of cells have shown many attractions in spinal cord repair studies. However, as the processes of purification and growth require time, they cannot be fully implemented in acute injuries. Furthermore, application of cell grafts from other human or animal sources are likely to provoke immune reactions in human patients. Immediate repairing or sealing the regions of compromised membrane with hydrophilic polymers or surfactants could retard or reverse the permeabilization of nerve fiber membranes. Biodegradable polymeric materials used for tissue engineering, made of either peptide or non-peptide components, are considered as potential candidates. Application of the hydrophilic polymer, polyethylene glycol (PEG), has showed to be effective in the repair of nerve membrane damage associated with severe spinal cord injury in adult rat. This class of large hydrophilic molecules can reverse the permeabilization of ruptured cell membranes as well as anatomically reconnect their severed processes. Our preliminary results showed rather rapid partial restoration of the declined magnitude of compound actions potentials (CAPs) in injured spinal cords, varying as a result of the introduction of PEG's with different molecular weights.

*Keywords:* Biodegredation; Neural membrane; Polymer; Spinal cord.
*Received:* December 4, 2007; *Accepted:* January 10, 2008

## 1. Introduction

Damage to neural membrane as a result of mechanical damage induces a series of events that result in tissue disruptions and subsequent long-term disabilities. Destruction of membrane leads to the failure in maintaining ionic differences (mainly $Ca^{2+}$ and $K^{+}$) between intracellular and extracellular medium [1-8]. These changes produce both oxidative stress [9] and blockage of the

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transmission of compound action potential (CAP) [7, 8]. Following the spinal cord injury, because of risen intracellular $Ca^{2+}$ the production of reactive oxygen species (ROS) increases and leads to tissue damage via several different cellular and molecular pathways [10]. ROS may damage components forming molecules such as lipid peroxide [11]. Cells normally have a number of mechanisms to defend against damages induced by ROS [12]. However, problems occur when production of free radicals exceeds the elimination power of antioxidant protective system [10], the process that initiates secondary phase of injury [9, 13, 14].

Disruption of the membrane and myelin sheath at the site of injury disturbs the activity of $K^+$ channels. These channels provide a pathway for the exodus of $K^+$ ions that quenches the nerve impulse when it reaches the damaged site [15-17].

There are some defined approaches to inhibit the progressive destruction of nervous system caused by ROS including protection of nerve tissues against free radicals by application of free radical scavenger, and blockage of the receptors, to control the liberation of endogenous toxin from damaged cells [3, 6, 18]. Another approach is to repair damaged neural membrane immediately after injury [19, 20].

Immediate recovery from spinal cord injury and inhibition of free radicals production can be accomplished partially by polyethylene glycol (PEG) molecules [21]. PEGs also mediate in the recovery of CAP conducted through the damaged section of spinal cord, within minutes [21]. Thus, PEGs are capable to reseal damaged neural membrane rapidly, leading to both inhibition of ROS production and recovery of nerve impulse [21, 22].

The electrophysiological conduction across damaged site of spinal cord has been investigated *in vitro* and *in vivo*. In an *in vitro* study, CAP traveling across strips of injured spinal cord, reappeared rapidly in response to PEG introduced following the compression of the spinal cord [23]. *In vitro* studies of damaged neural membrane repair by PEGs has been studied by means of the following three approaches through application of biodegradable polymers to recover the amplitude of CAP. In the first approach, the possible immediate molecular repair of nerve membranes in injured spinal cord was evaluated using polyethylene glycol [22]. The second investigation dealt with the analysis of recovery of spinal cord injury studied 6-8 h after application of PEG [24] and finally as the third mean, the effects of subcutaneous injection of the PEG polymers after ~6 h post injury has been evaluated [25]. The recovery of CAP has been monitored at different time scales (min. to days), as a result

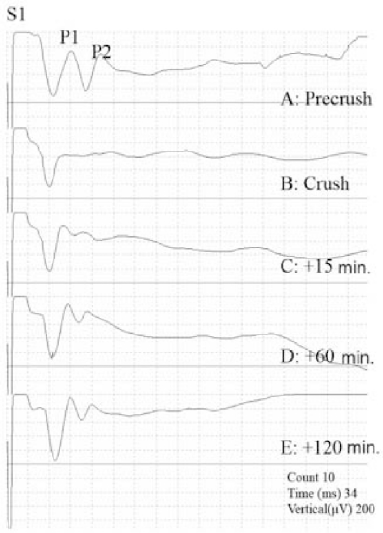


**Figure 1.** Recovery of amplitude of CAP traces in PEG-treated animals with injured spinal cord. PEG 600Da was introduced to the site of the injury. SA = stimulus artifact; P1= first arriving SCEP; P2= late arriving potentials. SCEP signals recorded in uninjured rats (A) were declined following crushing the spinal cord (B) and the effects of the introduced PEG were recorded 15 min. (C), 60 min. (D) and 120 min. (E) after removal of PEGs.

of PEG polymers application.

Here, we applied aqueous solution of PEG to recover CAP amplitude decline caused by intentional compression as a model of spinal cord injury. Aqueous solution of PEGs (400, 600 and 1000 Da, 50% by weight in distilled water) was introduced for 2 min. into injured site in experimental animals and different neural responses were detected.

## 2. Materials and methods

### *2.1. Spinal cord evoked potential (SCEP) recording*

Spinal cord evoked potential (SCEP) technique was applied to measure the amplitude of CAP conducted along the intact and injured spinal cord. Stimulating at T1-2, the resulted CAP was recorded at T10 to L1.

### *2.2. Animal preparation*

Adult rats (250-300g) treated as two separate experimental groups (PEG treated group and control group), were anesthetized with an intramuscular injection of 90 mg/kg ketamine HCl and 10 mg/kg xylazine. Keeping the animal fixed on the stage, its spinal cord was exposed by dorsal laminectomy.

### *2.3. Spinal cord injury (SCI) formation*

Subsequently, a standardized 30s compression of the spinal cord was performed using a Titanium aneurysm clips (closing force: 1.16N, blade length: 5 mm, maximum opening 4mm) that produced an immediate and total loss of CAP conduction through the injured site.

### *2.4. PEG application*

Within 10 min. of spinal cord compression, an aqueous solutions of PEG polymer (400, 600 or 1000 Da, 50% by weight in distilled water) was introduced by pipette to the exposed damaged site for 2 min. in PEG treated animals and then removed by aspiration. The site of PEG application was immediately lavaged with isotonic Krebs' solution (NaCl 124 mM, KCl 2 mM, $KH_2PO_4$

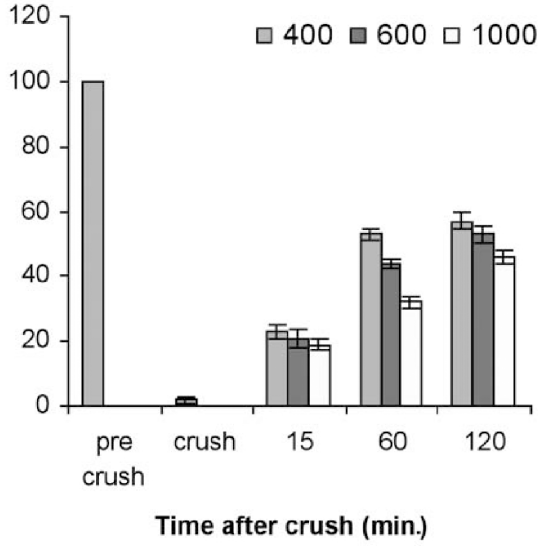


**Figure 2.** The effects of PEG 400, 600 and 1000 Da, on the recovery of the amplitude of early arriving peak (P1) in SCEP signals recorded from injured spinal cords of 8 rats. The mean and standard error of amplitude of P1 are shown for each treatment and compared with that of recorded at pre crush phase.

1.24 mM, $CaCl_2$ 1.2 mM, $MgSO_4$ 1.3 mM, $NaHCO_3$ 26 mM, dextrose 10 mM, sodium ascorbate 10 mM) and the excess of PEG and Krebs' solution were removed by aspiration. Control animals were just treated with isotonic Krebs solution, which was subsequently removed by aspiration. The amplitude of CAP within; 5, 15, 60 and 120 min. of application were recorded.

## 3. Results

SCEP conduction prior to spinal cord injury and within 10 min. after injury was recorded and analyzed. The effects of PEG polymer with three different molecular weights on SCEP conduction were recorded and the results were compared with that of shams that have only been treated with Krebs solution. In uninjured animals two peaks of SCEP signals were observed from which the first one was observed in all animals though the second pick was not that common.

Following standardized compression of spinal cord, an immediate lack of SCEP conduction through the injury site was observed in all animals. Control group did not show any recovery in SCEP conduction after 15, 60, and 120 min. On the other hand, a significant partial recovery was monitored in all PEG-treated injured animals (Figure 1).

After PEG application with molecular weigh of 400 Da in 8 injured animals, the corresponding SCEP was recorded within 15, 60 and 120 min. The recorded SCEP amplitude in all treated animals showed a SCEP amplitude recovery of about 23%, 53%, and 57% after 15, 60, and 120 min., respectively. The corresponding recovery following the application of PEG 600Da was 21%, 44%, and 53%, respectively. After PEG application with molecular weigh of 1000 Da, the least recovery in the amplitude of SCEP was monitored and showed to be about 19%, 32%, and 46%, respectively (Figure 2).

The effects of PEGs with different sizes (weights) were different at various recording time. Although, the amplitude of SCEP had been raised by about 20% after 15 min., due to the application of all three PEGs, irrespective of their sizes, there were no significant changes between them.

Within 120 min. after application of PEG with different molecular weight, the effects of three different size PEGs on the recovery of SCEP amplitude were recorded and compared with each other. There was no significant difference (in level of $p \leq 0.05$) detected in the responses to PEGs with molecular weights of 400 and 600 Da ($p$=0.070). On the other hand, the effects of PEG 400 and PEG 600 were significantly different from that of PEG 1000 (Figure 2). We detected a significant difference of about 11% ($p$=0.0025), between PEG 400 and PEG1000 and about 7% ($p$=0.012) between PEGs with MW of 600 and 1000 Da.

It was obvious that the main difference amongst PEGs treated cases appeared after 6 min. Further, after 60 min., the effect of PEG 400 did not change significantly, though it showed a linear ascending trend for PEG 600 and PEG1000.

## 4. Discussion

It is known that mechanical damage to neural membrane leads to local failure of the ability of the membrane in maintaining ionic differences across it. Evoked potential signal is blocked at the site of damaged neural membrane because of unregulated ionic differences (principally $K^+$, and $Ca^{2+}$) [1, 8] Damage to neural membrane is compromised by the local increase in the levels of $Ca^{2+}$ in the cytosol of the cells at the site of injury, leading to activation of a wide range of intracellular, including, $Ca^{2+}$-dependent catabolic enzyme activities, production of ROS, and eventual cellular damage [18]. One approach to minimize oxidative stress of ROS is to increase scavenging ROS by antioxidants

[26, 27]. This approach has been shown to be largely ineffective in spinal cord injury repair [26]. Since most of the ROS have a very short half-life scavengers can not eliminate them before the damage is done. Thus it is more effective to prevent ROS formation than lowering its level in cell. Therefore, it has been suggested to directly interfere with the generation of ROS by rapid repairing of damaged neural membrane [9].

Hydrophilic polymers such as PEGs and related compounds such as non-ionic detergents (poloxamer and poloxamin) have shown the ability to reseal damaged neural membranes [22, 28-33]. Non-ionic detergents such as copolymers that can form micellar films can also cover the breach formed in the damaged membrane [26, 31]. The hydrophobic tail of these molecules may insert to the hydrophobic core of exposed plasmalemma due to the injury and the hydrophilic heads integrate into the outer leaflet of plasmalemma [31]. PEGs may also repair damaged neural membrane via acute dehydration of the damaged region. Dehydration of plasmalemma leads to the reduction of the polar forces arising from proteins, glycoprotein and lipids, leading to proper accommodation of these molecules in membrane. When PEG is removed and the membrane is rehydrated, the aqueous phase helps membrane to maintain its organization. Therefore, PEGs restore the anatomic integrity of the damaged neural membranes and cause immediate recovery of nerve impulse conduction through the lesion site [18]. In general, hydrophilic polymers have the ability to reseal permeabilized damaged cells, ceasing them from progressive destruction, degeneration and death.

Here, we have shown that PEG solutions with lower molecular weight are more effective in immediate recovery of SCEP amplitude. It seems logical that PEGs with lower molecular weight penetrate into the injured site of cells quicker than PEGs with higher molecular weight. Hence PEGs with molecular weight of 400 Da can be more effective in short term dehydration of closely oriented approximate membranes, allowing their structural components to be rearranged properly. Immediate sealing function of PEGs with lower molecular weight implies that they would be more effective in repair of damaged neural membranes required at the early stage of the accident. However, one should not ignore the more efficient effects of PEGs with high molecular weights of 600 and 1000 Da that showed the ability of reestablishing the declined amplitude of the damaged spinal cord to higher mplitudes. Consequently, it is wise to think of a rather complex treatment using PEGs of different molecular weights at different stages. The current data appeared in this article are based on our preliminary data, however, our complementary experiments are on the way and the effects of molecular weight, concentration, timing and length of PEGs incubation, and the application means are of the main points that are being further studied.

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