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Original research

Local administration of prostaglandin E1 combined with silicone chamber improves peripheral nerve regeneration

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ABSTRACT

The aim of this study was to assess the effect of locally administered prostaglandin E1 on peripheral nerve regeneration and functional recovery. Sixty male healthy white Wistar rats were divided into four experimental groups ($n = 15$), randomly: In transected group (TC), left sciatic nerve was transected and stumps were fixed in the adjacent muscle. In treatment group defect was bridged using silicone graft (SIL/PE) filled with 10 μL prostaglandin E1. In silicone graft group (SIL), the graft was filled with phosphate-buffered saline alone. In sham-operated group (SHAM), sciatic nerve was exposed and manipulated. Each group was subdivided into three subgroups of five animals each and regenerated nerve fibers were studied 4, 8 and 12 weeks after surgery. Behavioral testing, sciatic nerve functional study, gastrocnemius muscle mass and morphometric indices confirmed faster recovery of regenerated axons in SIL/PE than SIL group ($p < 0.05$). In immunohistochemistry, location of reactions to S-100 in SIL/PE was clearly more positive than that in SIL group. When loaded in a silicone graft, prostaglandin E1 improved functional recovery and morphometric indices of sciatic nerve. Local application of prostaglandin E1 improved functional recovery and morphometric indices of sciatic nerve.

1. Introduction

Where there is considerable nerve tissue loss, peripheral nerve regeneration is still a concern in regenerative medicine.[1] When an axon is crushed or severed, changes occur on both sides of the lesion.[2] Where a gap is present between the severed ends of the nerve, proliferating Schwann cells emerge from the stumps (mainly the distal stump) and form a series of nucleated cellular cords (the bands of Bungner) which bridge the interval.[3]

Following nerve transection Wallerian degeneration occurs which is a sequential pattern of axonal degeneration, myelin degradation and supporting glial cell proliferation. During this process, various events take place, including blood-nerve barrier dysfunction, endoneural space reorganization, and most importantly the induction of an intense inflammatory response, constituted by inflammatory mediator release and production.[4,5]

Axonal degeneration recruits this response activating Schwann cells and macrophages that proliferate and activate, clearing myelin debris and producing cytokines that perpetuate an inflammatory state. Axonal regeneration is then regulated by the interactions between all the involved cell types and by cytokines, chemokines, growth factors, and other inflammatory mediators.[5] All these events culminate in the promotion of an environment suitable for subsequent regeneration, repair, and axon regrowth. Arachidonic acid and its metabolites are known to modulate neuronal function and survival. There is also evidence that arachidonic acid derivatives, such as prostaglandins, are centrally involved in Wallerian degeneration and in axonal regeneration.[5]

Regarding the association that exists between arachidonic acid derivatives and nerve degeneration and regeneration, the therapeutic modulation of this pathway emerges as a novel strategy aimed at increased motor, sensory, and structural recovery after nerve injury.[6]

Aimed to study local effects of prostaglandin E1(PGE 1) on peripheral nerve regeneration, the present study was designed to attempt to determine if topical PGE 1 do in fact reduce dysfunction after small gap nerve transection injury in the rat sciatic nerve transection model. Assessment of nerve regeneration was based on behavioral, functional (walking track analysis), histomorphometrical and immunohistochemical (Schwann cell detection by S100 expression) assessment at 4, 8, and 12 weeks after surgery.

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2. Materials and methods

2.1. Study design and animals

Sixty male Wistar rats weighing approximately 290 g were divided into four experimental groups ($n = 20$), randomly: sham-operation group as normal control (SHAM), transected control (TC), silicone graft (SIL) and prostaglandin E1 treated group (SIL/PE). Each group was further subdivided into three subgroups of five animals each and surveyed 4, 8 and 12 weeks after surgery. Two weeks before and during the experiments, the animals were housed in individual plastic cages with an ambient temperature of (23 ± 3) °C, stable air humidity and a natural day/night cycle. The rats had free access to standard rodent laboratory food and tap water. All measurements were made by two blinded observers unaware of the analyzed groups.

2.2. Surgical procedure

Animals were anesthetized by intraperitoneal administration of ketamine-xylazine (ketamine 5%, 90 mg/kg and xylazine 2%, 5 mg/kg). The procedure was carried out based on the guidelines of the Ethics Committee of the International Association for the Study of Pain.[7] The University Research Council approved all experiments.

Following surgical preparation in the sham-operation group, the left sciatic nerve was exposed through a gluteal muscle incision and after careful homeostasis the muscle was sutured with resorbable 4/0 sutures, and the skin with 3/0 nylon. In TC group, the left sciatic nerve was transected proximal to the tibio-peroneal bifurcation where a 7 mm segment was excised, leaving a 10 mm gap due to retraction of nerve ends. Proximal and distal stumps were fixed in the adjacent muscle with 10/0 nylon epineurial suture. No graft was interposed between the stumps. In the SIL group, a 7 mm nerve segment was resected to produce a 10 mm nerve gap after retraction of the nerve transected ends. The gap was bridged using a silicone graft (Polyerubb Industries, Ahmedabad − 380023, Gujarat, India), entubulating 2 mm of the nerve stump at each end. The graft was 14 mm in length, 2 mm in inner diameter and 2 mm in thickness. A subtle retraction of 1 mm was already expected. Two 10/0 nylon sutures were used to anchor the graft to the epineurium at each end. In prostaglandin E1 treated group (SIL/PE) the graft was filled with 10 μl prostaglandin E1 (100 ng/mL) (Sigma–Aldrich Chemie GmbH, Steinheim, Germany). The animals were anesthetized and euthanized with transcardiac perfusion of a fixative containing 2% paraformaldehyde and 1%glutaraldehyde buffer (pH 7.4) 4, 8 and 12 weeks after surgery.

2.3. Behavioral testing

Functional recovery of the nerve was assessed using the Basso, Beattie, and Bresnahan (BBB) locomotor rating scale for rat hind limb motor function.[8] Although BBB is widely used to assess functional recovery in spinal cord injured animals, however, it has been demonstrated that it could be most useful in assessment of never repair processes in peripheral nerve injuries.[9] Scores of 0 and 21 were given when there were no spontaneous movement and normal movement, respectively. A score of 14 shows full weight support and complete limbs coordination.[8,9] BBB recordings were performed by a trained observer who was blinded to the experimental design. The animals were observed and assessed within a course of a 4-min exposure to an open area of a mental circular enclosure. BBB scores were recorded once before surgery in order to establish a baseline control and again weekly thereafter to assess functional recovery during 12 weeks.

2.4. Functional assessment of reinnervation

2.4.1. Sciatic functional index (SFI)

Walking track analysis was performed 4, 8 and 12 weeks after surgery based on the method of others.[10] The lengths of the third toe to its heel (PL), the first to the fifth toe (TS), and the second toe to the fourth toe (IT) were measured on the experimental side (E) and the contralateral normal side (N) in each rat. The sciatic function index (SFI) of each animal was calculated by the following formula:

$$\mathrm{SFI} = -38.3 \times (\mathrm{EPL} - \mathrm{NPL})/\mathrm{NPL} + 109.5 \times (\mathrm{ETS} - \mathrm{NTS})/\mathrm{NTS} + 13.3 \times (\mathrm{EIT} - \mathrm{NIT})/\mathrm{NIT} - 8.8$$

In general, SFI oscillates around 0 for normal nerve function, whereas around −100 SFI represents total dysfunction. SFI was assessed in the NC group and the normal level was considered as 0. SFI was a negative value and a higher SFI meant the better function of the sciatic nerve.

2.4.2. Static sciatic index (SSI)

SSI is a time-saving digitized static footprint analysis described by others.[11] A good correlation between the traditional SFI and the newly developed static sciatic index (SSI) and static toe spread factor (TSF), respectively, has been reported by others.[11] The SSI is a time-saving and easy technique for accurate functional assessment of peripheral nerve regeneration in rats and is calculated using the static factors, not considering the print length factor (PL), according to the equation:

$$\mathrm{SSI} = [(108.44 \times \mathrm{TSF}) + (31.85 \times \mathrm{ITSF})] - 5.49$$

where:

$$\mathrm{TSF} = (\mathrm{ETS}-\mathrm{NTS})/\mathrm{NTS}$$
$$\mathrm{ITSF} = (\mathrm{EIT}-\mathrm{NIT})/\mathrm{NIT}$$

Like SFI, an index score of 0 was considered normal and an index of −100 indicated total impairment. When no footprints were measurable, the index score of −100 was given.[31]

2.5. Muscle mass

Recovery assessment was also indexed using the weight ratio of the gastrocnemius muscles 12 weeks after surgery. Immediately after sacrificing of animals, gastrocnemius muscles were dissected and harvested carefully from intact and injured sides and weighed while still wet, using an electronic balance.

2.6. Histological preparation and morphometric studies

Nerve mid-substance in SIL group, nerve mid-substance in prostaglandin E1 treated group, midpoint of normal sciatic nerve (SHAM) and regenerated mid substance of TC group were harvested and fixed with glutaraldehyde 2.5%. They were post fixed in OsO4 (2%, 2 h), dehydrated through an ethanol series and embedded in Epon. The nerves were cut in 5 μm in the middle, stained with toluidine blue and examined under light microscopy. Morphometric analysis was carried out using an image analyzing software (Image-Pro Express, version 6.0.0.319, Media Cybernetics, Silver Springs, MD, USA). Equal opportunity, systematic random sampling and two-dimensional dissector rules were followed in order to cope with sampling-related, fiber-location-related and fiber-size related biases.[12]

2.7. Immunohistochemical analysis

In this study, anti-S-100 (1:200, DAKO) was used as marker for myelin sheath. Specimens prior to immunohistochemistry were post fixed with 4% paraformaldehyde for 2 h and embedded in paraffin. Then the nerve sections were dewaxed and rehydrated in PBS (pH = 7.4). They were incubated by 0.6% hydrogen peroxide for half an hour to neutralize endogenous peroxide. After that the sections were incubated with normal swine serum (1: 50, DAKO, Germany) for blocking of non-specific immunoreactions and then were incubated in S-100 protein antibody solution for 1 h at room temperature. They were washed three times with PBS and incubated in biotynilated anti-mouse rabbit IgG solution for 1 h. Horseradish peroxidase-labeled secondary antibody (1:100 swine anti-rabbit diluted in 5% normal rat serum) was applied for 1 h. All sections were then incubated with diaminobenzidine tetrahydrochloride chromogen (DAB, DAKO) substrate solution for 10 min. The sections were mounted in corbit balsam with coverslip. The results of immunohistochemistry were examined under a light microscope.

2.8. Statistical analysis

The results were expressed as means ± SD. Statistical analyses were performed using PASW 18.0 (SPSS Inc., ChicSILo, IL, USA). Model assumptions were evaluated by examining the residual plot. Results were analyzed using a factorial ANOVA with two between-subjects factors. Bonferroni test for pairwise comparisons was used to examine the effect of time and treatments. The differences were set at $P < 0.05$.

3. Results

3.1. BBB recovery

In order to assess hind limb recovery the open field locomotor was used. Fig. 1 shows BBB scores compared to the baseline. All experimental groups, except for sham, showed the greatest degree of functional deficit one week after surgery. The prostaglandin E1 treated group showed significant improvement in locomotion of the operated limb compared to the SIL group during the study period ($P < 0.05$).