Effects of local administration of ibuprofen on sciatic nerve regeneration and reinnervation after egg shell membrane conduit repair in rat

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ABSTRACT

BACKGROUND: The objective of this study was to assess the effect of locally administered ibuprofen (IBU) on transected peripheral nerve regeneration and functional recovery.

METHODS: Seventy-five male Wistar rats were divided into five experimental groups (N=15), randomly: In autograft group (AUTO) a segment of sciatic nerve was transected and reimplanted reversely. In transected group (TC), left sciatic nerve was transected and stumps were fixed in the adjacent muscle. In treatment group defect was bridged using an egg shell membrane conduit (ESM/IBU) filled with 10 µL ibuprofen (100 ng/mL). In ESM conduit group (ESM), the conduit 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.

RESULTS: Behavioral testing, biomechanical studies, sciatic nerve functional study, electrophysiological, gastrocnemius muscle mass and morphometric indices confirmed faster recovery of regenerated axons in ESM/IBU than ESM group (P<0.05). In immunohistochemistry, location of reactions to S-100 in ESM/IBU was clearly more positive than that in ESM group.

CONCLUSION: Ibuprofen accelerated and improved functional recovery and morphometric indices of sciatic nerve. This study is expected to set a stage for testing the ibuprofen in the human patients.

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Regeneration of the damaged peripheral nerve depends on the microsurgical procedure performed. Currently, there are several operating techniques used to repair injured nerves such as direct epineural repair, grouped fascicular repair, fascicular repair, and nerve grafting. The results following nerve repairs are influenced by many parameters, such as the nature, location, and extent of the injury, the level and timing of the repair, the fascicular anatomy, and appropriateness of re-alignment of the injured nerve, and the surgical technique, as well as patient factors.1, 2

In addition to these factors in the regeneration of nerve repair, some pharmaceutical agents which are used locally at the site of nerve repair also have an effect. Several studies have shown that the most frequently applied topical substances are tacrolimus (FK506), hyaluronic acid and its derivatives, melatonin, and methylprednisolone. These substances contribute to fibroblast proliferation suppression at the site of the peripheral nerve repair thus reducing scar formation in the injured peripheral nerve.3

Experimental studies and clinical reports indicate that
insertion of a conduit could be an interesting alternative to direct end-to-end suturing of nerve stumps or interposition of an autograft.4-6 The conduits act to guide axons growing from the regenerating nerve stump, provide a microenvironment for dissemination of neurotrophic and neurotropic factors secreted by the injured nerve end, and prevent infiltration of fibrous tissue.7

In a study, chicken eggshell membrane (ESM) is shown to be a suitable material to be utilized in nerve regeneration.8 The ESM is a resorbable biomaterial for implant applications, a secondary surgical site is not needed to obtain ESM and there is no donor site morbidity. In addition, ESM can be obtained in large quantities, being inexpensive, sterilized with ethylene oxide and eAESMy stored.9, 10 Another advantage of using the ESM as a nerve guide conduit is that the degradation speed of this biological material can be controlled by manipulating the thickness of the tube.11

The exact physiological and molecular signals involved in inducing the regenerative process are largely unknown. Induction of transcription factors, adhesion molecules, growth associated proteins and structural components are required for axonal elongation and intracellular signaling molecules that control cell cycle and differentiation. These seem to play a key role in nerve regeneration process.12

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a group of drugs widely used as cyclooxygenase selective and nonselective inhibitors. Ibuprofen has recently been found to reduce the formation of amyloid-42, a critical peptide leading to pathogenic cascade in Alzheimer’s disease, via RhoA inactivation. Rho is a member of the Ras superfamily of small GTP-binding proteins that play a central role in diverse biological processes such as Actin cytoskeleton organization, microtubule dynamics, gene transcription, oncogenic transformation, cell cycle progression, adhesion and epithelial wound repair.13, 14

Treatment with ibuprofen stimulates the axonal growth in spinal cord below the lesion and significantly increases the fiber number of descending corticospinal and raphespinal tracts, which play a role in locomotor recovery.15, 16 To the best knowledge of the authors, the literature is poor regarding the beneficial local effects of ibuprofen on transected sciatic nerve.

Gastrointestinal effects, ranging from relatively mild dyspepsia to potentially lethal gastrointestinal bleeding and perforated ulcers of chronic administration of NSAIDs are well-known adverse effects of these compounds.17 In order to avoid systemic adverse effect of NSAIDs in the process of nerve repair, the present study was conducted to evaluate possible local effect of ibuprofen on peripheral nerve regeneration in rat sciatic nerve transection model. Assessment of the nerve regeneration was based on behavioral, functional (walking track analysis), electrophysiological, biomechanical, muscular mass measurement, histomorphometric and immunohistochemical (Schwann cell detection by S-100 expression) criteria 4, 8 and 12 weeks after surgery.

Materials and methods

Study design and animals

Seventy-five male Wistar rats weighing approximately 250g were divided into five experimental groups (N.=15), randomly: sham-operation group as normal control (SHAM), autograft group (AUTO), transected control (TC), egg shell membrane conduit (ESM) and ibuprofen treated group (ESM/IBU). Each group was further subdivided into three subgroups of five animals each and studied 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.

Preparation of egg shell membrane conduit

The conduit was prepared based on a method described by others.9 In brief, Fresh raw eggs were washed with water and methanol. The fluid contents were poured out. The remaining calcareous cups were rinsed inside and out with water and then submerged completely in 5% acetic acid for about 8 days. As the decalcification was proceeding, the residual sacs of shell membrane were rinsed daily in tap water and the proteinaceous residue of decalcified shell was gently removed mechanically insofar as possible. The decalcifying membranes were then returned to fresh 5% acetic acid. The procedure was repeated daily during a 6 to 8-day period until the membranes were soft and completely free of brittle
eggshell remnants. The membranes were immersed in phosphate buffered saline of pH 7.4 for a period of 30 min. They were then taken out and rotated over a mandrel manually under sterile conditions to achieve a longitudinal orientation. The formed conduit was removed a washed exhaustively with double distilled water for a period of 3 h and dried at 37°C for 24 hr in a sterile laminar flow hood. The conduits were then individually packed and sterilized with ethylene oxide for 24 hr at room temperature.

**Surgical procedure**

Animals were anesthetized by intraperitoneal administration of ketamine-xylazine (ketamine 5%, 90mg/kg and xylazine 2%, 5mg/kg). The procedure was carried out based on the guidelines of the Ethics Committee of the International Association for the Study of Pain. 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 hemostasis the muscle was sutured with resorbable 4/0 sutures, and the skin with 3/0 nylon. In AUTHO group the transected nerve segments were reimplemented reversely. 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 conduit was interposed between the stumps. In the ESM 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 an ESM conduit, entubulating 2 mm of the nerve stump at each end and filled with 10 μl phosphate-buffered saline. A 14-mm segment of the ESM conduit was prepared. In ibuprofen treated group (ESM/IBU) the conduit was filled with 10 μl ibuprofen (100 ng/mL). Because of local administration, this dose was a reduced dosage of ibuprofen that was used in another study systemically. Because of local administration. 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.

**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. 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. 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. BBB recordings were performed by a trained observer who was blinded to the experimental design. The testing was performed in a serene environment. The animals were observed and assessed within a course of a 4-minute 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 twelve weeks.

**Functional assessment of reinnervation**

**Sciatic Functional Index**

Walking track analysis was performed 4, 8 and 12 weeks after surgery based on the method of others. 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:

\[ SFI = -38.3 \times (EPL - NPL)/NPL + 109.5 \times (ETS - NTS)/NTS + 13.3 \times (EIT - NIT)/NIT - 8.8 \]

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.

**Static Sciatic Index**

Static Sciatic Index (SSI) is a time-saving digitized static footprint analysis described by others. A good