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Ketoprofen combined with artery graft entubulization improves functional recovery of transected peripheral nerves

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ABSTRACT

The objective was to assess the local effect of ketoprofen on sciatic nerve regeneration and functional recovery. Eighty healthy male white Wistar rats were randomized into four experimental groups of 20 animals each: In the transected group (TC), the left sciatic nerve was transected and nerve cut ends were fixed in the adjacent muscle. In the treatment group the defect was bridged using an artery graft (AG/Keto) filled with 10 microliter ketoprofen (0.1 mg/kg). In the artery graft group (AG), the graft was filled with phosphated-buffer saline alone. In the sham-operated group (SHAM), the sciatic nerve was exposed and manipulated. Each group was subdivided into four subgroups of five animals each and regenerated nerve fibres were studied at 4, 8, 12 and 16 weeks post operation. Behavioural testing, sciatic nerve functional study, gastrocnemius muscle mass and morphometric indices showed earlier regeneration of axons in AG/Keto than in AG group ($p < 0.05$). Immunohistochemical study clearly showed more positive location of reactions to S-100 in AG/Keto than in AG group. When loaded in an artery graft, ketoprofen improved functional recovery and morphometric indices of the sciatic nerve. Local usage of this easily accessible therapeutic medicine is cost saving and avoids the problems associated with systemic administration.

1. Introduction

Peripheral nerve regeneration where there is considerable nerve tissue loss is still a concern in regenerative medicine (Elizabeth et al., 2005). Changes occur on both sides of the lesion when an axon is damaged (Zhang et al., 2000). Where a gap occurs between the cut ends of a nerve, proliferating Schwann cells develop from the ends, mostly the distal end, and form sequences of nucleated cellular strands which fill the gap (Malizos et al., 1997).

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 (Quarles, 2002). An artery graft provides laminin and some collagen. These substances can be found in Schwann-cell basal membranes and are known as factors for axonal outgrowth (Itoh et al., 1996; Wang et al., 1993, 1995; Ferrari et al., 1999; Ide et al., 1983; Lander et al., 1985; Valentini et al., 1987).

Laminin, one of the main basal membrane components, encourages neurite outgrowth and induces Schwann-cell mitosis, which plays an important role in peripheral nerve repair (Tohyama and Ide, 1984; Bryan et al., 1993).

Regular Schwann-cell differentiation needs interaction with a connective tissue matrix or some related material such as collagen and nerve fibre contact (Bunge and Bunge, 1978).

The inflammatory process and its mediators have been implicated in the regulation of the axonal regenerative processes after injury (Hirschberg et al., 1994; Richardson and Lu, 1994). Gastrointestinal effects, ranging from relatively slight dyspepsia to possibly lethal gastrointestinal (GI) haemorrhage and perforated GIT ulcers of chronic administration of NSAIDs are well-known adverse effects of these compounds (Bidaut-Russell and Gabriel, 2001).

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This study was conducted to evaluate possible local effect of ketoprofen on peripheral nerve regeneration in a rat model.

2. Materials and methods

2.1. Study design and animals

Eighty male Wistar rats weighing approximately 280 g were divided into four experimental groups ($n = 20$), randomly: sham-operation group as normal control (SHAM), transected control (TC), artery graft (AG) and ketoprofen treated group (AG/Keto). Twenty rats were used as artery graft donors. Each group was further subdivided into four subgroups of five animals each and surveyed 4, 8, 12 and 16 weeks after surgery. Two weeks before and during the experiments, the animals were housed in individual plastic cages with an ambient temperature of $(23 \pm 3)\ ^\circ C$, 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 analysed groups.

2.2. Surgical procedure

Animals were anaesthetized 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 (Zimmermann, 1983). The University Research Council approved all experiments.

Following surgical preparation in the sham-operated group, the left sciatic nerve was exposed through a gluteal muscle incision and after careful haemeostasis the muscle was sutured with resorbable 4/0 sutures, and the skin was closed 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 a 10/0 nylon epineurial suture. No graft was interposed between the stumps. In the AG 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 artery graft, entubulating 2 mm of the nerve stump at each end. The artery graft was harvested from the abdominal aorta of the donor animal. The abdominal aorta artery was exposed through a midline abdominal incision and canulated. Then, a 15 mm segment was harvested on the cannula. After harvesting of the graft the donor animals were sacrificed with overdose of an anaesthetic agent. Harvested grafts were washed in physiological solution and left at room temperature for 40 min. A subtle retraction of 1 mm was already expected. Allografts did not receive preliminary treatment to reduce their antigenicity. Two 10/0 nylon sutures were used to anchor the graft to the epineurium at each end. In ketoprofen treated group (AG/Keto) the graft was filled with 10 μl ketoprofen (0.1 mg/mL). The animals were anaesthetised and euthanized with transcardiac perfusion of a fixative containing 2% paraformaldehyde and 1% glutaraldehyde buffer (pH 7.4) 4, 8, 12 and 16 weeks after surgery.

2.3. Behavioural testing

Functional recovery of the nerve was assessed using the Basso, Beattie, and Bresnahan (BBB) locomotor rating scale for rat hind limb motor function (Basso et al., 1995). Although BBB is widely used to assess functional recovery in spinal cord injured animals it has been demonstrated that it can be very useful in assessment of never repair processes in peripheral nerve injuries (Dinh et al., 2009). 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 limb coordination (Basso et al., 1995; Dinh et al., 2009). 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 over 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 16 weeks.

2.4. Functional assessment of nerve regeneration

Walking track analysis was performed 4, 8, 12, 16 weeks after surgery based on the method of others (Bain et al., 1989). 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.

2.5. Muscle mass

Recovery assessment was also indexed using the weight ratio of the gastrocnemius muscles 16 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 AG group, nerve mid-substance in ketoprofen 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 analysing 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, fibre-location-related and fibre-size related biases (Geuna et al., 2003).

2.7. Immunohistochemical analysis

In this study, anti-S-100 (1:200, DAKO, USA) was used as marker for myelin sheath. Specimens were post fixed with 4% paraformaldehyde for 2 h and embedded in paraffin. Prior to immunohistochemistry nerve sections were dewaxed and rehydrated in PBS (pH 7.4). Then the nerve sections were incubated with 0.6% hydrogen peroxide for 30 min. To block non-specific immunoreactions the sections were incubated with normal swine serum (1:50, DAKO, USA). Sections were then incubated in S-100 protein