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Split-sciatic nerve surgery: A new microsurgical model in experimental nerve repair

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Summary *Introduction:* Sciatic experimental surgery in rat often leads to hindlimb autophagy, with considerable ethical and research issues. In this work, the distal part of the sciatic nerve was split following the natural bifurcation between tibial and peroneal branches, before applying regenerative stem cells in a fibrin conduit on the peroneal segment. The new microsurgical model was tested in terms of animal morbidity and consistency of research outcomes, particularly comparing to the standard total sciatic axotomy procedure.
Materials and Methods: After dissection of sciatic the nerve, the tibial and peroneal fibres were split upwards and a total axotomy was performed in the peroneal side. The 1 cm nerve gap between the proximal sciatic nerve and peroneal nerve was crossed using fibrin conduits. The tibial nerve was not included. Experimental groups involved either empty or fibrin conduit seeded with Schwann cell-like differentiated adipose derived stem cells (dASC) (Fib + dASC). Autografts and sham rats were used as controls (total n = 20). At 12 weeks post-implantation, an extensive histomorphometric analysis was performed. Functional aspects of regeneration were analysed by walking track analysis.
Results: No major autophagy occurred using the split-sciatic technique. A detailed histomorphometric analysis showed consistent results with previous literature using fibrin conduits in a full sciatic axotomy experimental setting. Walking track analysis reflected the histological regeneration pattern, displaying superior regeneration in both autograft and dASC groups.

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Conclusion: Split-Sciatic nerve surgery reduced animal morbidity, while being representative of the whole nerve as regeneration outcomes were consistent with previous data obtained on the whole sciatic nerve. The decreased autophagy rate allowed for a more efficient functional evaluation.

Introduction

Up to 5% of patients admitted to level one trauma centres have a peripheral nerve injury.[1] In clinical practice, current repair of nerve injuries is represented by either direct coaptation of distal and proximal stumps, or by autologous nerve grafting. However, autograft harvest requires a second operative site with donor site sensory loss, potential formation of neuroma and neuropathic pain.[2]

In the regenerating nerve, the link between Schwann cells (SCs) and basal lamina plays a crucial role in nerve regeneration. If this structure remains intact after injury, axons return to their targets with greater precision. However, when this connection is lost, inappropriate targets may be innervated causing dysesthesia and poor recovery.[3] Improving bioengineered neural conduits with regenerative stem cells represents a promising strategy for recreating a permissive environment, supporting the potential of host SCs.[4] Adipose-derived stem cells (ASCs), have been recognised as one of the most effective[4–6] adult stem cell lineage and are potentially easily translatable into a clinical setting.[7]

The most common experimental model to evaluate peripheral regeneration is the sciatic nerve injury model. Main drawbacks of this experimental surgery are the difficulty to create "critical" gaps (longer than >10 mm, according to an average rat size, 250 g) and the concrete possibility to induce hindlimb scratching or self mutilation, due to initially firing fibres or neuropathic pain.[8,9] A clear aetiology of autotomy after sciatic nerve transection has not been detected yet, as this phenomenon seems to be mostly caused by different interacting biological factors. Hyperalgesia can be present from regenerating sprouts supporting the reinnervation[10] and on the other hand a potentially insensate hindlimb may induce animals to automutilation.[11] Firing of neuropathic pain fibres may be an index of pathological activity in traumatised nerves[8] and a persistent tendency to automutilate has been recently put in relation with chaotic regeneration and reduced myelination.[9]

Despite potential triggering causes, autotomy after sciatic nerve surgery limits peripheral nerve research in rats, potentially leading to the impossibility to assess the functional aspects of reinnervation (especially when considering sciatic functional index or gate analysis, modifying footprints or walking behaviour). Moreover, this can lead to a significant number of drop out, up to 20% when using a neural tube,[12] conflicting with the 3Rs principles of experimental animal surgery.[13]

In this paper, we established a new microsurgical procedure aiming to improve the standard rat sciatic model. Overcoming autotomy and its consequences, extending the potential experimental nerve gap, while ensuring reliable histological results, were the main goals of this work. In order to fully validate the effectiveness of this procedure, we used a well-established *in vivo* long-term experimental setting,[4,14] where fibrin conduits are coupled with regenerative stem cells. Differences in regeneration among groups enabled us to detect different degrees of myelination and to compare the effectiveness of the split-sciatic model with previous data on full sciatic transection and repair.

Materials and methods

Experimental animals

Male Sprague–Dawley rats (Janvier, France) weighing in average 250 g were used for this study. All animal protocols were approved by the local veterinary commission in Lausanne, Switzerland and were carried out in accordance with the European Community Council directive 86/609/ECC for the care and use of laboratory animals.

Cell cultures and differentiation and fibrin conduit preparation

All cells were obtained from Sprague–Dawley rats (Janvier, France). ASCs were harvested and treated as previously described.[15] The fibrin conduit was prepared from two compound fibrin glue (Tisseel® Kit VH 1.0, Baxter SA, USA) as previously described.[16]

Experimental design and surgical procedure

Four experimental groups were included: fibrin conduit without cells (Fib), fibrin conduit seeded with dASC (Fib + dASCs) and two control groups composed by Autografts and Sham nerve. In all groups (each one formed by five animals for a total n = 20), conduits were left in place for 12 weeks and subsequently harvested together with the proximal and distal nerve stumps.

A few hours before implantation in the rats, cells were centrifuged and then trypsinised, 2×10^6 cells were suspended in 50 μl of growth medium and injected into the fibrin tubes as we previously reported.[16] The fibrin tubes without cells only contained just growth medium. The operation was performed on the left sciatic nerve under aseptic conditions using a power focus surgical microscope (Carl Zeiss, Germany). A skin incision from the left hip to knee was made for exposure of the underlying muscles, which were then retracted to reveal the sciatic nerve.[16,17] This was gently divided by splitting the tibial and peroneal branches, avoiding any nerve injury during the micro-dissection procedure