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Enhancement of nerve healing with the combined use of amniotic membrane and granulocyte-colony-stimulating factor

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Summary Healing of a transected nerve is not always optimal even if it is repaired with an ideal microsurgical technique. We studied the effects of solitary and combined usage of amniotic membrane (AM) wrapping and granulocyte-colony-stimulating factor (GCSF) injections after primary repairs of transected sciatic nerves of Wistar rats.

No repair was performed in group 1, primary nerve repairs were performed in group 2, AMs were wrapped around repair sites in group 3, in addition to AM wrapping G-CSF injections were performed in group 4, and only G-CSF injections were performed in group 5. Nerve regeneration was assessed by clinical tests at the 4th, 8th, and 12th weeks and by electrophysiological studies and histological evaluations at the end of the 12th week.

Group 4 rats gave earlier responses to clinical tests that showed a stable increase throughout the study. In electrophysiological studies, the mean amplitude values were higher in group 4 whereas mean latency and duration values were higher in group 1. At the end of the 12th week, the morphology of the distal nerve segment was similar to healthy nerves in the absence of fibrosis in group 4. The comparison of mean scores of axonal counting under the light microscope revealed that scores of group 4 were higher than the other groups in a statistically significant manner. Electron microscopy revealed that samples from groups 3 and 4 had high numbers of axons possessing myelin sheaths of normal thickness, as well as less inter-axonal fibrosis. In terms of both axonal diameter and myelin thickness, groups 2, 3, and 4 had higher values than groups 1 and 5.

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As a conclusion, both AM wrapping and G-CSF injection have a supportive effect on nerve regeneration, and this effect is further potentialized by their combined use.

Introduction

Even after an early and proper primary repair of a transected peripheral nerve, regeneration may not proceed in the desired optimal quality and time. In most cases, such inconvenience in regeneration is due to the presence of a poor healing environment, and this is reflected to the clinic as excessive scar and neuroma formation.[1]

Neurotrophic factors used to enhance nerve regeneration do not necessarily prevent the formation of fibrosis and neuroma. This fact led scientists to look for an appropriate model of neural tube to guide regenerating axons towards their appropriate targets and to prevent the interference of surrounding tissue. Synthetic neural tubes did not gain much popularity due to the increased costs and their potential to provoke foreign reaction around the regenerating axons. On the other side, there is a certain amount of donor site morbidity when veins or arteries were used as neural tubes of autogenic origin.[2–4] In the search for an alternative, no ideal material or product has been found that combines the advantages of low donor site morbidity and maintenance of a secure healing environment.[5,6]

Amniotic membrane (AM) has long been used for the enhancement of healing in various wound models. Previous research has also documented its success in the regeneration of peripheral nerves. Early studies concerning the effects of AM matrix on the regeneration of peripheral nerves were conducted in the late 1980s.[7] The first use of AM as a peripheral nerve conduit was reported by Mohammad et al., in 2000, and a number of studies on the subject were conducted since then.[8–11]

The granulocyte-colony-stimulating factor (G-CSF) is a member of hematopoietic growth factor family and it supports the formation, survival, and differentiation of hematopoietic progenitor cells. The influence of G-CSF on inflammation along with its anti-apoptotic effects has been reported. It can also act on neuronal cells as a neurotrophic factor, and its receptor is expressed by neurons in the brain and spinal cord.[12] Furthermore, it was also shown that when G-CSF is used in combination with AM it enhances the survival of stem cells within the AM.[13]

In light of the knowledge obtained from the current literature, we wanted to evaluate the clinical, electrophysiological, and histological effects of AM and G-CSF application on nerve regeneration in a primary nerve repair model.

Materials and methods

The study proposal was approved by the Ethics Committee of Mersin University Medical Faculty (HADYEK) with the given number 2009/23. All animals used received humane care in compliance with the guidelines established by the Ethical Committee, and were caged two by two in standard environmental conditions at room temperature with a 12 h light/dark cycle, and maintained on commercially available balanced rodent food ad libitum. Seventy adult, male Wistar rats of 250 $\pm$ 25 g were randomized into five groups.

Groups

In every group, right sciatic nerves were transected 1 cm above their trifurcation points.

Group 1 (n = 10): No other intervention was carried out.

Group 2 (n = 15): Primary nerve repair was carried out.

Group 3 (n = 15): Primary nerve repair and AM wrapping were carried out.

Group 4 (n = 15): Primary nerve repair, AM wrapping, and G-CSF application were carried out.

Group 5 (n = 15): Primary nerve repair and G-CSF application were carried out.

Preparation of AM

The placental tissues were collected from healthy human donors, whose serological analysis revealed no viral infections and who were not using any steroids. After the placental tissues were washed with sterile saline, the chorion layers were removed. The obtained AMs were kept in sterile saline solution at +4 °C under sterile conditions until further surgical interventions.[14]

Surgical technique

Anesthesia was performed by intramuscular injections of ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg). In every rat, hair on the right gluteal region was removed using a hair clipper (Wahl-Moser Acro Professional Animal Clipper; WAHL®, Sterling, IL, USA), followed by meticulous rinsing of cut hair pieces. Surgical cleansing was performed with 10% povidone—iodine solution.

The right sciatic nerve of every rat was exposed via a gluteal muscle-splitting incision, and transected using a pair of microsurgery scissors 1 cm above the trifurcation point. In groups other than 1, primary nerve repairs were performed with four epineural sutures (9/0 Ethilon®, Ethicon, Johnson—Johnson Intl, Cincinnati, OH, USA). In groups 3 and 4, AMs of 1.5 $\times$ 1.5 cm size were wrapped around the repair sites and fixed with two epineural sutures. In all groups, the repair sites were closed with 4/0 silk sutures (4/0 Silk®, Ethicon, Johnson—Johnson Intl).

Injections

Starting on the day before surgery, rats in groups 4 and 5 received intraperitoneal G-CSF (Neupogen®, Roche, Basel,