Neurolysis for nerve adhesion in rats affected the paraneurium and reduced macrophages in the nerve, reducing intraneural scarring and contributing to recovery of nerve function

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INTRODUCTION:

Peripheral neuropathy caused by nerve adhesion is associated with scarring resulting from perineural inflammation caused by trauma or surgical manipulation. The paraneurium and macrophages play an important role in nerve adhesion. The paraneurium, which attaches the nerve to its surrounding neural bed, facilitates low-friction nerve gliding. By contrast, macrophages secrete inflammatory cytokines, resulting in an inflammatory reaction that causes intraneural scarring.

Neurolysis is generally used to treat neuropathy, but there are few reports regarding the physiological effects of neurolysis on the peripheral nerves. In the present study, a rat sciatic nerve adhesion model was used to investigate the role of the paraneurium and macrophages in neurolysis on neuropathy caused by adhesion.

METHODS:

The adhesion procedure was performed according to the method described by Ikeda et al.(2003). Briefly, after the left sciatic nerve was exposed and the surface of the neural bed coagulated over a 15-mm length, the nerve was fixed to the neural bed using nylon sutures.

Two experiments were performed in this study. For Experiment 1, to assess neuropathy caused by the adhesion procedure, evaluation was performed 6 weeks after the adhesion procedure and the non-operated (control group) and the operated side (adhesion group) were compared. For Experiment 2, to assess the effectiveness of neurolysis, nerve adhesion was first completed over 6 weeks using the adhesion procedure. The control group was evaluated 6 weeks after the completion of nerve adhesion without treatment. The neurolysis group underwent neurolysis after the completion of nerve adhesion (Figure 1) and was evaluated 6 weeks later.

The evaluated parameters were as follows: (1) Tensile strength until the nerve was detached from the neural bed. (2) Nerve strain, which is the rate of nerve stretching by limb movement at the adhesion site, proximal site, and distal site of adhesion. (3) Tibialis anterior muscle weight ratio. (4) Distal latency of the compound muscle action potential of the tibialis anterior muscle (5) mRNA expression levels of CD68 (macrophage marker), iNOS (M1 macrophage marker), Arginase-1 (M2 macrophage marker), and TGF-β in nerves. (6) Histological evaluation.

The Mann-Whitney U-test was used for Experiments 1 and 2. Differences were considered statistically significant at $p < 0.05$.

RESULTS:

In Experiment 1, nerve strain decreased at the adhesion site and increased at the distal site because of the increase in tensile strength in the adhesion group (Figure 2). Muscle weight ratio and distal latency were significantly worse in the adhesion group. All mRNA expression levels were significantly higher in the adhesion group. Histopathology showed that the cell-infiltrated paraneurium adhered to the nerve, and there was more intraneural cellular infiltration and scarring in the adhesion group (Figure 3).

In Experiment 2, the nerve strain increased at the adhesion site and decreased at the distal site because of the decrease in tensile strength in the neurolysis group. Muscle weight ratio and distal latency were significantly better in the neurolysis group. mRNA expression levels of Arginase-1 and TGF-β were significantly lower in the neurolysis group, and those of CD68 and iNOS tended to be similarly low in the neurolysis group. Histopathology showed that the paraneurium did not adhere to the nerve and there was less intraneural cellular infiltration and scarring in the neurolysis group.

DISCUSSION AND CONCLUSION:

In Experiment 1, high tensile strength prevented the nerve from sliding at the adhesion site and instead increased nerve strain at the distal site, indicating traction damage to the nerve. Such traction damage is caused by the adhesion of the paraneurium to the nerve. It also results in cellular infiltration from the adhered paraneurium into the nerve. It has been reported that M1 macrophages increase first, followed by M2 macrophages, which secrete TGF-β, during neuropathy. TGF-β is a cytokine involved in fibrosis and excess TGF-β has been reported to lead to pathological scarring. In the present study, M1 and M2 macrophages migrated to nerves and elevated TGF-β levels in nerves, resulting in intraneuronal scarring, which may have caused further neuropathy.

In Experiment 2, neurolysis reduced tensile strength, resulting in nerve gliding, similar to a normal nerve. This is a result of neurolysis, which affects the paraneurium. It is possible that TGF-β, which was lowered because of the decrease in M2 macrophages, reduced intraneuronal scarring. Neurolysis improved nerve gliding on nerve adhesion and reduced intraneuronal scarring, leading to the recovery of nerve function.

Figure 1　Procedure of experimental model preparation
A: As the adhesion procedure, the sciatic nerve was fixed to the coagulated neural bed using nylon suture.
B: The nerve was exposed again 6 weeks after the adhesion procedure. The nerve was adherent to the scarring around nerve. C: Neurolysis was performed. Neurolysis was performed by blunt dissection, and sharp dissection was applied to the areas where adhesions were extremely strong. The nerve was then circumferentially removed from the neural bed.

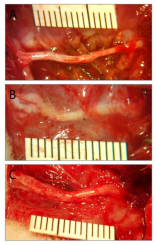

Figure 2　Results of Experiment 1 and 2. (A) Tensile strength. (B) Nerve strain. (C) Tibialis anterior muscle weight ratio. (D) Distal latency. (E) CD68 expression. (F) iNOS expression. (G) Arginase-1 expression. (H) TGF-β expression. *p<0.05 **p<0.01 ***p<0.001.

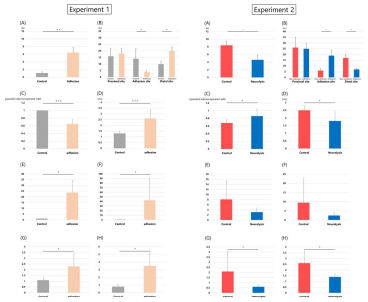

Figure 3　Results of Experiment 1 and 2. Histological evaluation with hematoxylin-eosin stain (H-E stain) and Masson's trichrome stain (M-T stain).
A: The cell-infiltrated pannecurium adhered to the nerve, and there was more intraneural cellular infiltration and scarring in the adhesion group than in the control group. B: The pannecurium did not adhere to the nerve and there was less intraneural cellular infiltration and scarring in the neurolysis group than in the control group.