Validation of a model using fibrin glue after peripheral nerve transection injury

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INTRODUCTION: Following traumatic injury to the nerves in the peripheral nervous system (PNS), full functional recovery is poor. The slowness and inefficiency of axon regeneration are most often attributed to this poor outcome. The slowness and inefficiency of axon regeneration are most often attributed to this poor outcome. Enhancing axon regeneration has emerged as a therapeutic target for improving functional recovery after PNS injuries. Several experimental animal models have been used to evaluate axonal regeneration and functional recovery. Sciatic nerve crushed model is one of them. Crushed model is known for natural recovery over time. On the one hand surgical method is easy, on the other hand crushed model has the problem that the effects of various interventions are difficult to verify. To reduce problems in natural recovery, we use a more damaging model which sciatic nerve transected. Transected models require therapeutic indications, such as nerve sutures. However, the suture technique is difficult and causes more damage to the injured nerves. So that we use the fibrin glue model, which is one of transected models, resolve the challenges. In this study, we created the sciatic nerve transected and fibrin glue model used in previous studies, and examined the validity from evaluation of motor function, electrophysiological analysis, and histological observations.

METHODS: This study was approved by the Animal Research Committee of Saitama Prefectural University (approval number: 2022-09). We used 12-week-old male C57BL/6j mice (8animals and 12limbs). Mice were divided into three groups. The first group had no operations (Intact). The left hindlimbs were used in this group. In the second group, the right sciatic nerve was transected, and it was ensured that the proximal and distal stumps were in proximity and without disturbing the surrounding fascia or muscle (Simple Transection: ST). The third group was fibrin glue repair, which thrombin and fibrinogen in the ratio of 2:1, respectively, were added to the nerve stumps (Simple Transection and Glue: STG). The sciatic functional index (SFI) and compound motor action potential (CMAP) were measured 28 days postoperatively. After SFI and CMAP were performed, the gastrocnemius muscle (GAS) was quickly sampled and observed. Freeze-embedded GAS was sliced into 10μm thickness. The samples were used for Hematoxylin and Eosin (HE) staining. Statistical analysis was performed using the Mann-Whitney U test.

RESULTS SECTION: SFI of ST group was -80.7±13.3 and STG group was -50.7±12.3 (Fig.1). STG group had a significantly higher SFI than the ST group (p<0.05). The maximum amplitude of CMAP of ST group was 0.75±0.24mV and STG group was 9.57±4.01mV (Fig.2). STG group had a significantly higher CMAP than the ST group (p<0.05). From observation of GAS, muscle atrophy occurred in both the ST and STG groups (Fig.3A). However, atrophy was suppressed in the STG group. In the section of GAS was smaller which diameter of muscle fibers (Fig.3B).

DISCUSSION: The results of SFI and maximum amplitude of CMAP showed that the STG group had significant reinnervation and motor function recovery compared to the ST group. Secondary muscle atrophy after denervation occurred in the ST and STG groups. This was inferred from the observation of muscle sections. The nerve transection injuries used in this study were indicated for surgical intervention, and it is possible that motor function recovery and reinnervation did not occur in the ST group because of persistent denervation. In the STG group, the neuromuscular axon extension after the transection injury may have allowed reconnection with the muscle, resulting in the recovery of motor function.

SIGNIFICANCE/CLINICAL RELEVANCE: The fibrin glue model of peripheral nerve transection and junction used in this study has high validity as a model that promotes recovery even after transection injury.

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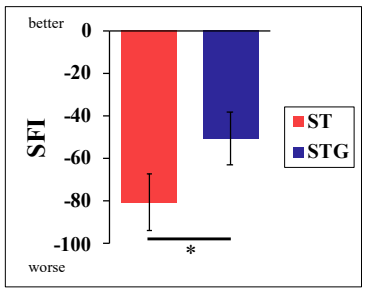

Fig.1 SFI of post-operative day at 28

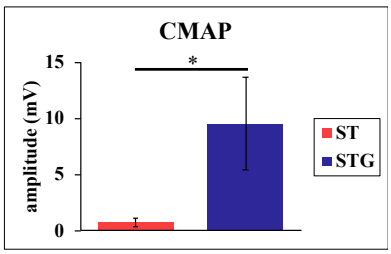

Fig.2 CMAP of post-operative day at 28

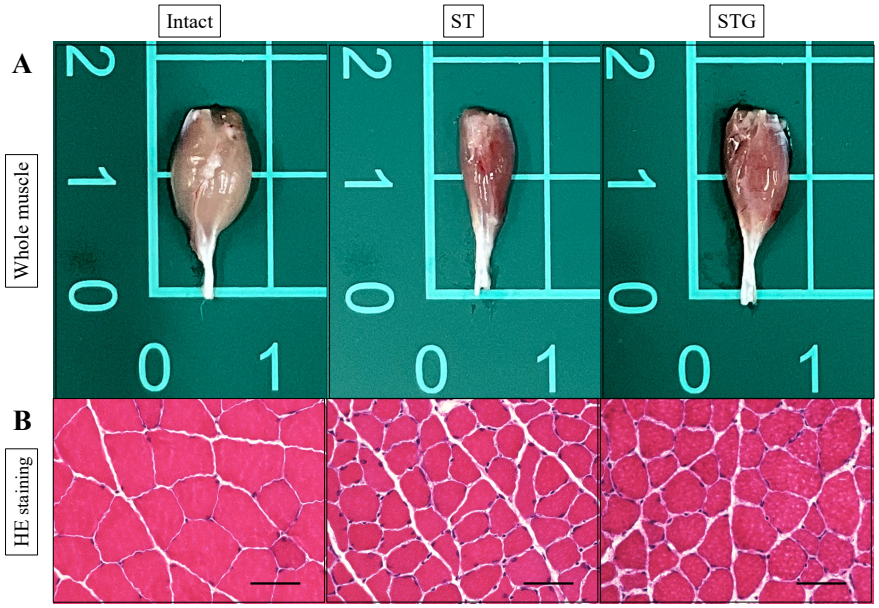

Fig.3 Whole muscle (A) and HE staining (B)