Comparison of peripheral nerve repair using ethyl-cyanoacrylate and conventional suture technique in a rat sciatic nerve injury model

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Objective: The aim of this study was to compare the outcomes of primary nerve repair using either ethyl-cyanoacrylate or conventional microsuture technique in a rat peripheral nerve injury model.

Methods: In this study, a total of 30 Wistar Albino rats weighing between 220 and 275 g were used. The rats were randomly divided into three groups (10 in each), including one control (group 1) and two experimental groups (group 2, conventional microsuture repair; group 3, cyanoacrylate repair). In each group, the sciatic nerve was identified and transected. No further intervention was performed in group 1. The nerve was repaired using the epineural technique with a 10/0 atrumatic nylon in group 2 and synthetic cyanoacrylate adhesive in group 3. At the fifth postoperative week, needle electromyography (EMG) was performed to measure distal latency, combined muscle action potential (CMAP), and motor nerve conduction velocity (MNCV). Following the EMG recordings, animals were euthanized. Nerve samples were collected to evaluate vacuolar degeneration, fibrosis, and foreign body reaction histopathologically.

Results: In the EMG analysis, mean distal latency was significantly shorter in group 1 (0.85±0.09 ms) than in groups 2 (1.17±0.25 ms) (p=0.0052) and 3 (1.14±0.14 ms) (p=0.0002) and while no significant differences existed between groups 2 and 3 (p=0.9999). The mean CMAP was greater in group 1 (10.5±1.28 mV) than in groups 2 (2.16±1.34 mV) (p=0.0002), but there was no significant difference between groups 2 and 3 (p>0.9999). The mean MNCV was 53.5±5.95, 39.62±7.31, and 39.84±4.73 mm/sec in groups 1, 2, and 3, respectively. There was a significant difference between groups 1 and 2 (p=0.0052) and between 1 and 3 (p=0.0026), but not between 2 and 3 (p=0.9999). In the histopathological evaluation, the mean vacuolar degeneration score was 0, 2.12, and 1.88 in groups 1, 2, and 3, respectively. There was no obvious difference was observed between groups 2 and 3 (p=0.743). The mean fibrosis score was 0, 1.62, and 1.77 in groups 1, 2, and 3, respectively. There was no no significant difference between groups 2 and 3 (p=0.888). The mean foreign body reaction score was 0, 2.5, and 2.44 in groups 1, 2, and 3, respectively. No difference was present between groups 2 and 3 (p=0.743).

Conclusion: Primary nerve repair using the cyanoacrylate adhesive may provide similar electrophysiological and histopathological results as compared to the conventional microsuture repair.

Peripheral nerve injuries are a leading cause of permanent disability. These injuries are frequently a result of work and traffic accidents and primarily affect the young population and are frequently accompanied by other musculoskeletal injuries.

The classical treatment of complete dissection of a peripheral nerve involves perineural or epineural coaptation with microsutures. Despite being the preferred technique, this is a time-consuming procedure and misplaced sutures can potentially impair blood flow to axons thereby interrupting nerve regeneration.

Various alternative techniques have been proposed for nerve repair, including adhesive techniques that involve application of an adhesive material to both ends of the transected nerve for coaptation. An ideal adhesive should be easy to apply, have high binding strength, and be compatible with the surrounding tissue. Fibrin glue has been used as an adhesive for nerve repair and for microvascular anastomosis.
It is most often used in combination with nylon sutures since alone it does not have sufficient strength to hold the anastomosis in place. The major advantage of fibrin glue over other adhesives is its biocompatibility. However, since it is a blood product, it carries the risk of blood-borne disease transmission.

Alternatively, previous studies have proposed cyanoacrylate glue as an adhesive for microvascular anastomosis and nerve injuries (9, 10). Experimental studies on rats found no difference with respect to recovery of the peripheral nerve between nerve repair using synthetic cyanoacrylate adhesive and repair using microsutures, but most of these studies relied on histological evaluation of the results, which could be misleading or incomplete in the absence of an electrophysiological study (10-13).

The aim of this study was to compare the use of cyanoacrylate adhesive with conventional microsuturing in primary nerve repair using electrophysiology and histological analysis.

Materials and Methods

The study was conducted in Düzce University Medical School Animal Research Laboratory with the approval of the local ethics committee of Düzce University (Protocol number: 2010-01). Thirty Wistar albino rats weighing between 220 and 275 g were used. Ten animals were selected randomly for each group.

Rats were anesthetized with an intraperitoneal injection of 5 mg/kg xylazine hydrochloride and 75 mg/kg ketamine. Cefazolin was used as a prophylactic antibiotic. The skin was shaved prior to surgery and prepared with povidone-iodine solution.

A longitudinal incision was performed on the anterior thigh and hip to visualize the thigh muscles. The muscles were split to gain access to the sciatic nerve, which was dissected free from the surrounding tissues, and the tibial and peroneal branches were dissected away from each other. The rats were divided into one control and two experimental groups:

Group 1 (control): The nerve was transected (Figure 1) approximately 15 mm proximal to the sural, peroneal, and tibial nerve trifurcation using microscissors (Figure 2). No further intervention was made in this group.

Group 2 (conventional microsuture repair): The transected nerve was repaired under an operating microscope with 10/0 atraumatic nylon sutures. The epineural technique was used for the repair and approximately four sutures were placed for each repair (Figure 3).

Group 3 (cyanoacrylate repair): The proximal and distal ends of the nerve were held in place temporarily with a single 10/0 nylon suture while ethyl cyanoacrylate (Permabond, Permabond Engineering Adhesives Ltd., Hampshire, UK) was applied to both ends of the nerve to complete the repair (Figure 4). The suture was immediately removed following the application of the adhesive material.

Muscle repair and closure of the skin incision was performed in all groups with 5/0 nylon sutures at the end of surgery. An-
algia was administered following the surgery. Results were evaluated at the fifth postoperative week using macroscopic, electrophysiological, and histological examinations.

Electrophysiological evaluation
Needle electromyography (EMG) evaluation was performed by a neurologist who was blinded to the experimental groups. An Esaote Myto II EMG (Esaote, Istanbul, Turkey) device was used for the evaluation. Room temperature was kept at 24-25 °C for the evaluation. Rats were anesthetized using an intraperitoneal injection of 75 mg/kg ketamine (Ketalar, Pfizer, New York, USA). The electrical stimulus was given 2 cm proximal to the repair site of the nerve. Bipolar needle electrodes placed 2.5 cm distal to the repair site were used as a detector. Combined muscle action potentials (CMAPs) were recorded. Maximum CMAP amplitude, latency (the time delay between stimulation and CMAP onset), and motor nerve conduction velocity (MNCV) were calculated. In healthy resting muscles, two types of electrical activity can be recorded. The first occurs with movement of the needle inside the muscle. The second is due to the muscle end plate potential, which can be a low-amplitude electronegative activity (endplate noise) or can be composed of spikes (endplate spikes). During muscle contraction, motor-unit action potentials are recorded. A decrease in the amplitude and duration of the spikes and abnormal electrical activity at rest are usually indicative of a pathological condition. The distal latency difference is the interval measured from the moment of stimulation of the distal-most accessible site on the nerve. For MNCV calculation, the conduction distance between the stimulating electrodes and the latency of CMAP is measured. To calculate the MNCV, the proximal latency is subtracted from the distal latency and divided by the conduction distance.

Histological evaluation
Following the EMG study, the rats were euthanized using a lethal-dose intraperitoneal phenobarbital injection and nerve samples for histopathological examination were obtained. Samples were fixed in 10% formaldehyde solution, embedded in paraffin, and 3-4 µ slices were obtained. H&E, Toluidine Blue, and Masson's Trichrome stainings were performed. An Olympus BX50 (Tokyo, Japan) microscope was used to evaluate the samples. Parameters including vacuolar degeneration, fibrosis, and foreign-body reaction were evaluated (Figure 5). Vacuolar degeneration characterized by the presence of cytoplasmic vacuoles and fibrosis is consistent with degeneration following nerve injury. For the foreign-body reaction score and inflammatory response, the presence of activated macrophages, foreign-body giant cells in the epineural space, and morphometric changes were evaluated.

Statistical analysis
Statistical evaluation was performed to establish a descriptive and comparative analysis of the results. The Kruskal-Wallis test and Mann-Whitney U tests were used to compare the groups. Multiple comparisons were performed using Dunn’s test.

Results
Distal latency difference, action potential amplitude, and MNCV results
Mean distal latency difference values in milliseconds were 0.85±0.09, 1.17±0.25, and 1.14±0.14 for the control, conven-
tional suture, and cyanoacrylate groups respectively (p values: control vs conventional: 0.0052 control vs cyanoacrylate: 0.0026 conventional vs cyanoacrylate: >0.9999). Mean action potential amplitudes in millivolts were 2.86±1.28, 2.16±1.34, and 10.5±0.35 for the control, conventional suture, and cyanoacrylate groups respectively (p values: control vs conventional: 0.0110, control vs cyanoacrylate: 0.0002, conventional vs cyanoacrylate: >0.9999). Mean MNCV values in millimeters per second were 53.5±5.95, 39.62±7.31, and 39.84±4.73 for the control, suture, and cyanoacrylate groups, respectively (p values: control vs conventional: 0.0052, control vs cyanoacrylate: 0.0026, conventional vs cyanoacrylate: >0.9999). The differences between the conventional suture and cyanoacrylate groups were statistically insignificant. Differences between the conventional suture group and the control group and between the cyanoacrylate group and the control group were all statistically significant (Figure 6).

Vacuolar degeneration, fibrosis and foreign-body-reaction results

The mean numbers of vacuolar degeneration scores were 0, 2.12, and 1.88 for the control, suture, and cyanoacrylate groups, respectively (p value: conventional vs cyanoacrylate: 0.606). The mean numbers of fibrosis scores were 0, 1.62, and 1.77 for the control, suture, and cyanoacrylate groups, respectively (p value: conventional vs cyanoacrylate: 0.888). The mean numbers of foreign-body-reaction scores were 0, 2.5, and 2.44 for the control, suture, and cyanoacrylate groups, respectively (p values: conventional vs cyanoacrylate: 0.743).
The differences between the conventional suture and cyanoacrylate groups were statistically insignificant. The differences between the conventional suture group and the control group and between the cyanoacrylate group and the control group were statistically significant (p <0.05) (Figure 6).

Discussion

Peripheral nerve injuries that can result in long-term functional impairment are common in clinical practice. Despite ongoing experimental and clinical research, nerve regeneration results are often incomplete and can result in permanent disabilities. The rat sciatic nerve model is commonly used in experimental research. Advantages of this nerve-injury model include the fact that the sciatic nerve is a mixed sensory and motor nerve and that this model is cost effective (14).

Human peripheral nerve axons have a high regeneration capacity after trauma (15). Despite this, various problems after nerve repair can result in impaired nerve regeneration. The outcome of nerve regeneration depends on the balance between Schwann cell nerve regeneration and scar tissue (16). Excessive scar tissue can be related to technical difficulties regarding nerve coaptation during surgery. In our study, we found that the fibrosis scores of the conventional suture and cyanoacrylate repair groups were similar. Despite these results, the lack of skill of inexperienced surgeons in performing conventional-suture repair is probably more likely to result in scar formation. Cyanoacrylate repair could therefore be advantageous for surgeons who are inexperienced in microsurgical procedures.

Electrophysiological studies are one of the most valuable tools to evaluate recovery following peripheral nerve injury. Despite this, early EMG studies cannot provide sufficient information since axons are still regenerating in the early postoperative period (17). In our study, we allowed five weeks of regeneration to ensure that the electrophysiological study was reliable. The electrophysiological results revealed no significant difference in the CMAP amplitude, MNCV values, or distal latency times of the cyanoacrylate and conventional suture groups. Regeneration after a nerve injury can be assessed based on the number of axons and the nerve diameter, but these histological parameters are often insufficient to determine the quality and functional status of the axons (18). Some axons may be disrupted and dysfunctional (17, 19). Electrophysiological studies that provide combined action potential information are valuable in this respect (20). A delay in surgery after nerve injury is an important factor that affects functional outcomes. In our study, immediate nerve repair was performed. In clinical settings, possible delays in repair might correspond to differences in results (21-23). One of the shortcomings of combined-action-potential measurement is that the results are dominated by the nerve fascicles that are myelinated and fast. Amplitude and speed measurements do not correspond to the whole nerve but rather to the fastest, most myelinated fascicles (24). Electrophysiological study results also correlate with the numbers and diameters of axons and the numbers of dorsal root ganglion cells. One of the limitations of this study was that it lacked tests to evaluate the functionality of the rats, such as walking. Future studies involving further histology and electron microscopy could be valuable to demonstrate nerve regeneration and the efficiency of nerve repair using adhesives.

Synthetic tissue adhesives have been used previously in studies of vessel anastomosis and nerve repair. Cyanoacrylates are synthetic adhesives that react quickly with biological tissues when in contact with air, water, or blood. Their toxicity depends on the rate of elimination. Formaldehyde and heat are produced once they are in contact with air, which are responsible for their toxicity (25). Short-chain acrylate adhesives such as ethyl-2-cyanoacrylate have higher tissue toxicity (26, 27). Long-chain acrylates such as 2-octyl-cyanoacrylate have proven to be much less toxic in comparison and have been approved for topical use by the FDA (28). Hall et al. and Ang et al. used octyl cyanoacrylate for microsurgical vessel anastomosis with success (29, 30). At three weeks post-surgery, they found that there was no foreign-body reaction and excess adhesive had been completely resorbed from surrounding tissues. However, ethyl-cyanoacrylate repair has been shown to induce Wallerian degeneration, macrophage density, and an inflammatory reaction that can increase the regeneration capacity of the nerve (9). In a previous study that used a pig model, Rickett et al. found no acute toxicity from ethyl cyanoacrylate, and the bonding strength was sufficient for nerve repair (31). In our study, histological examination revealed no significant difference in foreign-body-reaction findings between the conventional suture and cyanoacrylate groups.

Microsuture anastomosis is the conventional technique used in nerve repairs. This technique has the potential to disrupt the fascicular blood flow and delay nerve regeneration and tissue repair, especially in the hands of surgeons inexperienced in microsurgery. The skills needed for cyanoacrylate repair, in contrary, are easy to learn and have a shallow learning curve. Cyanoacrylate nerve repair can be performed in tight spaces such as the intraoral region where conventional repair would be very difficult.

In conclusion, in our study, there were no significant differences between the conventional suture and cyanoacrylate techniques in terms of CMAP peak amplitude, distal latency, and MNCV measurements. In addition, the histological findings of the two groups regarding vacuolar degeneration, fibrosis, and foreign-body reactions were similar. Nerve separation was not seen in any of the repairs using cyanoacrylate, indicating the adequate adhesive strength of the material. In conclusion, our results imply that cyanoacrylate nerve...
repair can achieve similar results to conventional suture techniques in certain situations. The cyanoacrylate technique is inexpensive, less time consuming, and easy to learn. It can be used safely in inconvenient and tight surgical spaces with basic microsurgical training.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Local Ethics Committee of Düzce University (Protocol number: 2010-01).

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