

ORIGINAL ARTICLE

**Electrophysiological Comparison of Peripheral Nerve
Repair Methods: An Animal Study**

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OBJECTIVES: The aim of this study was to increase the experience with N-butyl-2-cyanoacrylate in peripheral nerve anastomosis, to compare this group with primary suture, cyanoacrylate and control groups and to present our results and experience.

MATERIALS AND METHODS: In the study, 49 adult male Sprague Dawley species rats were used, in the years 2004 and 2005. The study was planned as 4 groups; control group (n=10), suture group (n=12), cyanoacrylate group (n=14) and N-butyl 2-cyanoacrylate group (n=13). The right sciatic nerves of the rats were operated under general anesthesia in all groups. To measure the nerve conduction velocity (NCV), electrical stimulations were given by a stimulator using electrodes which were placed to the proximal end of the sciatic nerves and then the nerve conduction speeds were calculated by using a computer program.

RESULTS: In the control group NCV was 58.6±0.7 m/s at the beginning and 55±1.3 m/s three months after the procedure and there was no statistically significant difference between them (p>0.05). In the suture group, NCV at the beginning of the experiments was 57.1±0.6 m/s and, after three months, it was found to be 36.2±0.6 m/s (p<0.01). In the cyanoacrylate group, the NCV before and three months after the experiments were 58.4±0.4 m/s and 44.7±1.1 m/s, respectively (p<0.01). The NCV in the N-butyl 2-cyanoacrylate group was 58.5±0.4 m/s at the beginning and, after three months, it was found 47.4±1.2 m/s (p<0.01).

CONCLUSIONS: Our results support that, in the peripheral nerve repair, it is possible to get better electrophysiologic results by using N-butyl 2-cyanoacrylate and cyanoacrylate compared to the traditional microsuture technique.

Various methods are in use for the repair of peripheral nerve injuries. To date, suturing is the predominant technique to anastomose nerves.^[1-3] However, this technique is time consuming and can cause crushed, misaligned endoneural tissue.^[1-4] Misalignment of the regenerated axonal fibers, in turn, decreases functional gain of the nerve.^[1-5]

Therefore, there has been attempts to develop new repair techniques with various materials or instruments, with limited or without suturing.^[1,6-10] The goal is less tissue contact, less tissue trauma and perfect alignment of many nerve fascicles.^[1,6-8] However, unfavorable results including high rate of dehiscence, marked coagulopathy, hypotensive responses, foreign body reactions, allergic reactions and tissue necrosis have been reported following these procedures.^[1,8-13]

Despite good results with the use of fibrin glue in nerve anastomosis,^[14,15] inadequate tissue tension to accomplish peripheral nerve anastomosis, strictures in the line of anastomosis and viral transmission with human fibrin have also been reported.^[16,17]

Unsuccessful results with cyanoacrylate, a synthetic tissue adhesive used for nerve repair have been attributed to short chain acrylate toxicity. To prevent this, cyanoacrylate polymers are being manufactured and there is an ongoing attempt to increase tissue tolerance.^[1,13,18] We planned to investigate the effects of N-butyl 2-cyanoacrylate, one of the cyanoacrylate polymers on post-nerve repair function by comparing the results with those of the control group. Butyl-cyanoacrylate adhesives have been used for years in selected cases, for the purposes of tissue repair and adhesion, embolization, sclerotherapy and hemostasis. Further, FDA recommends the use of cyanoacrylate by experienced physicians in life-threatening conditions.^[1]

The aim of this study was to enhance our experience in nerve anastomosis with N-butyl 2-cyanoacrylate and to present the functional results and our experience by comparing N-butyl 2-cyanoacrylate group with primary suture, cyanoacrylate and control groups.

MATERIAL AND METHOD

This study has been carried out at the Electrophysiology Laboratory of the Department of Physiology of Uludağ University Faculty of Medicine using 49 adult male Sprague Dawley rats (310-460 g), in the years 2004 and 2005. Rats were kept in cages under 18-22 °C, with 4 rats in a cage. Animals were fed standard rat diet and they had ad libitum access to food and water.

The study was designed in 4 groups, namely, the control group, suture group (group-I), cyanoacrylate group (group-II) and N-butyl 2-cyanoacrylate group (group-III). Procedures were performed in all rats on the right sciatic nerves under general anesthesia.

In the rats of control group (n=10), the sciatic nerves were isolated, NCV was measured and the wounds were closed, with the layers in anatomical plane, without performing any other procedure. At 3 months, same procedure was repeated and NCV was recorded.

Following the measurement of the NCV, the sciatic nerves of the rats in suture group (n=12), were transected totally. Right after the transection, the ends of the nerve were reapproximated without tension and anastomosed under the magnifying glass by 2 sutures with 8/0 monofilament suture thread (Prolen®). The incision was closed, with the layers in anatomical plane. At 3 months, nerve conduction velocity was measured and recorded again in the area encompassing the region of repair.

Once the NCV was measured, the sciatic nerves of the rats in Cyanoacrylate Group (n=14), were transected totally. The ends of the nerve were reapproximated without tension and glued with Cyanoacrylate (Crazy Glue®) solution under the magnifier, taking care not to let the glue seep into the nerve ends. The incision was closed, with the layers in anatomical plane. At 3 months, NCV was measured and recorded again in the area encompassing the region of repair.

The NCV was measured and the sciatic nerves of the rats in N-Butyl 2-Cyanoacrylate Group (n=13), were transected totally. The ends of the nerve were reapproximated without tension and glued with N-Butyl 2-Cyanoacrylate (Histoacryl Blue™) solution under the magnifier, taking care not to let the glue seep into the nerve ends. The incision was closed, with the layers in anatomical plane. At 3 months, NCV was measured and recorded again in the area encompassing the region of repair.

Prior to the surgical procedure, rats had intravenous anesthesia with 40 mg/kg Thiopental Sodium (Pentotal Sodium-Abbott). Skin incisions were closed in all rats with 4/0 absorbable sutures.

To measure the NCV, the stimulating electrode (Biopac MP 100 Data Acquisition and Analysis System Stimulator- Biopac Systems, Inc. Santa Barbara) was placed on the proximal portion of the sciatic nerve and electrical stimuli were given (8 V, 0.5 ms duration). The electrical impulse was detected by the recording electrode placed 2 cm distal to the stimulating electrode (extracellular recording method) and transferred to a computer by an analog/digital converter. The NCV was calculated using a software installed on the computer and supplied by the manufacturer of the system (Biopac MP 100 Data Acquisition and Analysis System, Biopac Systems, Inc. Santa Barbara).

Kruskal-Wallis Test was used to compare the baseline sciatic nerve measurements between groups while Paired Student-T test was used to compare baseline velocities with those recorded at 3 months within groups. Data are presented as Mean (\pm SD). Changes in velocity over time among groups were calculated as percent changes. Differences among groups with respect to the percent changes in 3 months were compared with Kruskal-Wallis test and pairwise comparisons were made by Student-T test. Significance was determined at $p < 0.05$. SPSS v10.1 statistical package was used for statistical analyses.

RESULTS

Baseline sciatic NCV and those at 3 months in all groups are presented in Table 1. There were no statistically significant differences between groups in baseline velocities (Table 1) (Figure-1).

In the control group, NCV was 58.6 ± 0.7 m/sec at the beginning and 55 ± 1.3 m/sec at 3 months and the difference was not statistically significant ($p > 0.05$). In the suture group, NCV before and 3 months after the procedure were 57.1 ± 0.6 m/sec and 36.2 ± 0.6 m/sec, respectively, and this difference was statistically significant ($p < 0.01$). The difference in the cyanoacrylate group was also significant, with a NCV of 58.4 ± 0.4 m/sec and 44.7 ± 1.1 m/sec before and 3 months after the procedure, respectively ($p < 0.01$). The NCV decreased from 58.5 ± 0.4 m/sec to 47.4 ± 1.2 m/sec in the N-butyl 2- Cyanoacrylate group ($p < 0.01$) (Table 1) (Figure-1).

After 3 months, the lowest and highest percent changes in the NCV's compared to the baseline values were observed in the control and suture groups, respectively. When compared to the control group, there were significant decreases in the NCV in all experiment groups. Percent improvements in the NCV's in the cyanoacrylate and N-butyl 2-cyanoacrylate groups were significantly higher than the suture group. However, the cyanoacrylate and N-butyl 2- cyanoacrylate groups did not differ from each other in terms of improvement in the NCV (Table 1) (Figure-1).

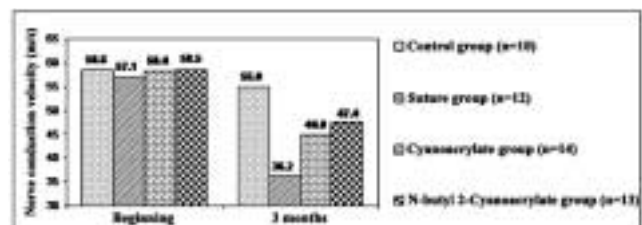


Figure-1: Baseline and at 3 months, sciatic nerve conduction velocity (NCV), in m/sec, in all groups, by diagrams.

Table-1: Demographic and some clinical parameters of the patients

GROUPS	Baseline	At 3 months	p-value*
Control group (n=10)	58,6±1,6	55,0±3,1	p>0,05
Suture group (n=12)	57,1±2,5	36,2 ±2,2	p<0,01
Cyanoacrylate group (n=14)	58,5±1,7	44,8 ±4,4	p<0,01
N-butyl 2-Cyanoacrylate group (n=13)	58,5±1,8	47,4 ±4,4	p<0,01
p-value**		^x p<0,001 ^a p<0,001 ^b p<0,001 ^c p<0,001 ^d p<0,001 ^e p<0,001 ^f p>0,05	

* p-Paired Student t-test

**p- Kruskal-Wallis test

^a p- Control -Suture^b p- Control - Cyanoacrylate^c p- Control - N-butyl 2-Cyanoacrylate^d p- Suture - Cyanoacrylate^e p- Suture - N-butyl 2-Cyanoacrylate^f p- Cyanoacrylate - N-butyl 2-Cyanoacrylate

DISCUSSION

The results of this study support previous studies which demonstrated that synthetic adhesives such as cyanoacrylate and N-butyl 2-cyanoacrylate yielded better electrophysiological responses in peripheral nerve lesions than microsuture technique.^[19,20]

Cyanoacrylate is an ester of cyanoacrylic acid that polymerizes by interacting with basic substances such as air, blood, water and provides a rapid and permanent adaptation in biological tissues^[15] To date, many cyanoacrylate derivatives are produced for medical uses.^[21,22] Methyl 2-cyanoacrylate was the first cyanoacrylate used in closing surgical wounds, as an alternative to suture.^[19] As the number of carbons increase in the ester chains, the molecule becomes more biocompatible and less histotoxic, compared to cyanoacrylates with short alkyl chain.^[23] In biological

tissues cyanoacrylate adhesives slowly degrade and the rate of degradation decreases with longer alkyl chain.^[19,24] Besides, adhesion power generally decreases with the length of the chain.^[19] Chemical structure of the cyanoacrylates is being modified to reduce toxicity and enable its use in various clinical applications. On the other hand, there is currently no universal agreement on which cyanoacrylate is to be used in peripheral nerve injury repair.

Unsuccessful results in peripheral nerve repair with methyl 2-cyanoacrylate and alkyl 2-cyanoacrylate have been reported.^[19] In an animal experiment, Wlodarczyk showed that the electrical activity was obliterated when butyl-cyanoacrylate was injected in the gap on the peripheral nerve.^[25] On the other hand, there are also studies on butyl-cyanoacrylate that reported functionally and morphologically successful results.^[19,20]

To the best of our knowledge, no study exists assessing normal cyanoacrylate, N-butyl 2-cyanoacrylate, and microsuture technique with the control group and presenting the electrophysiological results. Meanwhile, there are studies that compared cyanoacrylate derivatives with microsuture technique.^[19] In the present study, we preferred N-butyl 2-cyanoacrylate which has been developed to increase tissue tension and decrease histotoxicity. Even though it has been claimed that N-butyl 2-cyanoacrylate is less toxic like ethyl-cyanoacrylate and that butyl form is a less potent adhesive,^[19] we did not observe any sign of dehiscence in any of the nerve endings that has been repaired. In line with previous studies, this procedure is not more time-consuming than microsuturing.^[19] In the same study^[19], the authors argued that the duration of the procedure can be shortened further if the synthetic adhesives are available in single-use packages or if the adhesive can be administered with a dropper capable of delivering in micro-droplets.

It has been known that cyanoacrylate-based adhesives are more potent than fibrin glue for nerve repair, serve as a carrier for biological active materials and compression and nourishment problems due to mechanical effects is less than microsuture repair.^[19,26] Cyanoacrylates in human body break down into cyanoacetate and formaldehyde which are toxic for tissues and excreted via the renal and gastrointestinal systems.^[26] Considering that cytotoxicity is dose dependent, it is critical to use the minimum amount.^[27]

A gap occurs as a result of elastic retraction of the transected nerve ends. This results in a tension when the nerve ends are approximated to put the suture. Further, epineural suturing can result in additional axonal damage and dehiscence of the sutures under tension. Therefore an adhesive applied to the line of incision would be helpful in approximating the proximal and distal ends and facilitating the initial budding process.^[19,28] Our results support this view. Compared to microsuturing, statistically better improvements in NCV have been observed with N-butyl 2-cyanoacrylate and cyanoacrylate. Though

improvement in NCV was better with N-butyl 2-cyanoacrylate than cyanoacrylate, the difference was not statistically significant. Supporting these findings with future histopathological studies is important not only for histotoxicity but also for clinical usage.

We are ongoing on the histological part of the study and would like to present in a new study. In this study our initial aim was to determine the electrophysiological recovery of neural repair.

Our results lend support to previous studies which reported that electrophysiologically better outcome could be achieved in peripheral nerve repair by N-butyl 2-cyanoacrylate than conventional microsuturing. Further, clinically oriented experimental models can be developed to explore their effects on the repair of delayed nerve damage and demonstration of local inflammatory reaction in the region of repair, observation of cytotoxicity in the long term may be appropriate.

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