

## Effects of low dose trapidil on electrical properties of a rat peripheral nerve after crush injury

Sıçan periferik sinirinin elektriksel özellikleri üzerine hasar sonrası düşük doz trapidil tedavisinin etkileri

Belgin BÜYÜKAKILLI,<sup>1</sup> Bahar TAŞDELEN,<sup>2</sup> Sevgi GÜNEŞ<sup>1</sup>

### BACKGROUND

Trapidil has been shown to possess the protective effects in the treatment of ischemia and reperfusion injury in the peripheral nervous system. The purpose of this study was to determine the effects of low dose trapidil on peripheral nerve regeneration with electrophysiological method.

### METHODS

The sciatic nerve was compressed for 20 sec by using a jeweler's forceps. Trapidil treatment groups were administrated a single dose of trapidil (8 mg/kg) intraperitoneally just after the injury. Electrophysiological recordings were performed in crush and crush + trapidil treatment groups on the 2nd, 7th, 15th, 30th and 45th days following the nerve injury.

### RESULTS

EMG recordings on the second day following the crush injury showed low values of compound motor action potentials in the gastrocnemius muscle when compared to normal values obtained in intact animals; also, the values on the second day following the crush injury were significantly different between control and trapidil-treated groups. The action potential values for both groups did not yet reach baseline values at the end of the experiment. There was no difference in the action potential amplitude, area and distal latency values between rats with crush and crush+trapidil on all days.

### CONCLUSION

We could not prove a neuroprotective effect of a single low dose of trapidil in rat crush injury model using electrophysiological method.

**Key Words:** Compound motor action potential; crush injury; neuroprotection; peripheral nerve; trapidil.

### AMAÇ

Bazı çalışmalarda, trapidil'in periferik sinir sisteminde iskemi ve reperfüzyon hasarındaki koruyucu etkileri gösterilmiştir. Ancak, periferik sinir hasarı sonrası trapidil'in etkileri değerlendirilmemiştir. Çalışmamızın amacı, düşük doz trapidil'in sinir regenerasyonu üzerindeki etkilerini elektrofizyolojik yöntemle saptamaktır.

### GEREÇ VE YÖNTEM

Siyatik sinir, bir "jeweler forceps" ile 20 saniye sıkıştırıldı. Trapidil tedavi gruplarına, sinir hasarı sonrası tek doz trapidil (8 mg/kg) intraperitoneal olarak enjekte edildi. Sinir hasarını takiben 2., 7., 15., 30. ve 45. günlerde sadece hasar uygulanan gruptan ve hasar+trapidil grubundan elektrofizyolojik kayıtlar yapıldı.

### BULGULAR

Sinir hasarını izleyen ikinci günde, trapidil tedavisi uygulanmayan gruptan kaydedilen EMG kayıtlarından elde edilen gastrocnemius kasına ait bileşik motor aksiyon potansiyellerinin, kontrol hayvanlarından elde edilene göre daha düşük genlikte olduğu bulundu; keza, aynı sonuç kontrol ve trapidil grubu arasında da gözlemlendi. Her iki gruptan kaydedilen (sadece hasar uygulanan grup ile hasar ve trapidil uygulanan grup) aksiyon potansiyelleri parametreleri, deneyin sonunda (45. günde) kontrol değerlerine ulaşmadı. Hasar uygulanan ile hasar ve trapidil uygulanan sıçanlardan 2., 7., 15., 30. ve 45. günlerde kaydedilen aksiyon potansiyellerinin genlik, alan ve distal latans parametreleri arasında anlamlı farklılık bulunmadı.

### SONUÇ

Sinir hasarı uygulanan sıçanlarda, bir düşük doz trapidil uygulamasının, elektrofizyolojik yöntem kullanılarak sinir koruyucu bir etkisi olmadığı sonucuna varıldı.

**Anahtar Sözcükler:** Bileşik motor aksiyon potansiyeli; sinir hasarı; sinir koruyucu; periferik sinir; trapidil.

Departments of <sup>1</sup>Biophysics and <sup>2</sup>Biostatistics, Medicine Faculty of Mersin University, Mersin, Turkey.

Mersin Üniversitesi Tıp Fakültesi <sup>1</sup>Biyofizik Anabilim Dalı, <sup>2</sup>Biyoistatistik Anabilim Dalı, Mersin.

Correspondence (İletişim): Belgin Büyükakıllı, M.D. Mersin Üniversitesi Tıp Fakültesi Yenışehir Kampüsü Biyofizik Anabilim Dalı, Mersin, Turkey. Tel: +90 - 324 - 341 28 15 Fax (Faks): +90 - 324 - 341 24 00 e-mail (e-posta): bbuyukakilli@mersin.edu.tr

It is known that crush in peripheral nervous system results in damage of intraneural microcirculation by direct mechanical injury.<sup>[1,2]</sup> Demyelination and remyelination, axonal degeneration and regeneration, focal, multifocal or diffuse nerve fiber loss and endoneural edema may be encountered due to this effect.<sup>[2,3]</sup> It is also known that free oxygen radicals increase and cause tissue damage due to the tissue destruction after the injury.<sup>[3,4]</sup> A variety of new therapeutic agents have been evaluated for their ability to improve nerve regeneration. Trapidil is one such example. Trapidil's therapeutic effect is shown in nervous tissue as well as in other tissues. It is reported that this effect of trapidil is caused both by decreasing the inflammatory response and free oxygen radicals release from neutrophils due to the inhibition of thromboxane A2 synthesis.<sup>[3]</sup> Furthermore, it is also reported that trapidil's effects on growth factors inhibit the oligodendrocyte and astrocyte proliferation after the central nervous system injury.<sup>[5,6]</sup> Under experimental conditions, the neurotrophic activity of a therapeutic agent was shown on rats either with histological analysis, sensorimotor, functional, biochemical or electrophysiological methods.<sup>[3,7-9]</sup> It may act specifically on motor or sensory fibers or both. Few experimental studies have been published concerning the effects of trapidil on peripheral nerves. The protective effects of trapidil in the treatment of ischemia and reperfusion injury in the peripheral nervous system were reported by Bagdatoglu et al. with histopathological and biochemical methods<sup>[3]</sup> and Kurtoglu et al.<sup>[8]</sup> investigated the effects of trapidil on crush injury by monitoring nitric oxide, malondialdehyde and transforming growth factor- $\beta$ 2 levels and by transmission electron microscopy in the rat sciatic nerve.

However, electrophysiological studies on trapidil's effects on regeneration in the peripheral nerves after crush injury have not been reported yet. Nerve action potential recording method has been useful in providing objective data about therapeutic agents-mediated effects on the peripheral nervous system. Measurements of action potential amplitude, area and latency may provide information about membrane  $\text{Na}^+$  and  $\text{K}^+$  transport. Compound motor action potential (CMAP) amplitude, area and latency are positively correlated with sodium transport and amplitude and area of action potential can be used to estimate the number of activated nerve fibrils.<sup>[10]</sup> Therefore, in the present study we evalu-

ated the effect of trapidil on nerve regeneration after a crush injury in the rat sciatic nerve electrophysiologically by recording CMAP.

## MATERIALS AND METHODS

Experiments were performed on 81 adult female albino rats weighing 200-225 g at the time of crush injury. Animals were housed and cared for pre- and postoperatively according to the Institutional Animal Care and Use Committee at the Mersin University Medical Faculty. They were housed in plastic cages at room temperature in a 12:12 h light-dark cycle. The rats had free access to food and water.

In this study, eight non-treated rats without a crush injury were used as the control (intact animals). Each of the crush and crush+trapidil groups was divided into 5 subgroups after the crush injury based on the regeneration period, on the 2nd, 7th, 15th, 30th and 45th days. Rats were anesthetized by ketamin HCl at a dose of 50 mg/kg intramuscularly. The sciatic nerve was exposed at the right gluteal region without any damage to the muscle tissue and crushed for 20 sec with a jeweler's forceps (no: 5). Crush level was marked on the muscle by a 4/0 non-absorbable silk suture and then the incision site was closed. All surgical procedures were conducted under sterile conditions. Rats in the therapeutic groups were administrated a single dose of trapidil (8 mg/kg) (Rocornal; Rentschler Biotechnologie GmbH, Laupheim, Germany) intraperitoneally just after the injury. The dose of trapidil was chosen on the basis of the daily human dose and previous experiments that reported substantial benefits.<sup>[11]</sup>



Fig. 1. BIOPAC MP 100 general EMG recording system.

**Table 1.** Descriptive statistics for action potential parameters studied

Day	Group	CMAP peak-to-peak amplitude (mV)	Area (mV.ms)	Distal latency (ms)
2	Control	10.44±0.90	0.01466±0.00458	0.24±0.02
	Cr	3.63±1.82	0.00430±0.00213	3.06±1.41
	Cr + Tr	3.56±1.21	0.00402±0.00251	2.27±0.79
7	Control	10.06±0.95	0.01459±0.00247	0.23±0.04
	Cr	3.96±1.75	0.00711±0.00241	1.74±0.34
	Cr + Tr	4.39±1.31	0.00559±0.00177	2.19±0.79
15	Control	10.22±0.62	0.01615±0.00303	0.22±0.03
	Cr	2.98±1.02	0.00579±0.00223	2.21±0.65
	Cr + Tr	3.69±2.13	0.00470±0.00354	2.11±1.09
30	Control	10.08±0.91	0.01767±0.00183	0.24±0.02
	Cr	4.38±0.58	0.00777±0.00330	2.29±1.33
	Cr + Tr	5.24±0.97	0.00597±0.00186	1.45±0.23
45	Control	10.11±1.08	0.01489±0.00395	0.25±0.03
	Cr	5.52±0.53	0.00809±0.00141	2.07±0.79
	Cr + Tr	5.12±0.97	0.00672±0.00249	2.02±0.58

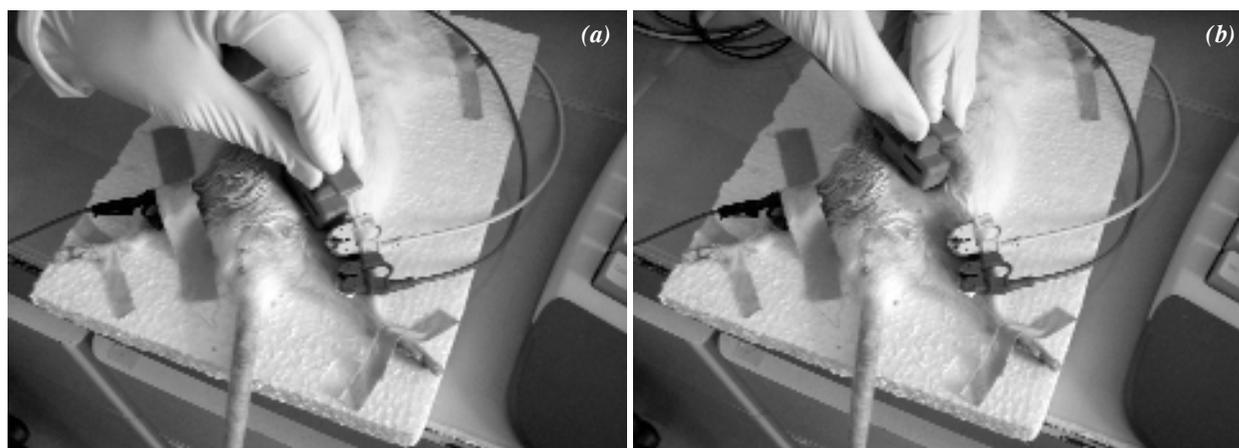
Cr: Crush applied; Tr: Trapidil applied; Values are mean ± SD.

### Electrophysiological recording

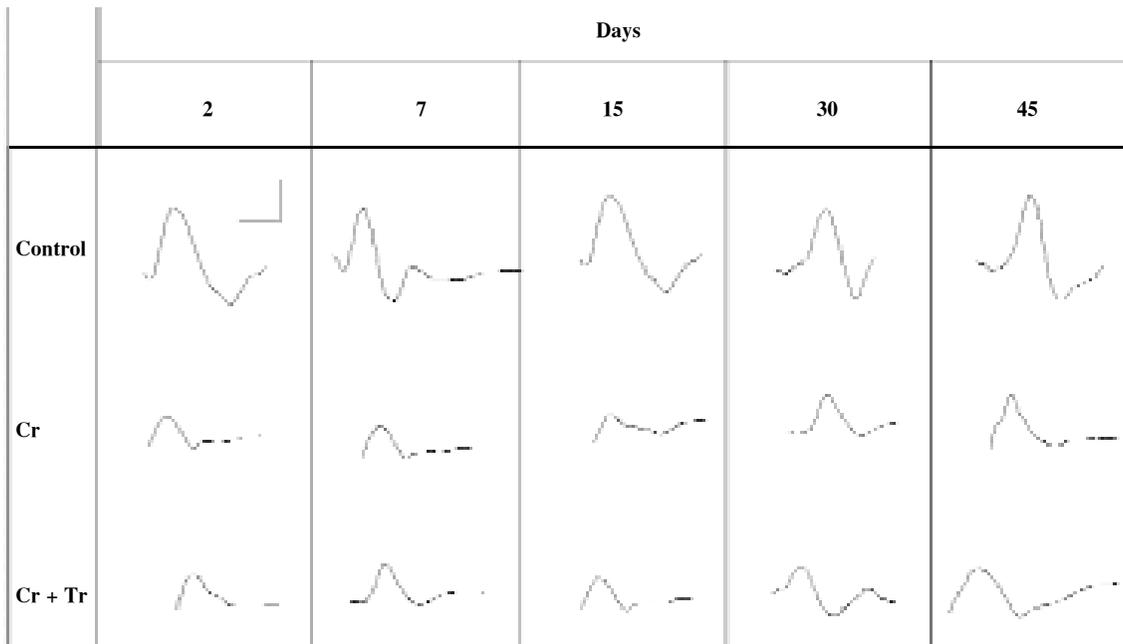
Electrophysiological recordings (CMAP) across the injured nerve segment were made using BIOPAC MP 100 acquisition system (Santa Barbara, USA) (Fig. 1 and Fig. 2). Rats were anesthetized by ketamin HCl at a dose of 50 mg/kg intramuscularly. CMAP were recorded from right side on the 2nd, 7th, 15th, 30th and 45th days after the crush injury in all five groups. CMAP that were recorded from controls on the 2nd, 7th, 15th, 30th and 45th days served as the baseline data. Bipolar

surface electrodes (Medelec small bipolar nerve electrodes, 6894T, Oxford, UK) were used for stimulation.

The ground electrode was placed on the thigh on the stimulation side. The supramaximal stimulus consisted of single square pulse (intensity 10 V, duration 0.5 ms). CMAPs were recorded from the distal end of gastrocnemius muscle with surface disc electrodes (Medelec, number 017K006, Oxford, UK) (Fig. 2a). BIOPAC Acknowledge Analysis Software (ACK 100 W) was used to measure CMAP



**Fig. 2.** Arrangement for measuring latency in the sciatic nerve. The nerve is stimulated distally (a) and proximally (b), and the evoked potentials are recorded from the gastrocnemius muscle.

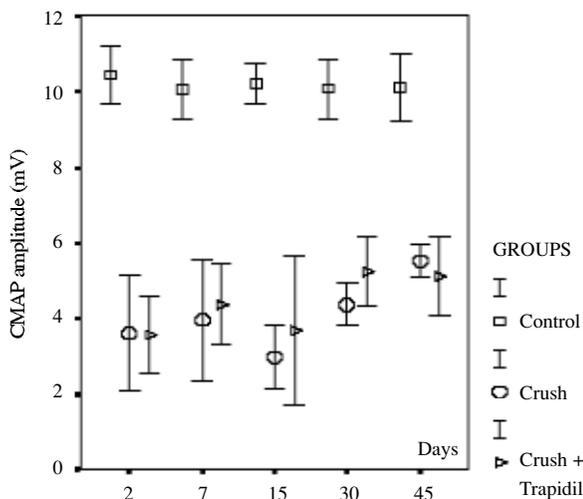


**Fig. 3.** Records of the compound motor action potential (CMAP) at control group and after crush lesion of the sciatic nerve observed through 45 days in rats non-treated (Cr) or treated with trapidil (Cr+Tr). Calibrations for all traces are shown in upper left; vertical bar = 4.5 mV; horizontal bar = 2.2 ms.

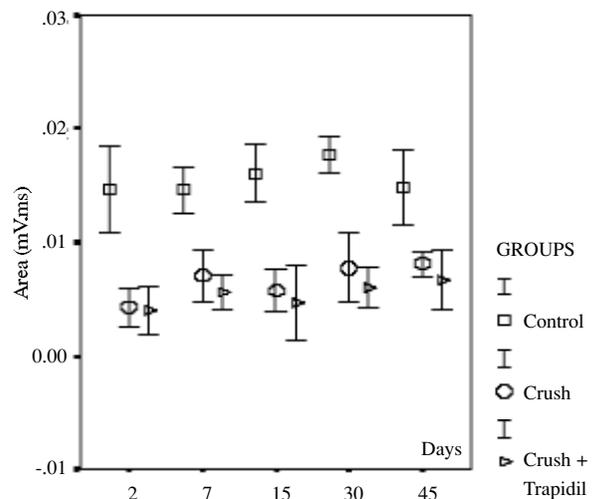
peak-to-peak amplitude, area and distal latency (DL). The distal latency (DL) of evoked potential was measured in milliseconds. DL includes the duration of motor nerve conduction between the stimulating and the recording electrodes.

**Statistical analysis**

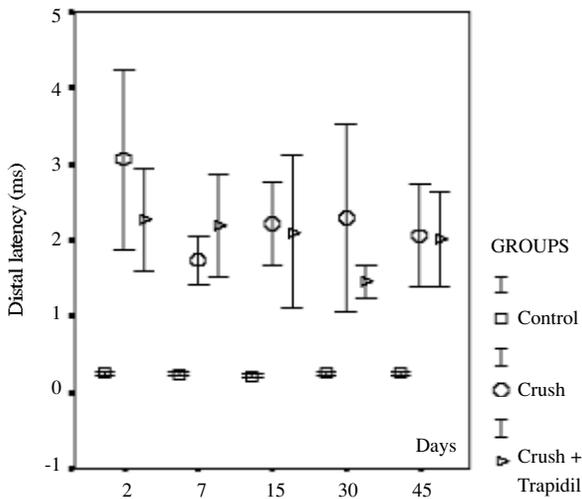
After testing normal distribution with Kolmogorov-Smirnov, the data were statistically analyzed by an analysis of variance (two-way ANOVA) by



**Fig. 4.** Recovery of the peak-to-peak amplitudes of compound motor action potentials (CMAPs) after crush lesion of the sciatic nerve observed through 45 days in rats non-treated or treated with trapidil. Data are means  $\pm$  SD.



**Fig. 5.** Mean area of compound motor action potentials after crush lesion of the sciatic nerve observed through 45 days in rats non-treated or treated with trapidil. Data are means  $\pm$  SD.



**Fig. 6.** Time course of the distal latency measured in parallel with CMAP in trapidil-treated or trapidil-non-treated rats after crush lesion of the sciatic nerve, and controls. Data are means  $\pm$  SD.

using SPSS 11.5 for Windows. Following these analyses, a Bonferroni's post hoc test was used to determine the significant differences. Data were analyzed for day/group interaction. Descriptive statistics of the results are shown in Table 1. The significance was set at  $p < 0.05$ . All data are given as mean  $\pm$  SD. The error bars were used for graphics (Figs. 4-6).

## RESULTS

### Electrophysiological data

**Compound motor action potential (CMAP).** Records of CMAP at control group and after crush lesion of the sciatic nerve observed through 45 days in rats non-treated (Cr) or treated with trapidil (Cr+Tr) are shown in Fig. 3. As seen in Table 1, Fig. 3 and Fig. 4, there were not significant differences between 2nd, 7th, 15th, 30th and 45th days on the control group regarding CMAP amplitude.

EMG recordings on the second day following the crush injury showed low values of CMAP in the gastrocnemius muscle when compared to normal values obtained in intact animals ( $3.63 \pm 1.82$  mV compared to about  $10.44 \pm 0.90$  mV normal values,  $p < 0.01$ ), suggesting a partially interruption of the signal through the nerve fibers (Fig. 3 and Fig. 4); also, the values were significantly different between the control and trapidil-treated groups

( $10.44 \pm 0.90$  mV and  $3.56 \pm 1.21$  mV, respectively,  $p < 0.01$ ). CMAPs increased significantly on rats with crush on the 45th day following the crush ( $p < 0.01$ ) but they did not increase on the rats with crush-trapidil even on the 45th day.

CMAP values for both groups did not yet reach baseline values at the end of the experiment. There was no difference in the CMAP amplitudes between rats with crush but non-treated and treated with trapidil on the 2nd, 7th, 15th, 30th and 45th days. So, CMAP amplitude was not influenced by treatment with trapidil.

**Area.** CMAP area between 2nd, 7th, 15th, 30th and 45th days on the control group did not show statistical difference (see Table 1 and Fig. 5).

EMG recordings on the second day following the crush indicated low values in CMAP area in the gastrocnemius muscle when compared with normal values obtained in intact animals ( $0.00430 \pm 0.00213$  mV.ms compared to about  $0.01466 \pm 0.00458$  mV.ms normal values,  $p < 0.01$ ), suggesting a partially interruption of the signal through the nerve fibers; also, the values were significantly different the control and trapidil-treated groups ( $0.01466 \pm 0.00458$  mV.ms and  $0.00402 \pm 0.00251$  mV.ms, respectively,  $p < 0.01$ ).

CMAP area increased significantly on the rats with crush on the 45th day following the crush ( $p < 0.05$ ) but they did not increase on rats with crush-trapidil even on the 45th day. CMAP values for both groups did not yet reach baseline values at the end of the experiment. There were no differences in the CMAP areas between rats with crush but non-treated and treated with trapidil on 2nd, 7th, 15th, 30th and 45th days. So, CMAP area was not influenced by treatment with trapidil.

**Distal latency (DL).** DL values did not show statistical difference for control group on all days (see Table 1 and Fig. 6). DL increased greatly after the lesion in both groups (crush:  $3.06 \pm 1.41$  ms; crush-trapidil:  $2.27 \pm 0.79$  ms) as compared with normal values of the rats in the control group ( $0.24 \pm 0.02$  ms) ( $p < 0.01$ ), suggesting a decrease of the motor nerve conduction velocity. DL values for both groups had not yet reached normal values at the end of the experiment. There was no significant difference in the DL values for both groups on all days.

## DISCUSSION

Experimental studies are important sources of information on peripheral-nerve regeneration and the sciatic nerve of the rat is probably the most used animal model due to its similarities to the human peripheral nerve. Peripheral nerve trunks are well-vascularized structures where a well-developed collateral system may compensate for local vascular damage. It is well known that pathological events such as trauma, compression and crushing directly cause mechanical injury to nerve fibers and deteriorate neuronal functions by impeding the intraneural microvasculature.<sup>[1,2]</sup> Impulse transmission and axonal transport are dependent on a continuous local energy supply provided by the intraneural microcirculation. Therefore, depletion of high-energy phosphates and resultant conduction failure ensue as soon as intraneural blood flow decreases. Morphologically, ischemic nerves reveal various pathological abnormalities, including demyelination and remyelination, axonal degeneration and regeneration, focal, multifocal or diffuse nerve fiber loss and endoneural edema.<sup>[3]</sup>

A neuroprotective effect of dose of 8 mg/kg of trapidil in rat crush injury model could not be observed during our study period using electrophysiological method. The dose of trapidil used in the present study, a single dose of trapidil (8 mg/kg) intraperitoneally, was chosen on the basis of the daily human dose and previous experiments that reported substantial benefits.<sup>[11]</sup> In view of the finding, because of CMAPs increased significantly on rats with crush injury on the 45th day following the crush ( $p < 0.01$ ) but they did not increase on rats with crush-trapidil even on the 45th day, we have demonstrated that regeneration was faster in the crush group but that a time window of 45 days was probably still too short to witness complete regeneration. So, in this study, it was shown that trapidil retards myelin regeneration. Also, Kurtoglu et al.<sup>[12]</sup> reported that trapidil has been retarding myelin regeneration and that this influence was devoted to its growth factor inhibiting effect.

In the present study, we used a time window of 45 days for the study of electrophysiological parameters under crush alone and crush with trapidil treatment. The curves for recovery of electrophysiological parameters of nerve function went almost exactly parallel in both groups, such that an

effect of trapidil could not be established. The study was stopped when the values of crush-trapidil group obtained at repetitive measurements did not change any more, i.e., when regeneration seemed to have reached a plateau.

Kurtoglu et al.<sup>[8]</sup> reported that treatment with a single dose of 8 mg/kg intraperitoneal trapidil prevented cell damage and edema at the injury site at a time window of 45 days. Therefore Kurtoglu et al.<sup>[8]</sup> indicated that trapidil had neuroprotective effect in rat crush injury model using histological method. In the present study using the same experimental model, we found that trapidil's neuroprotective effect on electrophysiological parameters could not be reflected. Kurtoglu et al.<sup>[8]</sup> reported that there was not a harmony between the histological and biochemical results of their study, so they hypothesized that the trapidil dose used in their study might have been below or just at the threshold of effect and higher doses or multiple injections might show detectable alterations in the NO, MDA and TGF-B2 levels. Bagdatoglu et al.<sup>[3]</sup> evaluated the efficacy of the antiplatelet and vasodilator agent trapidil in the amelioration of ischemia/reperfusion injury in rat sciatic nerves. They demonstrated that trapidil had protective effects in the peripheral nervous system in the treatment of ischemia and reperfusion injury.<sup>[3]</sup> They administered the trapidil to rats as a single dose and 8 mg/kg.

Distal latency, so conduction velocity across an injured nerve has often been used to determine the success of regeneration.<sup>[13]</sup> Recently, Meyer et al.<sup>[14]</sup> examined recovery of motor function by measuring the conduction properties of the rat sciatic nerve at 8 and 16 weeks. They found that the mean conduction velocity of the sciatic nerve in the crush group was significantly slower than that of controls at both 8 and 16 weeks, but had not yet reached normal values. Similarly, in the present study, we found that DL increased greatly after the lesion in crush group as compared with normal values in rats of control, suggesting a decrease of the motor nerve conduction velocity, but that DL values had not yet reached normal values on the 45 days. So our results were in agreement with study by Meyer et al.<sup>[14]</sup> Bischofs et al.<sup>[9]</sup> reported that electrophysiological studies carried out over a period of 3 months after sciatic nerve crush did not show major differences between topiramate-, a neuroprotective drug

in models of cerebral ischemia and facial nerve lesion, and saline-treated rats. They showed that CMAPs increased from day 35 in both groups but did not yet reach baseline values by the end of the experiment at 3 months. In the present study, CMAP values for both groups did not yet reach baseline values at the end of the experiment. So, our results of crush group were in agreement with their saline-treated group.

In conclusion we could not prove a neuroprotective effect of a single dose and 8 mg/kg trapidil in rat crush injury model using electrophysiological method. So, further studies that higher doses or multiple injections might show detectable alterations in the electrophysiological parameters are needed.

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### REFERENCES

1. Lundborg G. Intraneural microcirculation. *Orthop Clin North Am* 1988;19:1-12.
2. Zochodne DW, Ho LT. Endoneurial microenvironment and acute nerve crush injury in the rat sciatic nerve. *Brain Res* 1990;535:43-8.
3. Bagdatoglu C, Saray A, Surucu HS, Ozturk H, Tamer L. Effect of trapidil in ischemia/reperfusion injury of peripheral nerves. *Neurosurgery* 2002;51:212-20.
4. Arslan E, Milcan A, Unal S, Demirkan F, Polat A, Bagdatoglu O, et al. The effects of carnitine on distally-burned dorsal skin flap: an experimental study in rats. *Burns* 2003;29:221-7.
5. Takamiya Y, Kohsaka S, Toya S, Otani M, Mikoshiba K, Tsukada Y. Possible association of platelet-derived growth factor (PDGF) with the appearance of reactive astrocytes following brain injury in situ. *Brain Res* 1986;383:305-9.
6. McKay JS, Blakemore WF, Franklin RJ. The effects of the growth factor-antagonist, trapidil, on remyelination in the CNS. *Neuropathol Appl Neurobiol* 1997;23:50-8.
7. Azzouz M, Kenel PF, Warter J-M, Poindron P, Borg J. Enhancement of mouse sciatic nerve regeneration by the long chain fatty alcohol, N-Hexacosanol. *Exp Neurol* 1996;138:189-97.
8. Kurtoglu Z, Ozturk AH, Bagdatoglu C, Polat G, Aktekin M, Uzmsansel D, et al. Effects of trapidil after crush injury in peripheral nerve. *Acta Med Okayama* 2005;59:37-44.
9. Bischofs S, Zelenka M, Sommer C. Evaluation of topiramate as an anti-hyperalgesic and neuroprotective agent in the peripheral nervous system. *J Peripher Nerv Syst* 2004;9:70-8.
10. Daube JR. Alteration of wave forms and artifacts. In: Daube JR, editor. *Clinical neurophysiology*. Philadelphia: FA Davis; 1996. p. 65-8.
11. Okamoto S, Inden M, Setsuda M, Konishi T, Nakano T. Effects of trapidil (triazolopyrimidine), a platelet-derived growth factor antagonist, in preventing restenosis after percutaneous transluminal coronary angioplasty. *Am Heart J* 1992;123:1439-44.
12. Kurtoglu Z, Ozturk AH, Bagdatoglu C, Turac A, Camdeviren H, Uzmsansel D, et al. Effect of trapidil on the sciatic nerve with crush injury: a light microscopic study. *Neuroanatomy* 2004;3:54-8.
13. Fields RD, Ellisman MH. Axons regenerated through silicone tube splices. I. Conduction properties. *Exp Neurol* 1986;92:48-60.
14. Meyer RS, Abrams RA, Botte MJ, Davey JP, Bodine-Fowler SC. Functional recovery following neurotomy of the rat sciatic nerve by epineurial repair compared with tubulization. *J Orthop Res* 1997;15:664-9.