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Effect of trapidil on the sciatic nerve with crush injury: a light microscopic study

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Abstract

Trapidil's therapeutic effect is shown in nervous tissue in ischemia and reperfusion injury, but any study on trapidil's effects on regeneration in the peripheric nervous system after crush injury is not encountered in the literature.

In this study, 40 female albino wistar rats were used. The sciatic nerves were crushed for 20 seconds by a jeweler's forceps. A single dose of 8 mg/kg of trapidil was administered to the treatment group intraperitoneally. After the crush injury, the crush site was excised on 2nd, 7th, 15th, 30th, and 45th days, fixed in formalin and then prepared for routine histological evaluation. Each section was stained with toluidin blue. Myelin thicknesses were measured by an ocular micrometer.

Measurements were evaluated by factorial analysis of variance. Separation of myelin lamellae and vacuole formation, which are signs of axonal degeneration were seen on the 7th and 15th days of both groups, but were more prominent in the crush group. Regenerating myelinated fibers were increased on the 30th and 45th days in increasing density. Interaction for myelin thickness was statistically significant with Student-Neyman-Keuls Post Hoc test ($p=0.038$). In the trapidil group, myelin thickness was less on the 15th and 45th days according to the control group ($p<0.05$).

These findings were interpreted as trapidil was effective in preventing edema and myelin damage by preventing vasospasm, inactivating macrophages, inhibiting the inflammatory response and stabilizing the cell membrane. On the other hand, it is thought that trapidil had a retarding effect on myelin regeneration in the recovery period.

Key words: [trapidil] [crush injury] [myelin sheath] [sciatic nerve]

Introduction

It is known that crush in peripheral nervous system results in damaging of intraneuronal microcirculation by direct mechanical injury [1,2]. Demyelinization and remyelinization, axonal degeneration and regeneration, focal, multifocal or diffuse nerve fiber loss and endoneural edema may be encountered due to this effect [2,3]. It is also known that after the injury due to the tissue destruction, free oxygen radicals increase and cause tissue damage [3–6].

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 [3]. Furthermore, it is also reported that trapidil's effects on growth factors inhibit the oligodendrocyte and astrocyte proliferation after the central nervous system injury [7,8]. However, any study on trapidil's effects on regeneration in the peripheric nervous system after crush injury is not encountered in the literature.

In this study, trapidil which was reported to have protective effects in the central nervous system and many other tissues, is evaluated histologically and morphometrically in the peripheric nervous system after crush injury.

Material and Methods

The procedures were reviewed and approved by the Institutional Animal Care and Use Committee at Mersin University Medical Faculty. In this study 40 female albino wistar rats (ten weeks old and weighted 200–225 gr) were used. After the crush injury, crush and crush + trapidil groups were divided into 5 subgroups as 2nd, 7th, 15th, 30th and 45th days. Rats were anaesthetized by ketamin HCl of 50 mg/kg intramuscularly. The sciatic nerve was exposed at the right gluteal region without any damage to the muscular tissue and crushed for 20

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Table 1. Descriptive statistics of measurements. ***(SEM:** Standard Error Mean)*

Group	Day	n	Total Diameter	Myelin Thickness
			Mean±SEM	Mean±SEM
Trapidil+Crush	2	4	63.417±1.278	20.208±0.718
	7	4	72.857±1.323	22.098±0.744
	15	4	53.059±1.519	19.147±0.854
	30	4	40.676±1.628	19.730±0.915
	45	4	53.778±1.476	17.556±0.830
Crush	2	4	66.167±1.476	20.694±0.830
	7	4	56.703±1.468	19.225±0.825
	15	4	72.333±1.808	28.333±1.016
	30	4	66.833±1.278	20.375±0.718
	45	4	79.250±1.808	23.333±1.016

seconds by using a jeweler's forceps (no: 5) in sterile operative conditions [10]. Crush level was marked on the muscle by a 4/0 non-absorbable silk suture and then the incision site was closed. Rats of therapeutic groups were administrated a single dose of trapidil (8mg/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 [9]. Rats were sacrified by exsanguination after ketamin anaesthesia on the 2nd, 7th, 15th, 30th and 45th days. The right gluteal region is dissected and a 5 mm long sciatic nerve section including the crush site is cut, and then fixed in formalin.

After embedding in paraffin, 5μm thick slices were taken and stained by toluidin blue. The histological features were then evaluated in X400 and X1000 magnification by light microscope. For the myelin thickness measurements, an ocular micrometer was used for the X1000 magnification. In a randomly selected area, both external and internal diameters of the myelinated fibers were measured from the section of each rat's sciatic nerve (Figure 1A). Myelin thickness was calculated as (external diameter–internal diameter) / 2 (Table 1). Results were evaluated with factorial analysis of variance with two factors. Student-Neyman-Keuls Post Hoc test was used in order to determine the significant differences. Descriptive statistics as mean± SEM (standard error mean) is given in Table 1. Type I error rate was accepted as 0.05 in statistical calculation.

Results

Histological evaluation of the sections

2nd day. Diffuse erythrocyte infiltration in the epineurium was seen outside the vessels in both groups. Myelin degeneration was more prominent in large and medium sized myelinated fibers. Small sized myelinated fibers were protected. Degranulated mast cells were found in the connective tissue of both groups (Figure 1A).

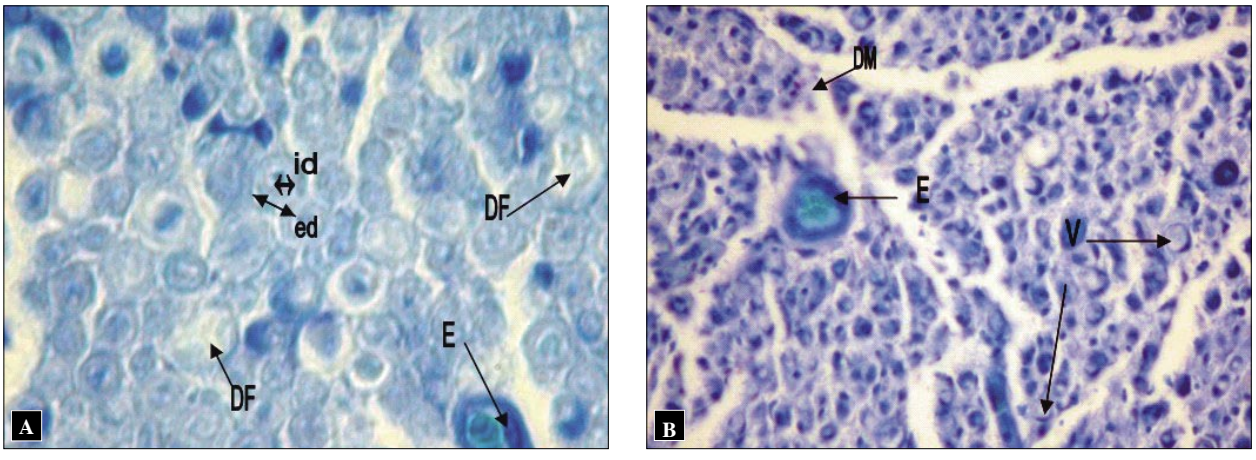

Figure 1. A–B: Illustrations show toluidin blue stained sections for 2nd day of crush group and external diameter (ed), internal diameter (id) measurements of a myelinated fiber [X1000] (**A**); 7th day of crush group [X400] (**B**). See next page for abbreviations.

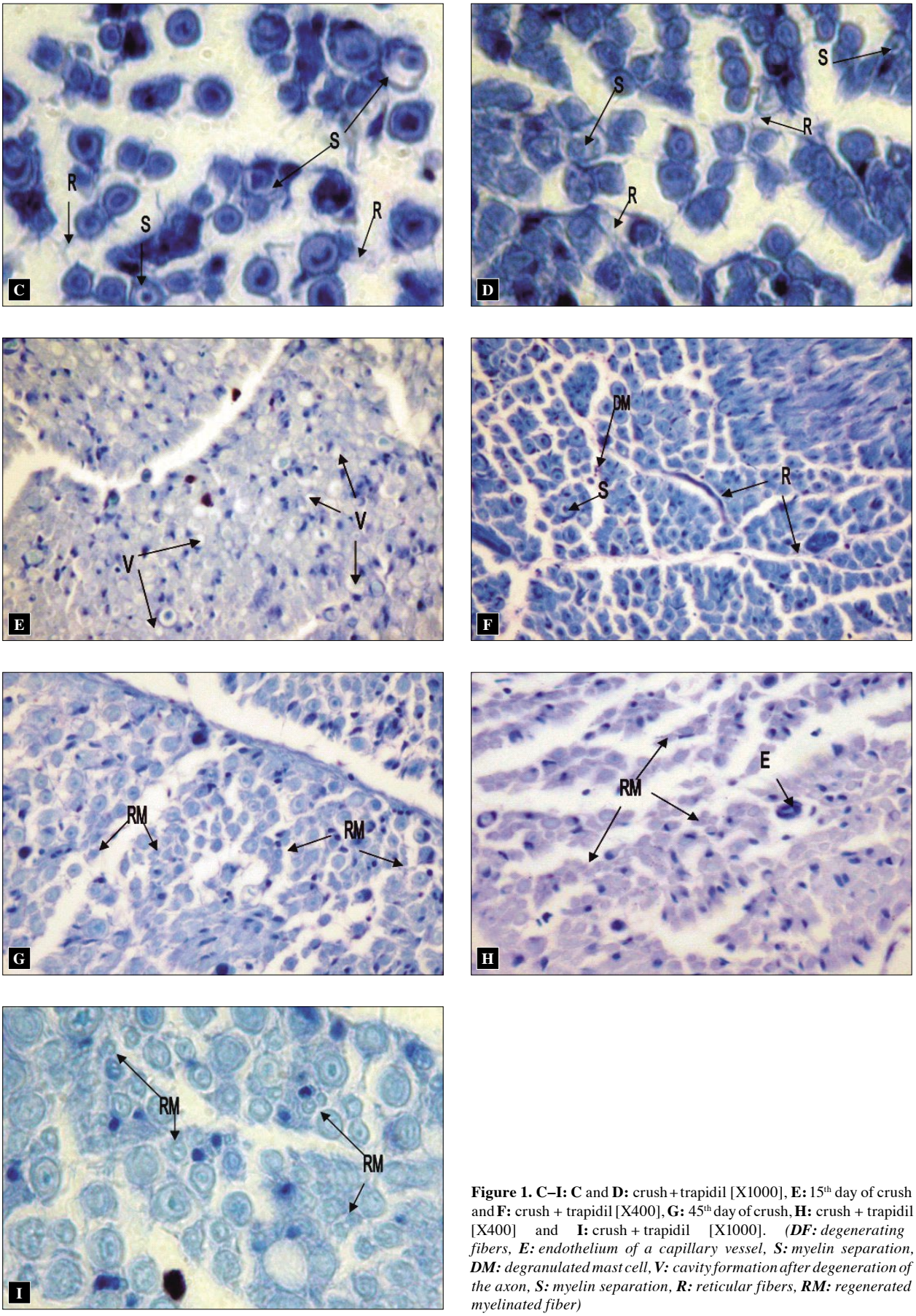

Figure 1. C–I: C and **D:** crush + trapidil [X1000], **E:** 15th day of crush and **F:** crush + trapidil [X400], **G:** 45th day of crush, **H:** crush + trapidil [X400] and **I:** crush + trapidil [X1000]. *(**DF:** degenerating fibers, **E:** endothelium of a capillary vessel, **S:** myelin separation, **DM:** degranulated mast cell, **V:** cavity formation after degeneration of the axon, **S:** myelin separation, **R:** reticular fibers, **RM:** regenerated myelinated fiber)*

7th day. There was diffuse myelin degeneration in both groups, but slightly less in the trapidil group. Small myelinated fibers were protected. Myelin separation was prominent in both groups. Vacuoles and endoneurial macrophages were more prominent according to the 2nd day results and the trapidil group. Again degranulated mast cells were seen in both groups (Figures 1B, C, D).

15th day. There were diffuse degeneration of the myelinated fibers, and diffuse myelin separation in both groups. According to the 7th day results which were less prominent, vacuole formation was seen only in some of the fibers. But they were more numerous and larger in the crush group. Degranulated mast cells and macrophages were seen especially in the crush group (Figures 1E, F).

30th day. Myelin separation was less prominent, when compared with the 7th and 15th days results. A few vacuole formations were seen in both groups. Regenerating myelinated axons were observed in the crush group and they were more numerous than the 15th day. In the crush group, there were thicker myelinated fibers. Remyelination of the new fibers were more prominent in the crush group than in the trapidil group.

45th day. Regenerated fibers were diffusely observed in both groups and were more prominent than the 30th day. Remyelination of the new fibers were more prominent in the crush group. Vacuoles were not observed in both groups. Myelin separation was nearly absent (Figures 1G, H, I).

Evaluation of measurements of the myelin thicknesses

Group/day interaction was found statistically significant for the myelin thicknesses ($p=0.038$) and according to this result, differences were as follows with Student-Neyman-Keuls post hoc test: When the days were compared; there was no statistically significant difference within the trapidil group. In the crush group, there were statistically significant differences only between the 7th and 15th, and 15th and 30th days ($p<0.05$). There was no statistically significant difference between the other days for this group.

When the groups were compared; differences between the two groups were found statistically significant on the 15th and 45th days ($p=0.05$). In those days, myelin thickness was higher in the crush group than in the trapidil group. There was no statistically significant differences between the groups on the other days.

Discussion

Edema in the peripheral nerve after the crush injury; impacts on the endoneural microenvironment by increasing the pressure, decreasing the blood flow or changing the electrolyte concentration in the endoneural fluid. Ischemia causes impairment of the axon and Wallerian like degeneration ensues if the restoration of the adequate circulation is delayed. Axonal and myelin degenerations are observed at the lesion site and distal to it a week later. After 3 weeks, most of the axons regenerate and remyelinate. Functional recovery is completed in nearly 4 or 5 weeks [10]. All these data are consistent with our findings.

External pressure to the peripheral nerve results in ranging from delayed venular flow in the epineurium to complete circulatory arrest according to the duration and intensity of the pressure [1]. In a serious trauma like crush, a short period of localized total or subtotal ischemia is followed by evident increase in the endoneural fluid pressure and impairment of the normal capillary blood flow in the endoneurium. All of these results in the release of the endogenous chemical mediators, increase in the vascular permeability and impairment of the blood-nerve barrier. Endoneural and intraneural edema with inflammatory response follows this process [1, 2, 11, 12]. Edema observed in the myelin sheath in both groups of the 2nd, 7th and 15th days is consistent with the above data.

Trapidil is well known as a vasorelaxant, which prevents tissue damage by diminishing the vasospasm due to the crush injury [13, 14]. Additionally, the tissue protective and edema preventive effects of trapidil in the injury site are explained by its membrane stabilizing effect due to the inhibition of tromboxane A2, and inhibition of inflammatory response by its macrophage inactivation effect [3, 15]. Tromboxane A2 is released in ischemia and injury of the tissue and causes free oxygen radical release by inducing neutrophils [3]. High level of the free oxygen radicals increase the tissue damage and lipid peroxidation in the lipid rich nervous tissue even more [3, 12]. Bagdatoglu et al. proposed that decrease in lipid peroxidation by trapidil was another way of tissue protection in ischemia/reperfusion injuries in the peripheral nerve [3]. In our study, less myelin damage, endoneural edema and myelin separation on the 7th and 15th days in the trapidil group are interpreted as the causes of tissue protective effect of trapidil.

Trapidil is defined as a specific platelet–derived growth factor (PDGF) antagonist. However, some of the mechanisms of its effects are not proven yet [7, 8, 16]. It is proposed that trapidil causes a general blockage in the growth factor related responses and so impacts on the nerve tissue healing process [8, 17].

Takamiya et al. showed that trapidil decreased the astrocyte proliferation by inhibiting platelet derived PDGF release in the central nervous system injuries [7]. McKay et al. reported that oligodendrocyte myelination in rats was evidently decreased. It was also proposed that PDGF affected on oligodendrocytes in the proliferation, migration, survival and differentiation processes. On the other hand, trapidil decreased the proliferation of the oligodendrocytes by inhibiting the production of the growth hormone by astrocytes, microglia and axons [8]. Decreased myelin thickness in the trapidil group when compared with the crush group on the 45th day in our study is thought to be due to trapidil's retarding effect on myelin regeneration. It is also thought that extra studies are necessary in order to evaluate the mechanisms of trapidil's effects on myelin regeneration and whether its effects are due to PDGF or other growth factors or not.

Conclusion

In this study, it is shown that the trapidil decreased myelin degeneration on the 7th and 15th days after crush injury in the peripheral nervous system. Unfortunately, this agent retards myelin regeneration, and this influence is devoted to its growth factor inhibiting effect.

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