CDP-choline and its endogenous metabolites, cytidine and choline, promote the nerve regeneration and improve the functional recovery of injured rat sciatric nerves

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**Objective:** Topical cytidine-5'-diphosphocholine (CDP-choline) has been shown to improve the functional recovery and promote the nerve regeneration of injured sciatic nerves in rats. The aims of this study were to test whether CDP-choline was effective at promoting nerve healing when the surgery to repair an injury was delayed and to determine whether the cytidine and/or the choline moieties of CDP-choline contribute to its beneficial actions.

**Methods:** One hundred and fifty Sprague-Dawley rats underwent a surgical procedure that involved damaging the right sciatic nerve and suturing the epineurium. The injured sciatic nerve was either repaired immediately (on the first day) or on the third day after surgery. Rats were assigned to one of five groups and received a topical application of either 0.4 ml of saline (control) or 0.4 ml of 100 \(\mu\)M CDP-choline, cytidine, choline, or cytidine + choline.

**Results:** The sciatic function index (SFI) of the rats in both groups (those who had their nerve repair immediately versus those on day 3) improved gradually by 4, 8, and 12 weeks after surgery. The percentage recovery in SFI score was significantly higher in rats treated with CDP-choline or cytidine + choline at all time points. Axon count increased by \(\sim 50\%\) in rats treated either with CDP-choline or cytidine + choline. Treatment with CDP-choline or cytidine + choline reduced scar formation and decreased nerve adherence when the sciatic nerve was repaired immediately, and rats treated with CDP-choline or cytidine + choline had better axonal organization than control rats. Treatment with choline or cytidine alone led to a less marked improvement in SFI score and failed to increase axon count.

**Conclusion:** Our results demonstrate that CDP-choline, as well as the combination of its metabolites, cytidine + choline, improves the functional recovery and promotes the regeneration of injured sciatic nerves treated with immediate or delayed surgical repair in rats.

**Keywords:** Citicoline, Peripheral nerve, Scarring

**Introduction**

Cytidine-5'-diphosphocholine (CDP-choline, also known as citicoline) has shown beneficial effects in a variety of models of central nervous system injury as well as in neurodegenerative diseases in humans.\(^1\)\(^-\)\(^3\) In a rat model of cerebral ischemia, citicoline was found to increase survival time, decrease cerebral infarct size, and prolong the duration of ischemia required to produce a specified behavioral deficit or infarct size.\(^4\)\(^-\)\(^6\) In a model of traumatic brain injury, CDP-choline was found to decrease neurobehavioral deficits\(^7\) and reduce brain edema and blood–brain barrier breakdown.\(^8\) CDP-choline was also found to reduce infarct size and decrease mortality in hypotensive rats with subarachnoid hemorrhages and basilar artery occlusion.\(^9\) A neuroprotective effect of citicoline has been also shown in clinical studies in which older subjects who were given citicoline had improvements in their cognitive and behavioral parameters.\(^10\)\(^,\)\(^11\) In patients who have had a stroke, citicoline has been found to improve neurologic outcomes\(^12\)\(^-\)\(^16\) and reduce the size of the ischemic injury.\(^17\)

Recently, we showed\(^18\) that CDP-choline improves functional recovery, promotes nerve regeneration, and reduces perineural scarring after immediate surgical repair in a rat model of peripheral nerve injury. In the present study, we first sought to determine whether CDP-choline was also effective...
when peripheral nerve injury was repaired several hours after injury, which may be more relevant to most clinical scenarios. Second, we sought to determine if the choline or cytidine moieties of CDP-choline, alone or in combination, had beneficial actions on nerve regeneration in a peripheral nerve injury model in rats. It is well known that exogenously administered CDP-choline is broken down into both choline and cytidine/uridine,19 which serve as substrates for the synthesis of phosphatidylcholine and promote cell membrane synthesis and repair20–23 and neurogenesis both in vitro24 and in vivo.19,25–27 These steps are essential for the process of recovery from neural injury.1–5 Choline derived from CDP-choline produces several pharmacologic effects that are cholinergic in nature, and thus by providing free choline, exogenously administered CDP-choline increases cholinergic neurotransmission.28–31 Choline also decreases the severity of the inflammatory response32 and prevents endotoxin-induced multi-organ injury.32,33

Material and Methods
Animals and general procedures
A total of 150 adult female Sprague-Dawley rats weighing 200–300 g were used in this study. All animals were housed in cages with four rats per cage and allowed free access to laboratory chow and tap water.

All parts of the study were performed in the Experimental Animals Breeding and Research Center of the Medical Faculty of Uludağ University. Animal care was conducted with the prior approval of the Animal Experimental Ethics Committee of Uludağ University.

Surgical procedures
Anesthesia was induced with an intraperitoneal injection of 30 mg/kg of thiopental sodium (Pental™ Sodyum; İ.E. Ululay, İstanbul, Turkey). After rats had their fur shaved and the skin at the surgical site disinfected with povidone-iodine solution (Glividon™; Bikar Ilaç San., Istanbul, Turkey), the right sciatic nerve of each rat was exposed through an incision that split the gluteal muscle. Following sharp transection of the sciatic nerve 10 mm from the sciatic foramen with a scalpel, immediate or delayed epineural repair of the free nerve ends was performed with 8-0 monofilament polypropylene suture (Prolene™; Ethicon/Prolene Ltd, Somerville, NJ, USA). Delayed nerve repair occurred 3 days after the initial surgery. Care was taken not to apply undue tension to the repair site. Since we thought that putting anchoring stitches to surrounding musculature could have caused additional scarring, we did not take any specific surgical action to prevent the transected nerve ends to be apart in the group of rats having their repair 3 days later.

In 75 rats, an immediate surgical repair was performed following sharp transection of the sciatic nerve and rats were randomly assigned either to the control group (n=15) or one of the four experimental groups (n=15 per group). Control animals had their surgical sites wrapped with an absorbable gelatin sponge that was impregnated with 0.4 ml of saline. Rats in the experimental groups had their surgical sites wrapped with absorbable gelatin sponges that were impregnated either with 0.4 ml of CDP-choline (100 µM), cytidine (100 mM), choline (100 µM), or cytidine (100 µM) + choline (100 µM).

In 75 rats, the wound was closed and the transected sciatic nerves were repaired on the third day after the sciatic nerve injury was induced. These rats were re-anesthetized with an intraperitoneal injection of 30 mg/kg of thiopental sodium (Pental Sodyum). Then, the wound was re-opened and an epineural repair of the free nerve ends was performed with 8-0 monofilament polypropylene suture (Prolene) as described above. Rats were randomly assigned either to the control group (n=15) or to one of the four experiment groups (n=15 per group) and treated as described above. Briefly, the control group of animals had their surgical sites wrapped an absorbable gelatin sponge that was impregnated with 0.4 ml of saline, whereas rats in the experimental groups had their surgical sites wrapped with absorbable gelatin sponges that were impregnated with 0.4 ml of CDP-choline (100 µM), cytidine (100 mM), choline (100 µM), or cytidine (100 µM) + choline (100 µM).

The wounds were closed in layers and rats were returned to their cages after they recovered from anesthesia. All operations were performed by the same neurosurgeon, who used micro-instruments and a surgical microscope (Zeiss Opmi 6; Carl Zeiss, Oberkochen, West Germany), under ×4 magnification.

Functional evaluation
Sciatic function index (SFI) scores were measured by walking track analysis that was performed both preoperatively and 4, 8, and 12 weeks after the operation, as described previously.18,34,35 The walking track was constructed of a 10 × 100 cm wooden corridor that was open on one end to a darkened compartment. The hind limbs of the rats were dipped in methylene blue and the rats were allowed to walk along a sheet of white paper through a crack parallel to the wall. Footprints were analyzed using the following four measurements: footprint length, toe spreading, intermediate toe spreading, and distance to the opposite foot. The SFI was calculated from these indices.36,37 The SCI was determined by the observer who was blinded to the intervention in rats.
Macrosopic evaluation
A gross macroscopic assessment of scar formation was made in all surviving rats at after 12 weeks. At the end of 12 weeks, rats were re-anesthetized and the surgical area was examined in a step-by-step fashion by micro-dissection. The sciatic nerve was exposed and examined to determine the amount of fibrous tissue surrounding the repair site. Skin closure, muscle fascia closure, nerve adherence to the surrounding muscle tissue, and the adherence and separability of nerves were assessed using a numerical grading scheme devised by Petersen et al. as described previously.18

Histologic evaluation
Sciatic nerves were prepared for histologic evaluation of peripheral nerve regeneration at the end of the twelfth post-operative week. At that point, 5 µm cross-sections of tissue that were originally located 10 mm distal to the repair site were obtained and stained for myelin using the Weil method. Nerve regeneration was examined using light microscopy. Transverse sections were created to allow assessment of axon counts and mean axon and fiber diameters. Axon counts were obtained at ×400 magnification by using the following sampling technique: of 100 ocular grid squares with a surface area of 0.01 mm² each, 20 were randomly selected and used to count myelinated axons. These grids were considered to be representative of the entire surface area and axon counts were normalized accordingly. Mean axon and fiber diameters were measured using an ocular micrometer (Olympus Eyepiece Micrometer Ax-0026; Olympus, Hamburg, Germany). Longitudinal 5-µm serial sections obtained from the repair site were stained with hematoxylin and eosin and the longitudinal organization of the regenerating nerve was evaluated according to the following scale described by Brown et al.: (1) failure (no continuity of axons from proximal to distal ends); (2) poor organization of the repair site; (3) fair organization of the repair site; (4) good organization of the repair site; and (5) excellent organization of the repair site (indistinguishable from normal). The histomorphologic evaluations were performed by the pathologist who was blinded to the intervention in rats.

Statistical analysis
Results are expressed as mean ± SEM. The change in SFI was analyzed with one- or two-way repeated measures analysis of variance (ANOVA) followed by Tukey’s multiple comparisons procedure. Axon count, fiber diameter, and axon diameter were compared between groups using one-way ANOVA followed by Tukey’s multiple comparisons procedure. Nerve adherence/separability scores and axonal organization scores were compared between groups using the Kruskal–Wallis one-way ANOVA by ranks test, followed by Dunn’s method for multiple comparisons. The relationships between SFI, axon counts, axon diameters, axonal organization, and the nerve adherence/separability score were determined using Pearson’s correlation analysis or Spearman’s rank order correlation procedure. For all statistical analyses, SigmaStat® statistical software, version 3.1, was used (SPSS Science GmbH, Erkrath, Germany). P values <0.05 was considered significant.

Results
Functional evaluations
The mean pre-operative SFI score of the five groups of rats was close to zero, indicating normal function. The mean SFI decreased to ~100 on the first day after the injury with a gradual recovery at 4, 8, and 12 weeks after treatment in all five groups of rats (Fig. 1A and B). At time points during the recovery, SFI scores were higher in rats treated topically with CDP-choline, cytidine, choline, or cytidine + choline than in the saline-treated control rats (Fig. 1A and B). The highest rates of increase in SFI score were observed in rats treated either with CDP-choline or cytidine + choline (Fig. 1A and B). Treatment with choline or cytidine alone was also effective, although to a lesser extent, at improving SFI scores (Fig. 1A and B). Overall, statistical analysis using two-way, repeated measures ANOVA revealed that treatment with CDP-choline [F(2,20)=9.61, P<0.001] or CDP-choline [F(2,16)=11.61, P<0.001], cytidine [F(2,22)=8.42, P<0.01] or CDP-choline [F(2,16)=8.17, P<0.01], choline [F(2,20)=5.73, P<0.05] or CDP-choline [F(2,16)=6.32, P<0.05], and cytidine + choline [F(2,24)=14.29, P<0.001] or CDP-choline [F(2,20)=14.25, P<0.001] improved SFI scores in rats whose sciatic nerves were repaired on the day of injury or the third day after injury, respectively.

The percentage recovery in SFI in rats whose sciatic nerves were repaired on the day of injury were slightly, but not significantly, higher than that in all of five groups of rats whose sciatic nerves were repaired on the third day of injury (Fig. 1A and B). However, statistical analysis using two-way, repeated measures ANOVA on pooled data from all of five groups of rats revealed significantly higher scores (P<0.01) in the SFI at 12 weeks, but not at 4 weeks (P=0.32) or at 8 weeks (P=0.055), in rats whose sciatic nerves were repaired on the day of injury.

Macroscopic evaluation
At the end of the twelfth post-operative week, rats were evaluated for scar tissue formation at the surgical site. Skin sutures were removed and sciatic nerves were exposed through at the original incision. There was no sign of infection or inflammatory reactions in any of the group. In rats whose nerves were repaired immediately who were treated with
Figure 1 Effect of topical application of CDP-choline, cytidine, choline, or cytidine + choline on SFI score 4, 8, and 12 weeks after sciatic nerve repair (repair took place either on the day of the nerve injury or 3 days after the nerve injury). The sciatic nerve was transected under thiopental sodium anesthesia and repaired surgically either immediately (A) or 3 days after the injury (B). The SFI score was determined 4, 8, and 12 weeks after surgery. The percentage recovery in SFI score at 4, 8, and 12 weeks was obtained by calculating the difference between pre-operative SFI scores and SFI scores observed 4, 8, and 12 weeks after sciatic nerve repair. *Significantly (P<0.05) higher than the respective value for 'saline' (control) rats. In all of five groups of rats, the percentage recovery in SFI scores at 4, 8, and 12 weeks was not significant between rats whose sciatic nerves were repaired either on the day of the nerve injury or 3 days later. When all data were pooled from five groups of rats, two-way repeated measures ANOVA revealed significant improvement in the percentage recovery in SFI score at 12 weeks (P<0.01), but not 4 weeks (P=0.32) or 8 weeks (P=0.055), after sciatic nerve repair on the same day of the injury.

Saline or choline, a thick, dense, collagenous, opaque scar surrounded the repair site (Fig. 2A and C), whereas in rats treated with CDP-choline, cytidine, or cytidine + choline combination, the surgical site was surrounded by only a thin, brownish, translucent membrane (Fig. 2B and D). In all of the five groups...
of rats whose sciatic nerves were repaired on the third day after surgery, the scar tissue surrounding repair site was denser than that in rats whose sciatic nerves were immediately repaired (data not shown).

Table 1 summarizes the results of the evaluation of nerve adherence and separability. Among rats whose sciatic nerves were immediately repaired, the nerve adherence and separability scores were significantly better in rats treated with CDP-choline, cytidine, or cytidine + choline than in the saline-treated control rats (Table 1). Among choline-treated rats, the score was also lower than that in the control rats, but failed to reach significance level (Table 1). In rats whose sciatic nerves were repaired on the third day after surgery, nerve adherence and separability scores were similar among all of the five groups of rats (Table 1). In rats treated with either CDP-choline or cytidine + choline, the nerve adherence and separability scores of rats whose sciatic nerves were immediately repaired were significantly lower than those of the rats whose sciatic nerves were repaired on the third post-operative day (Table 1).

**Histologic evaluation**

Table 2 summarizes the results of the histologic evaluation of the repaired sciatic nerves. Nerves that were treated with CDP-choline or cytidine + choline had significantly higher axon counts, thicker axon diameters, and better axonal organization than nerves treated with saline among all rats (i.e. those who underwent immediate versus delayed repair) (Table 2). Treatment either with cytidine or choline alone failed to alter axon counts. Nerves treated with cytidine, but not choline, had thicker axon diameters than nerves treated with saline (Table 2). Nerves treated with cytidine also tended to have better axonal organization than nerves treated with saline (Fig. 2E and F). This finding reached significance among rats that underwent immediate nerve repair, but not among rats that underwent delayed nerve repair. Treatment with choline alone failed to alter significantly either axon diameter or axonal organization (Table 2). Fiber diameters were similar in all groups who underwent surgery at both time points (Table 2).

**Relationship between functional and histomorphologic parameters**

There was a significant positive correlation between the percentage recovery in SFI scores 12 weeks after surgical repair and the observed values for axon count (r=0.456; P<0.01), axon diameter (r=0.482; P<0.01), and axonal organization scores (r=0.486; P<0.05) in rats treated with saline, CDP-choline, and cytidine + choline. Percentage recovery in SFI scores were inversely related (r=-0.364; P<0.05) with the nerve adherence and separability score. There was also an inverse relationship between the nerve adherence and separability score and observed values for axon count (r=-0.414; P<0.01), axon diameter (r=-0.395; P<0.01), and axonal organization scores (r=-0.488; P<0.001) in rats treated with saline, CDP-choline, and cytidine + choline. The nerve adherence and separability score were also inversely related with axonal organization in cytidine- (r=-0.307; P<0.05) and choline-treated (r=-0.348; P<0.05) rats. There was no significant relationship observed between fiber diameter and any of the measured parameters (data not shown). Additionally, no significant relationship was observed between SFI score and any of measured parameters in cytidine- or choline-treated rats (data not shown).

**Discussion**

These results show that the local application of CDP-choline itself or a combination of its endogenous metabolites, cytidine and choline, improves functional recovery and axonal organization in rats with sciatic nerve injuries and also increases axon counts and axon diameters of the injured sciatic nerves in rats that underwent immediate and delayed (3 days after the initial surgery) surgery to repair their transected nerves. Additionally, treatment with CDP-choline or cytidine + choline was found to reduce scar formation surrounding the repair site and to decrease nerve adherence when the sciatic

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**Table 1 Effects of CDP-choline and its metabolites on the nerve adherence and separability score 12 weeks after sciatic nerve repair**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group I (sciatic nerve repaired on first day)</th>
<th>Group II (sciatic nerve repaired on third day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (control)</td>
<td>3.0 (2.0−3.0)</td>
<td>3.0 (2.5−3.0)</td>
</tr>
<tr>
<td>CDP-choline</td>
<td>1.5 (1.0−2.0)</td>
<td>2.0 (2.0−3.0)</td>
</tr>
<tr>
<td>Cytidine</td>
<td>1.5 (1.0−2.0)</td>
<td>3.0 (2.0−3.0)</td>
</tr>
<tr>
<td>Choline</td>
<td>2.0 (2.0−3.0)</td>
<td>2.0 (2.0−3.0)</td>
</tr>
<tr>
<td>Choline + cytidine</td>
<td>1.5 (1.0−2.0)</td>
<td>2.0 (2.0−3.0)</td>
</tr>
</tbody>
</table>

1Repair took place either on the day of the nerve injury or 3 days after the nerve injury. Nerve adherence and separability were scored as described in the section on ‘Materials and Methods’. Data are presented as median (interquartile range). Data were analyzed with the Kruskal–Wallis one-way ANOVA by ranks, followed by Dunn’s method for multiple comparisons.

2Significantly different from the values for control rats.

3Significantly different from the values for the respective treatment in Group II (Mann-Whitney rank sum test).
nerve was repaired immediately. There were also positive correlations noted between the improvement in the SFI and the axon count, fiber diameter, and axonal organization score. However, the recovery in SFI scores, the axon count, and the axon diameters were found to be inversely related to the nerve adherence and separability score. Treatment with choline or cytidine alone was less effective at improving SFI scores and ineffective at altering axon counts, as compared to treatment with CDP-choline or cytidine + choline.

In our previous study, we showed that CDP-choline improves the functional recovery of the sciatic nerve after sharp transection and an immediate surgical repair in rats. Data from the current study confirm and extend these previous observations by demonstrating that CDP-choline is also effective at improving functional recovery of the transected sciatic nerve, even when it is surgically repaired surgically 3 days after transection. Furthermore, we observed that treatment with cytidine + choline, both of which are metabolites of CDP-choline, was as effective as an equimolar concentration of CDP-choline at improving functional recovery of transected sciatic nerves that were surgically repaired either immediately or 3 days after transection. Treatment with choline or cytidine alone was also effective, but the recovery in SFI score induced by choline or cytidine was relatively smaller and failed to reach significance at all of time points in both groups of rats (Fig. 1A and B). Taken together, these data indicate that of the mechanism by which CDP-choline improves SFI scores involves both of its endogenous metabolites, choline and cytidine, because providing both of these metabolites enhances their effects on SFI score improvements. This may be explained by the fact that cytidine enhances the utilization of choline during membrane phospholipid synthesis, which is believed to be essential for repairing injured neuronal membranes and for recovery from neuronal injury. Indeed, the improvement in SFI scores induced by CDP-choline and cytidine + choline was accompanied by increases in axon count and axon diameter, as well as improved axonal organization (Table 1), and there were significant positive correlations observed between these neuronal regeneration parameters and SFI score recovery.

The observed reduction in the perineural scar tissue associated with the local application of CDP-choline was in agreement with the previous study. In the present study, we further observed that this scar-reducing action of CDP-choline was mimicked by treatments either with cytidine + choline or with cytidine alone, but not choline alone, in rats whose sciatic nerves sutured were repaired immediately after injury. The reduction in scar tissue induced by CDP-choline, cytidine + choline, and cytidine was not seen in rats whose sciatic nerves were repaired 3 days after injury. These data indicate that the reduction in scar tissue formation induced by CDP-choline involves its endogenous metabolite, cytidine, and requires early surgical repair. Epineurial scar tissue acts as a mechanical barrier that impedes axonal outgrowth across the site of repair and leads to ischemia by causing tethering of nerves to adjacent tissue and preventing nerve mobility. This, in turn, leads to traction injury and vasospasm. Data from the present study revealed that the reduction in scar formation induced by treatment with CDP-choline or cytidine + choline was associated with a significant improvement in the nerve adherence and separability score, whereas there were inverse relationships noted between the nerve adherence/separability score and SFI, axon count, axon diameter, and the axonal organization score. Thus, the reduction in scar volume and improvements in nerve adherence/separability scores in rats treated either with CDP-choline, cytidine + choline, or cytidine may be involved, in part, in the observed improvements in nerve function.

Table 2 Effects of CDP-choline and its metabolites on axon count, axon diameter, fiber diameter, and axonal organization 12 weeks after sciatic nerve repair

<table>
<thead>
<tr>
<th>Group/treatment</th>
<th>Axon count</th>
<th>Axon diameter (µm)</th>
<th>Fiber diameter (µm)</th>
<th>Axonal organization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (sciatic nerve repaired on first day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline (control)</td>
<td>15720 ± 690</td>
<td>2.8 ± 0.1</td>
<td>7.9 ± 0.5</td>
<td>3.0 (2.0-3.0)</td>
</tr>
<tr>
<td>CDP-choline</td>
<td>23070 ± 1175</td>
<td>4.4 ± 0.3</td>
<td>8.5 ± 0.5</td>
<td>4.0 (4.0-5.0)</td>
</tr>
<tr>
<td>Cytidine</td>
<td>16520 ± 890</td>
<td>4.2 ± 0.3</td>
<td>8.2 ± 0.4</td>
<td>4.0 (3.0-4.0)</td>
</tr>
<tr>
<td>Choline</td>
<td>15860 ± 860</td>
<td>3.3 ± 0.3</td>
<td>8.6 ± 0.5</td>
<td>3.5 (3.0-4.0)</td>
</tr>
<tr>
<td>Choline + cytidine</td>
<td>23670 ± 920</td>
<td>5.4 ± 0.3</td>
<td>9.4 ± 0.6</td>
<td>4.0 (4.0-5.0)</td>
</tr>
<tr>
<td>Group II (sciatic nerve repaired on third day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline (control)</td>
<td>14700 ± 950</td>
<td>2.8 ± 0.2</td>
<td>8.2 ± 0.3</td>
<td>3.0 (2.5-3.0)</td>
</tr>
<tr>
<td>CDP-choline</td>
<td>21820 ± 860</td>
<td>4.1 ± 0.3</td>
<td>8.4 ± 0.4</td>
<td>4.0 (3.5-4.0)</td>
</tr>
<tr>
<td>Cytidine</td>
<td>14910 ± 760</td>
<td>3.5 ± 0.3</td>
<td>8.4 ± 0.4</td>
<td>4.0 (3.0-4.0)</td>
</tr>
<tr>
<td>Choline</td>
<td>14800 ± 690</td>
<td>3.1 ± 0.2</td>
<td>8.1 ± 0.2</td>
<td>3.0 (3.0-4.0)</td>
</tr>
<tr>
<td>Choline + cytidine</td>
<td>22850 ± 860</td>
<td>4.4 ± 0.3</td>
<td>8.9 ± 0.4</td>
<td>4.0 (3.0-4.75)</td>
</tr>
</tbody>
</table>

*Repair took place either on the day of the nerve injury or 3 days after the nerve injury. Data for axon count, axon diameter, and fiber diameter are presented as mean ± SEM. Data for the axonal organization score are presented as the median (interquartile range).
*Significantly different from respective value for 'saline (control)' group.
and regeneration observed with these treatments. The statistically significant inverse relationship noted between nerve adherence/separability scores and axonal organization in cytidine- or choline-treated rats suggest that increased nerve adherence to adjacent tissue may negatively affect axonal organization in these two groups of rats.

In addition to the reduction in scar formation observed in CDP-choline-treated rats, we observed that local application of CDP-choline has ability to increase axon counts significantly in rats, not only when their sciatic nerves are repaired immediately, but also when they are repaired 3 days later (Table 2). Moreover, the magnitude of the increase in axon counts observed in response to treatment with CDP-choline was similar between the two groups of rats (Table 2). Local application of cytidine or choline alone was ineffective at increasing the axon counts of sciatic nerves repaired surgically either immediately or 3 days after injury, whereas treatment with both cytidine and choline was as effective as CDP-choline. These data clearly indicate that the presence of increased concentrations (i.e. 100 μM) of both the choline and cytidine moieties of CDP-choline is essential for the increase in axon counts observed in response to treatment with CDP-choline. It is well known that the substrate binding sites of the enzymes involving in phosphatidylcholine synthesis (phosphatidylcholine is a major component of the phospholipid-containing neuronal membrane) via the Kennedy pathway by utilizing the phosphatidylcholine precursor molecules, cytidine/uridine and choline are unsaturated, and that treatments that increase the levels of phosphatidylcholine precursors simultaneously increase membrane synthesis and neurogenesis, both in vitro and in vivo.

In conclusion, data from the present study show that CDP-choline improves the functional recovery and promotes regeneration of the injured peripheral nerves of rats when it is locally applied to the surgical site on the day of surgery or the third day after injury. Treatment with cytidine + choline was as effective as treatment with an equimolar concentration of parent drug, CDP-choline, at improving the functional recovery of the injured sciatic nerves, when they were repaired either immediately or 3 days later. Choline or cytidine alone was less effective at improving SFI scores and did not increase axon counts. These data suggest that both CDP-choline and cytidine + choline may be useful in the surgical treatment of peripheral nerve injuries in humans.

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