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Mediation of Epigenetic Mechanisms in the Regenerative Effect of Uridine in a Rat Model of Sciatic Nerve Injury

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ABSTRACT

AIM: To investigate the possible mediation of epigenetic mechanisms underlying the regenerative effect of uridine in a sciatic nerve transection rat model.

MATERIAL and METHODS: Fifty adult male rats were randomized to sham, control, and uridine groups. After unilateral transection and primary anastomosis of the right sciatic nerve, a single daily dose of saline (1 ml/kg; sham and control groups) or uridine (500 mg/kg; uridine group) was injected intraperitoneally for a week. The sciatic nerves were removed en bloc on the eighth day and levels of histone deacetylase 1 (HDAC1), acetylated histone-H3, and acetylated histone-H4 were analyzed in nerve homogenates. The number of myelinated axons in the sciatic nerve specimens was analyzed histomorphologically.

RESULTS: The HDAC1 levels were significantly greater in the control group than in the sham ($p<0.001$) and uridine ($p<0.01$) groups. Compared to the sham group, the acetylated histone-H3 and histone-H4 levels decreased in the control group (by 81.49% and 79.98%, respectively for both; $p<0.001$) and increased significantly in the uridine group (by 62.54% and 51.68% respectively; $p<0.01$, $p<0.05$). The number of myelinated axons decreased significantly ($p<0.001$) in the control group, which was enhanced significantly by uridine administration.

CONCLUSION: Epigenetic mechanisms may partly mediate the regenerative effect of uridine treatment in a rat model of sciatic nerve injury. Our data provides novel insights in the management of peripheral nerve damage and suggests potential benefit of uridine for degenerative diseases in which epigenetic impairments are involved.

KEYWORDS: Uridine, Sciatic Nerve Injury, Peripheral Nerve Regeneration, Histone Deacetylase, Epigenetic

ABBREVIATIONS: HDAC1: Histone deacetylase 1, **PNI:** Peripheral nerve injury, **CDP-choline:** Cytidine diphosphate-choline, **HIE:** Hypoxic-ischemic encephalopathy, **ELISA:** Enzyme-linked immunosorbent assay, **PBS:** Phosphate-buffered saline, **SDS-PAGE:** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis, **PVDF:** Polyvinylidene fluoride, **TBST:** Tris buffer, tween 20, **OsO4:** Osmium tetroxide, **DNA:** Deoxyribonucleic acid, **HAT:** Histone acetylase

INTRODUCTION

Peripheral nerve regeneration is a challenge in regenerative medicine. Typically, the post-injury regeneration rate of a peripheral nerve is 1 mm/day, which is usually not sufficient for complete regeneration and functional recovery (30,46) owing to scar tissue formation, Schwann cell dysfunction, misdirection of axonal growth cones, or irreversible muscle atrophy after loss of innervation.

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In transection injuries, degenerative changes in the peripheral nerve occur on either side of the injury, resulting in partial or complete sensory/motor loss is observed (8,22,43,47). Following a peripheral nerve injury, the proximal segment undergoes degenerative changes and the distal segment undergoes Wallerian degeneration (30). Pathophysiological changes, such as apoptosis, oxidative stress, inflammation, destruction of the extracellular matrix, and several other events can worsen the extent of damage in a peripheral nerve injury (PNI) (29,48,49,52); however, these complex processes can be disrupted at every stage to prevent post-injury regeneration. Although several surgical and medical approaches targeting these processes have been developed, a treatment that can guarantee full functional recovery in PNI is yet to be discovered (8,30,43,47).

Uridine is the chief pyrimidine nucleoside in circulation (13,72), which is found in breast milk (68) in humans and other mammals. It increases the biosynthesis of cytidine diphosphate-choline (CDP-choline) as a rate-limiting compound both in vivo (12) and in vitro (60); it is also a precursor for synthesizing membrane phospholipids via the Kennedy pathway (37). Systemic treatment with uridine reportedly augments the synaptic connections of neurons (58), while exogenous treatment with uridine has been reported to provide neuroprotection and reduce long-term cognitive deficits by preventing apoptotic neuronal loss in experimental hypoxic-ischemic encephalopathy (HIE) (12,23,42) and hyperoxic (24) brain injury models. In the HIE model, uridine treatment promoted neuronal recovery by decreasing histone deacetylase (HDAC) activity and increasing levels of acetylated histone-H3 and acetylated histone-H4 (42). A recent study demonstrated the role of epigenetic mechanisms in treating peripheral nerve damage and enhancing functional recovery (28). The authors noted that an HDAC3 enzyme-dependent pathway was responsible for preventing peripheral myelin development and functional regeneration; therefore, inhibiting this pathway can potentially boost peripheral myelination (28).

Therefore, in continuation to our previous observations regarding the effect of uridine in rat sciatic nerve transection and primary anastomosis model (39,40), this study aimed to explore the mediation of epigenetic mechanisms (involving the HDAC1 enzyme, acetylated histone-H3, and acetylated histone-H4 levels) underlying uridine's effects in peripheral nerve regeneration.

MATERIAL and METHODS

Study Design and Experimental Animals

This experimental study was approved by the Local Ethics Committee on Animal Experiments, Bursa Uludag University, Bursa, Turkey (approval no: 2021-08/02; 15.06.2021), and carried out in accordance with the ARRIVE Guidelines Version 2.0 (56) and Guide for the Care and Use of Laboratory Animals – 8th edition (16).

Fifty male Sprague Dawley rats (3–6 months old and weighing 300–400 g) were purchased from the Bursa Uludag University Medical Faculty Experimental Animal Research Center, Bursa, Turkey. All rats were housed in single cages maintained at 22°C with a 12-hour light/dark cycle and free access to food and water.

Rats were randomized to Sham, Control and Uridine groups to further receive intraperitoneal injections of saline, saline and uridine, respectively. Before the treatments, rats in the Control and Uridine groups underwent unilateral sciatic nerve transection surgery and those in the Sham group only received sham surgery as a control for the effects of surgery. Two rats died following sciatic nerve transection surgery; hence, the study finally included 48 rats (n=16 in each group). Following surgery, treatments were initiated and lasted for 7 days. On the 8th day, rats were sacrificed and nerve samples were collected. Half in number (a total of 24 obtained from all three groups) of the nerve samples was used for enzyme and protein analyses while the other half (n=24) was used for histomorphological analyses.

Surgical Procedure

Rats underwent sciatic nerve transection surgery which was followed by immediate primary anastomosis. The rats were anesthetized using 2%-3% sevoflurane (Sevorane Liquid; Aesica Queensborough LLC., Queensborough Kent, UK), followed by the use of 1%-2% sevoflurane for anesthesia maintenance. The rats were placed on a dissection table in a left lateral decubitus position. To approach the sciatic nerve, the femoral head was palpated along the hind leg, and a 2-3 cm long posterolateral skin incision was made from the knee to the upper part of the trochanter major. Next, the plane between the biceps femoris and the gluteus muscle was dissected with the help of microdissection scissors and separated from the underlying fascia. Proceeding with blunt dissection, a retractor was placed between both muscle blocks, and 1-2 cm of the sciatic nerve was exposed extending from the sciatic foramen up to the origin of its tibial and peroneal branches (Figure 1). A full-thickness nerve cut was made in the sciatic nerve 1 cm away from the distal sciatic foramen in all the rats except those in the sham group. In the sham group, no transection was performed to explore normal nerve functions and activity. The distal and proximal nerve stumps were primarily sutured under a microscope with an aperture of 180° (Figure 2). The fascia and skin were closed, and the rats were returned to their cages. All operative procedures were performed by a single neurosurgeon using a surgical microscope (Zeiss Opmi; Carl Zeiss Meditec Inc., Jena, Germany).

Interventions

Before surgical procedures, the rats were randomized into the uridine, control, and sham groups (n=16 in each group). The treatments were initiated on the day of surgery; rats in the sham and control groups were administered daily with 1 ml/kg of saline intraperitoneally for seven days, while the uridine group received 500 mg/kg of uridine (Merck KGaA, Darmstadt, Germany) intraperitoneally daily for seven days. The uridine dose administered was determined as reported in previous studies (39,40).

On the 8th postoperative day, the rats were anesthetized again with sevoflurane (3%), and their sciatic nerves were excised

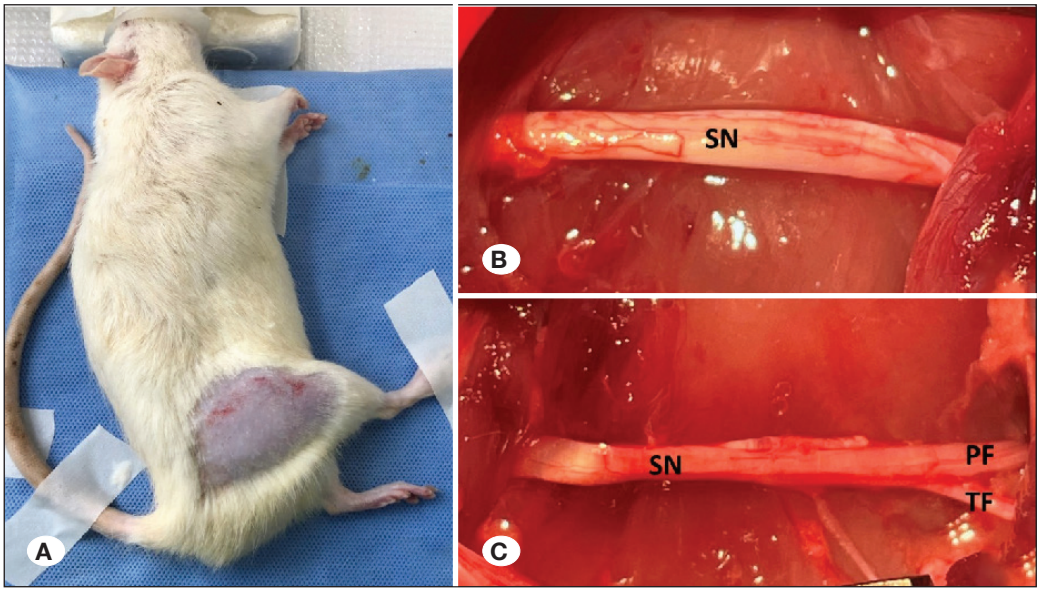

Figure 1: Rat placed in the left lateral decubitus position **(A)**; the right sciatic nerve **(B)** was exposed with blunt dissection. The sciatic nerve and distal fascicular branch point **(C)** were exposed. **SN:** Sciatic nerve, **PF:** peroneal fascicle, **TF:** tibial fascicle.

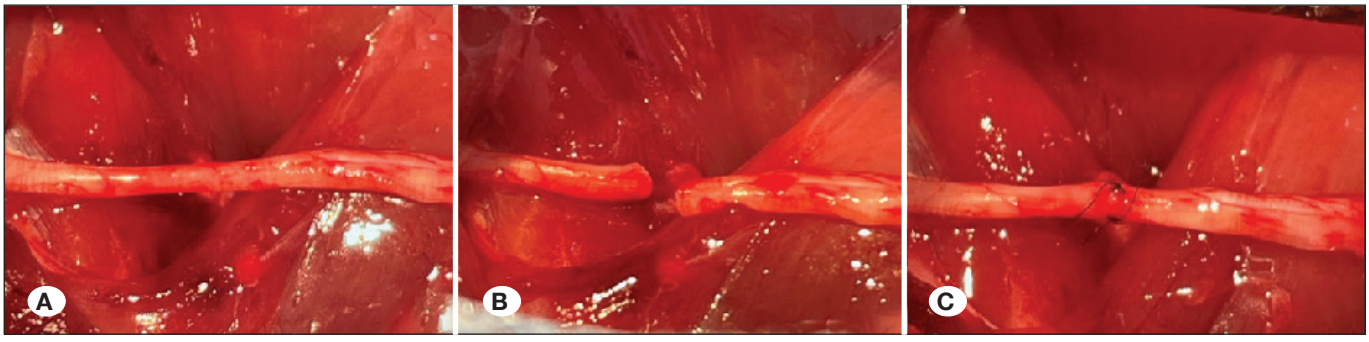

Figure 2: The unifascicular sciatic nerve segment was exposed **(A)**. Subsequently, a full-thickness nerve transection was made with a dermatome knife **(B)**. A primary anastomosis was achieved between the distal and proximal stump using two sutures made 180° apart using the epineural nerve suturing technique **(C)**.

unilaterally. Sciatic nerve samples from half of the rats in each group were homogenized to perform enzyme and protein analyses; the other half were used for histomorphological analyses. All rats were euthanized with a high dose of sevoflurane after tissue collection.

Macroscopic Evaluation

The operative site and surgical wound were assessed daily until the end of the experiment. The condition of the skin, muscle, and fascia, and the relationship of the sciatic nerve with its surrounding tissue were noted. The Petersen numerical rating score (57) was used to quantify the nerve's adherence to the surrounding soft tissue mass and its dissociation from these structures during macroscopic evaluation. The score uses two parameters: (i) skin and muscle fascia and (ii) nerve adherence and nerve separability. Considering skin and muscle fascia, rats were graded as Grade 1: skin or muscle fascia entirely closed, Grade 2: skin or muscle fascia partially open, or Grade 3: skin or muscle fascia completely open; based on the extent of nerve adherence and nerve separability, rats were graded as Grade 1: no dissection or mild blunt dissection required, Grade 2: some vigorous blunt dissection required, and Grade 3: sharp dissection required.

Tissue Sampling

On the 8th postoperative day, the surgical wound was re-opened, and the soft tissue was dissected. The sciatic nerves were removed en bloc for enzyme and protein analyses and stored at –80°C. For the enzyme-linked immunosorbent assay (ELISA) and Western blot analyses, the nerve samples were homogenized in 2 ml of ice-cold phosphate-buffered saline (PBS; pH 7.4). For the histomorphological studies, a sufficient amount of Trump's fixative (1.4 g formaldehyde, 1% glutaraldehyde in 1 × PBS, and 1.16 g $NaH_2PO_4 \cdot H_2O$ per 100 ml; Sigma-Aldrich, St. Louis, MO, USA) was added to the operative site, incubated for 10 minutes, and subsequently cleared from the surgical field; this step was repeated two more times. Nerve tissues were excised under microscopic guidance and stored at 4 °C in 15 ml tubes containing Trump's fixative.

Enzyme and Protein Analyses

The HDAC1 enzyme levels were determined using a rat-com-

patible ELISA kit (Bioassay Technology Laboratory, Shanghai, China) as per the manufacturer's instructions. Briefly, the procedure includes incubation of the HDAC colorimetric substrate with a compound containing HDAC activity, followed by treatment with lysine developer to produce a chromophore which was analyzed using spectrophotometry (BioTek Quant; BioTek Instruments Inc., Winooski, VT, USA) at a wavelength of 450 nm.

Levels of acetylated histone-H3 and acetylated histone-H4 were analyzed using Western blot analysis. The total protein levels were measured using the Bicinchoninic acid method; the homogenates were mixed with equal volumes of Laemmli buffer (0.0625 M Tris Base, 0.07 M Sodium Dodecyl Sulfate, 10% glycerol and 5% 2-mercaptoethanol) and boiled for five minutes. Equal amounts of the protein were loaded for all samples, which were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Mini Protean II; Bio-Rad, Hercules, CA, USA). The proteins were transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA), which were blocked for half an hour with tris buffer saline-tween 20 (TBST, Sigma-Aldrich, St. Louis, MO, USA), and 5% non-fat powdered milk solution (Carnation, Glendale, CA, USA). Subsequently, the membranes were washed three times with TBST and incubated overnight with anti-acetyl-histone H3 (1:1000; Cell Signaling Technology, Danvers, MA, USA) and anti-acetyl-histone H4 (1:1000; Cell Signaling Technology, Danvers, MA, USA) as primary antibodies, and anti-B-Actin (B-Actin, 1:1000; Cell Signaling Technology, Danvers, MA, USA) as the positive control. The following day, the membranes were washed with TBST again and incubated for 60 minutes with the appropriate secondary antibodies (1:5000; Cell Signaling Technology, Danvers, MA, USA). The intensity of the protein bands was digitized and analyzed using a CDigit scanner (Licor CDigit scanner, Lincoln, NE, USA).

Histomorphological Analyses

Tissue samples in the tubes containing Trump's fixative were fixed for one week by renewing the fixatives every 48 hours. Thereafter, the tissues were subjected to post-fixation procedures (2 h in 1% osmium tetroxide [OsO^4] with a phosphate buffer of 0.1 mol/L) before the histomorphological analysis. Spur's resin (Agar Low Viscosity Resin Kit; Agar Scientific, Essex, UK) was carefully added to the nerve samples (Figure 3A). The mold containing the nerve segments and resin were left overnight to polymerize at 68°C–70°C. The resin-embedded nerve blocks were placed in an ultramicrotome device (LKB Ultrotome Main Unit Type 4801 A, Vienna, Austria) set to 1–2 µm, to obtain transverse semi-thin sections. Subsequently, a drop of toluidine blue solution was added to the sections and left for 20–30 seconds; the slides were gently immersed in a jar of distilled water to rinse off the excess solution. This process was repeated several times until the sections were clear. The preparations were dried at 60°C for approximately 15 minutes and covered with a lamella (Objektträger, Isolab, Eschau, Germany). The slides were then analyzed microscopically and stored at room temperature (20).

Using the Zeiss Primo Star light microscope system and Zeiss Labscope software (Carl Zeiss Meditec AG, Jena, Germany), we obtained randomly selected images of the sections (5 MP resolution). The section images magnified to 100x were converted to BMP format using ImageJ 1.53t (Laboratory for Optical and Computational Instrumentation, University of Wisconsin, Madison, WI, USA) which was calibrated using the table micrometer before each examination and count. An Olympus micrometric slide was used for calibration. For all preparations, a count frame (1500 × 1000 pixels; 127.19 × 84.74 µm; 10772 µ2) was created using macros on the images that were obtained with a 100x objective. Additionally, myelinated axons in the field were counted using non-biased counting rules, and the number of axons per square millimeter (axonal density) was determined. For this purpose, axons in contact with the right and lower edges of the frame and all myelinated axons were included in the count, and those axons

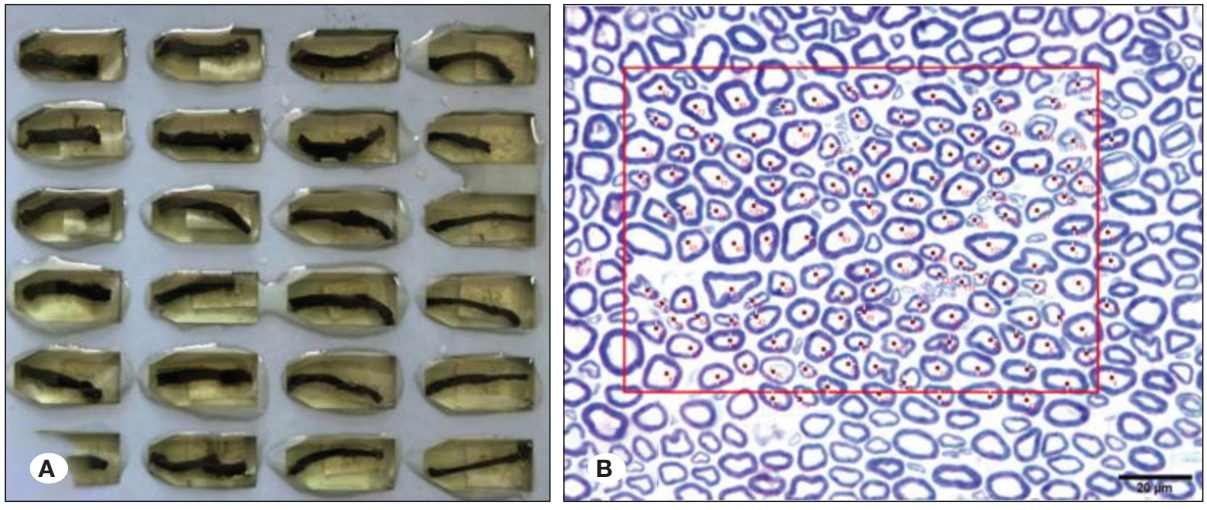

Figure 3: The post-fixation process of the excised sciatic nerve segments for histomorphological analyses was completed and the sections were placed in resin blocks **(A)**. A counting frame was created using ImageJ software before counting the axons **(B)**.

in contact with the left and upper edges of the counting area were excluded (Figure 3B).

Statistical Analysis

All analyses were performed using SigmaPlot (SYSTAT software, version 12.5, Bayshore Rd, Palo Alto, CA, USA). The variables were described as mean ± standard error of means (SEM). A one-way analysis of variance, followed by Tukey's post-hoc testing was used to compare multiple groups for enzyme and protein analyses. The histomorphological analysis results were evaluated using the Kruskal–Wallis test. A value of $p<0.05$ was considered statistically significant.

RESULTS

Operative and Macroscopic Evaluations

According to the Petersen numerical rating score (57) for the skin and muscle fascia around the wound, two rats had Grade 3 (completely open) wounds, three had Grade 2 (partially healed) wounds, and the remaining rats had Grade 1 (fully recovered) wounds; there was no statistically significant difference between the groups in terms of Petersen grading (Figure 4). In contrast, the groups were significantly different in terms of nerve adhesion and separability; nerve adhesion and fibrotic scar formation were significantly higher in the control group than in the sham group ($p<0.001$), while these pathologies were significantly improved in the uridine group ($p<0.01$) (Figure 4). Macroscopic examination of the excised nerve segments revealed that there were more neuromas and misdirection in the control group than in the sham and uridine groups. In contrast, the nerve segments were more intact and had a uniform appearance in the uridine group than in the control group (Figure 5).

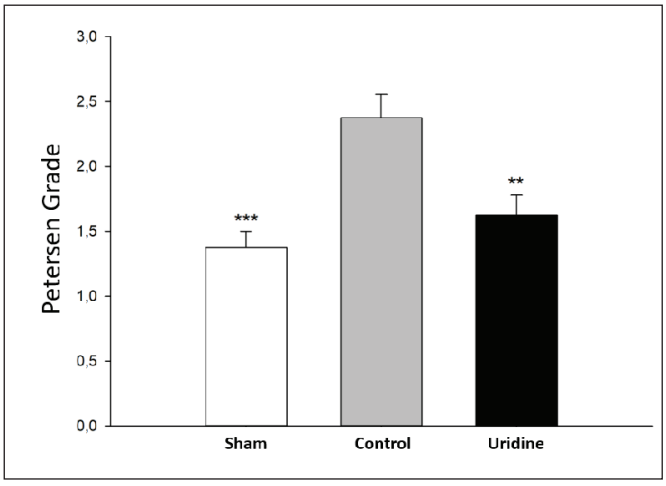

Figure 4: Quantitative results of Petersen's grading showing nerve adhesion and separability (n = 16 in each group). **p 0.01; ***p<0.001, compared to the control group.

Enzyme and Protein Analysis

In comparison to the sham group, the HDAC1 levels were significantly higher in the control group ($p<0.001$) and significantly lower in the uridine group ($p<0.01$) (Figure 6). Likewise, as compared to the sham group, the control group showed a significant decrease in the levels of acetylated Histone-H3 and acetylated Histone-H4 by 81.49% ($p<0.001$) and 79.98% ($p<0.001$), respectively, and the uridine group showed increased levels of acetylated Histone-H3 and acetylated Histone-H4 to 62.54% ($p<0.01$) and 51.68% ($p<0.05$), respectively (Figure 7).

Histomorphological Analyses

The mean ± standard error of measurements (SEM) number of axons counted within the counting frame was 32.52 ± 0.85, 116.37 ± 3.85, and 66.75 ± 1.73 for the control, sham, and uridine groups, respectively (Figure 8). The mean axonal density was noted as 3019.09 ± 79.01 in the control group, 10803.04 ± 358.24 in the sham group, and 6196.6 ± 161.48 in the uridine groups (Figure 9). Compared to the sham group, both the number of myelinated axons in the measurement area and axonal densities were significantly lower ($p<0.001$) in

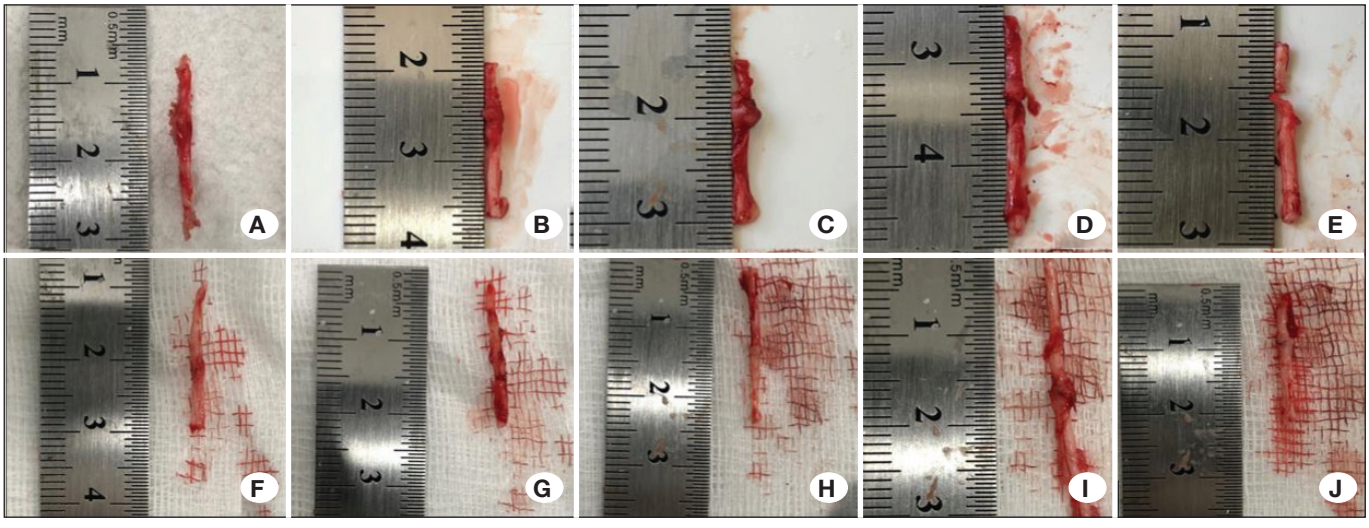

Figure 5: Excised nerve samples of the uridine and control groups. **A–E** are samples from the control group; **F–J** show the sciatic nerve segment samples of the uridine group.

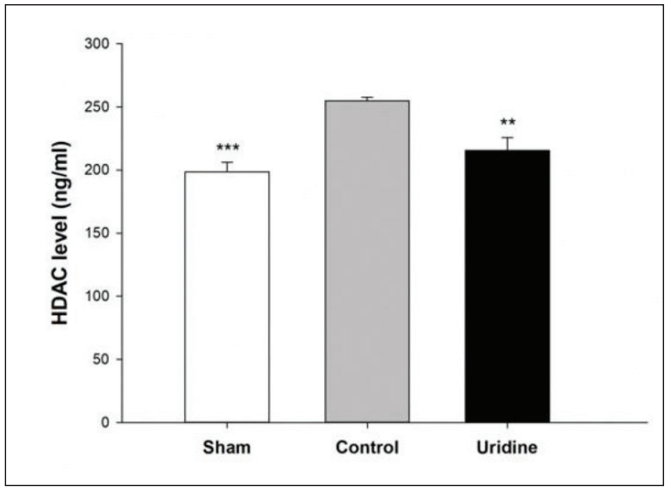

Figure 6: HDAC1 levels in the uridine, control, and sham groups. **p<0.01; ***p<0.001, compared to the control group (n=8). The data are expressed as mean ± standard error of measurement for each group.

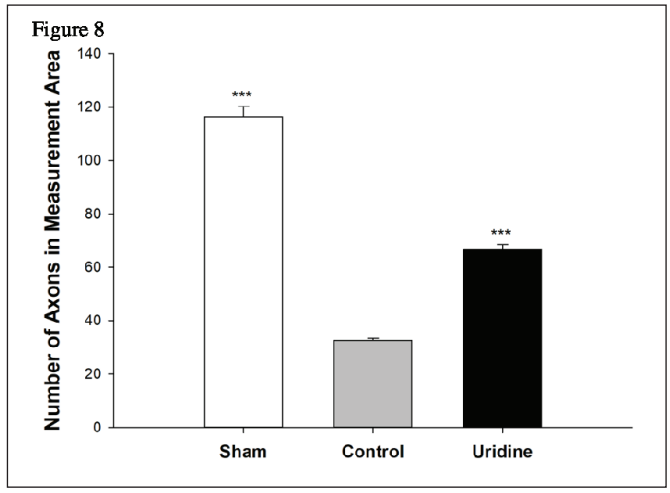

Figure 8: Number of axons in the measurement area. ***p<0.001 compared to the control group (n=8 in each group); mean ± standard error of measurement values are provided for each group.

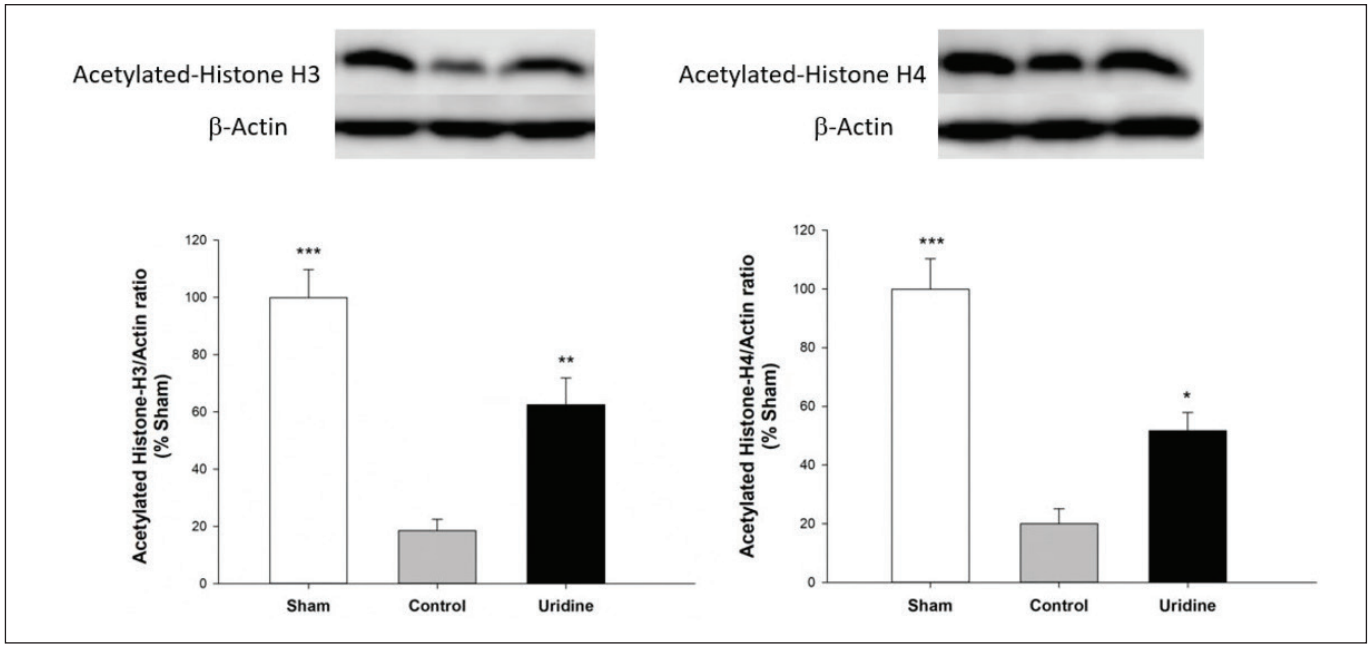

Figure 7: Acetylated histone-H3(**p<0.01; ***p<0.001) and histone-H4 (*p<0.05; ***p<0.001) protein levels in the uridine, control, and sham groups. p-values compared to the control group (n=8 in each group); data are expressed as mean ± standard error of measurement.

the control group and significantly more in the uridine group (p<0.001) (Figure 10).

DISCUSSION

These results confirm our previous findings regarding the neuroprotective effects of uridine in a rat model of sciatic nerve injury (39,40) and highlight the role of HDAC inhibition as a potential mechanism underlying the action of uridine.

PNI are common causes of severe morbidity with a reported prevalence of 2.8% (50) and an incidence of 13–23/100,000 (6) in developing countries. Surgery remains the gold standard for the treatment of PNI (27); however, despite the introduction of new suturing techniques (45) and other approaches, such as autogenous nerve grafting, vascularized nerve grafting, synthetic tube grafting, and end-to-side anastomosis, full functional recovery is not yet possible and the outcomes are far from being curative. This is because surgery alone cannot assuage the pathophysiological consequences of nerve damage, such as inflammation, oxidation, and apoptosis, which negatively affect the regeneration process. Therefore, previous studies have targeted one or more of the pathophysiological

parameters associated with PNI. So far, a variety of chemical agents, compounds, and other approaches have been tested for the treatment of PNI, which includes chemical agents; coenzyme Q10 and vitamin E (53), ginger and sesame oil (17), telmisartan (76), quercetin (69), hyperbaric oxygen therapy (38), ginkgo (1), pioglitazone (59), levetiracetam (2), lithium (7), surgical approaches; mesenchymal stem cells (35,63,74), the use of synthetic grafts to prevent misdirection of the growth cone (75), local treatment modalities like anti-transforming growth factor beta, hyaluronic acid, human amniotic fluid (55), aprotinin (25), 5-fluorouracil (51), low-dose radiation (21) and electrophysical modalities; electrical stimulation and pulse magnetic field (4,15,34).

Likewise, our previous study showed that treatment with CDP-choline (citicoline), a compound with anti-inflammatory, anti-oxidant, and anti-apoptotic properties (66), improved functional recovery, promoted nerve regeneration, and reduced postoperative scar formation in experimental rat models (3,9,26,33,54). CDP-choline is an endogenous compound formed in the rate-limiting step of phosphatidylcholine synthesis via the Kennedy pathway (37) which has cardiovascular (14,65), neuroendocrine (10,11,32), and metabolic (31) effects on exogenous administration. However, exogenously administered CDP-choline metabolizes to choline and cytidine while cytidine is rapidly converted to uridine in blood circulation (72). Treatment with uridine exhibits several beneficial effects on nerve tissue, such as increased nerve branching and axonal growth in cultured PC12 cells (58) and protection in HIE (12,23,42) and hyperoxia (24) models. Therefore, our recent studies used experimental models of sciatic nerve injury to test the effects of uridine in major pathophysiological consequences of PNI. We observed that uridine administration improved nerve regeneration and functional recovery (40) by exerting anti-apoptotic and anti-oxidant effects (39).

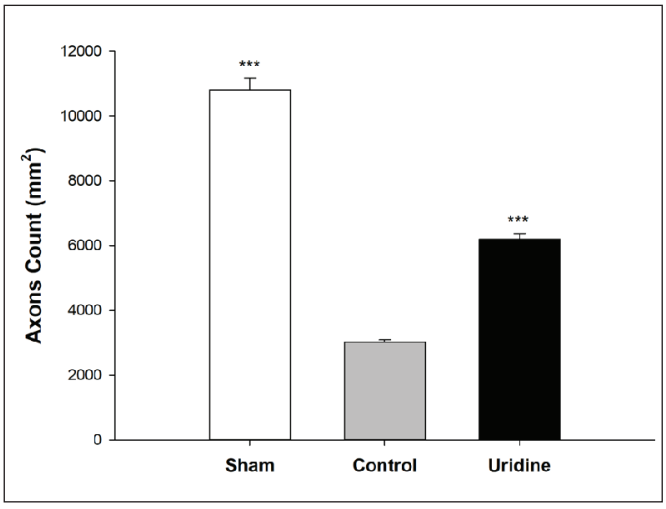

Figure 9: Axon count per square millimeter (axonal density) of the section. ***p<0.001 compared to the control group (n=8); mean ± standard error of measurement values are provided for each group.

Abnormal or dysfunctional histone acetylation underlies the pathophysiology of various neurological illnesses, including neurodegeneration (19,62). These acetylation mechanisms are dynamic events and are regulated by histone acetylase (HAT)-HDAC enzyme groups. HATs cause acetylation of lysine residues and relax the intact nucleosome structure between the DNA and histones, allowing transcription factors to reach the target gene and cause its expression. Conversely, HDACs cause deacetylation of the lysine residues; therefore, transcription cannot occur when DNA is firmly bound to the histones (36). Enzymes and proteins involved in histone metabolism are commonly found in the peripheral and central nervous systems. Thus, epigenetic mechanisms involving HDAC inhibition exert neuroprotective action in the pathophysiology of PNI (18,61,64,67).

It is known that inhibition of histone deacetylation is associated with reduced apoptosis (70), and uridine behaves as an anti-apoptotic HDAC inhibitor in a rat model of neonatal HIE (42). Therefore, the present study further explored the potential mechanism of action through which uridine exerts its neuroprotective effects in PNI. As reported previously (39,40), intraperitoneal administration of uridine (500 mg/kg) for seven consecutive days reduced nerve adhesion and fibrotic scar formation in our rat models of PNI. Moreover, the number of myelinated axons in the measurement area and axonal density (per mm^2) also enhanced significantly with uridine treatment. These findings were associated with decreased levels of HDAC1 and increased expressions of acetylated Histone-H3

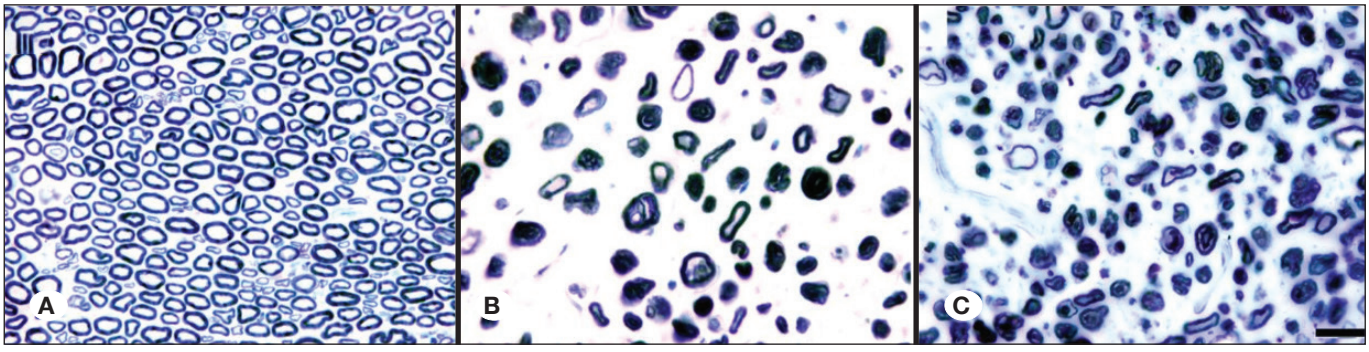

Figure 10: Thin sections of fixed sciatic nerve samples stained with 1% borax-toluidine blue. **A)** sham; **B)** control; and **C)** uridine group. Bar, 20 micrometers.

and acetylated Histone-H4, suggesting mediation of epigenetic mechanisms in sciatic nerve regeneration under the effect of uridine administration. Our findings concur with previous reports showing that HDAC inhibitors, such as trichostatin A, mocetinostat, sodium phenylbutyrate, and valproic acid, reduced axonal degradation (41), improved nerve regeneration (5), enhanced axonal regrowth and remyelination (73), as well as provided functional recovery (71) in models of PNI.

The regenerative effect of uridine was also confirmed by a recent study involving metabolomic analyses in the blastema cells of a vertebrate (axolotl) with high regenerative capacity and in deer antlers, which is the only organ with total regenerative capacity in mammals (44). This study identified uridine as a critical metabolic effector in the regenerative activity seen across species, in addition to other functions such as reactivating the aging stem cells and reducing fibrosis and inflammation (44). The study further stated that oral uridine treatment for two months was associated with increased cognitive function and locomotor activity in aged mice (44). These findings are in line with our previous and current findings in neonatal HIE (12,23,42) and hyperoxia (24) models.

The limitations of our study are that it does not cover all epigenetic mechanisms since it focuses on what has been observed before in the literature, and that it was observed with a small number of subjects. Larger experimental groups and broader enzyme protein analyzes will make a significant contribution in this sense.

CONCLUSION

This is the first study to demonstrate the mechanism underlying the neuroprotective effect of uridine in PNI. Using a rat model of sciatic nerve injury, we observed that uridine functions by modulating the epigenetic pathways associated with pathophysiological consequences of nerve damage to enhance regeneration. Our data provides novel insights into the management of PNI by confirming and extending the effects of uridine.

Declarations

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Availability of data and materials: The datasets generated and/or analyzed during the current study are available from the corresponding author by reasonable request.

Disclosure: The authors declare that they have no conflict of interest.

AUTHORSHIP CONTRIBUTION

Study conception and design: AB, MC
Data collection: AIO, CK, HU
Analysis and interpretation of results: MC, IMK, AB
Draft manuscript preparation: AIO, MC, IMK
Critical revision of the article: AB, MC
Other (study supervision, fundings, materials, etc...): CK, IMK
All authors (AIO, CK, HU, MC, IMK, AB) reviewed the results and approved the final version of the manuscript.

REFERENCES

1. Al-Adwani DG, Renno WM, Orabi KY: Neurotherapeutic effects of Ginkgo biloba extract and its terpene trilactone, ginkgolide B, on sciatic crush injury model: A new evidence. PLoS one 14:e0226626, 2019. https://doi.org/10.1371/journal.pone.0226626
2. Apaydin AS, Sahin C, Cayli S, Bal E, Bal C, Gokcem Yildiz F, Ayberk G: Levetiracetam treatment in an experimental model of sciatic nerve injury: A randomized controlled trial. Neurol Res 45:86-96, 2023. https://doi.org/10.1080/01616412.2022.2143617
3. Aslan E, Kocaeli H, Bekar A, Tolunay S, Ulus IH: CDP-choline and its endogenous metabolites, cytidine and choline, promote the nerve regeneration and improve the functional recovery of injured rat sciatic nerves. Neurol Res 33:766-773, 2011. https://doi.org/10.1179/1743132811Y.0000000004
4. Bademoglu G, Erdal N, Uzun C, Tasdelen B: The effects of pulsed electromagnetic field on experimentally induced sciatic nerve injury in rats. Electromagn Biol Med 40:408-419, 2021. https://doi.org/10.1080/15368378.2021.1907403
5. Brügger V, Duman M, Bochud M, Münger E, Heller M, Ruff S, Jacob C: Delaying histone deacetylase response to injury accelerates conversion into repair Schwann cells and nerve regeneration. Nat Commun 8:14272, 2017. https://doi.org/10.1038/ncomms14272
6. Brull R, Hadzic A, Reina MA, Barrington MJ: Pathophysiology and etiology of nerve injury following peripheral nerve blockade. Reg Anesth Pain Med 40:479-490, 2015. https://doi.org/10.1097/AAP.0000000000000125
7. Bu Y, Wang X, Li L, Hu X, Tan D, Li Z, Lai M, Qiu X, Sun F, Wang H, Yang F, Wu D, Guo J: Lithium loaded octa-poly(ethylene glycol) based adhesive facilitates axon regeneration and reconnection of transected peripheral nerves. Adv Healthc Mater 9:e2000268, 2020. https://doi.org/10.1002/adhm.202000268
8. Byrne PJ, Stuart RM, Fakhry C, Lehar M, Flint PW: An electrophysiologic model for functional assessment of effects of neurotrophic factors on facial nerve reinnervation. Arch Facial Plast Surg 7:114-118, 2005. https://doi.org/10.1001/archfaci.7.2.114
9. Caner B, Kafa MI, Bekar A, Kurt MA, Karli N, Cansev M, Ulus IH: Intraperitoneal administration of CDP-choline or a combination of cytidine plus choline improves nerve regeneration and functional recovery in a rat model of sciatic nerve injury. Neurol Res 34:238-245, 2012. https://doi.org/10.1179/1743132812Y.0000000003

10. Cansev M, Ilcol YO, Yilmaz MS, Hamurtekin E, Ulus IH: Choline, CDP-choline or phosphocholine increases plasma glucagon in rats: involvement of the peripheral autonomic nervous system. Eur J Pharmacol 589:315-322, 2008. https://doi.org/10.1016/j.ejphar.2008.05.017
11. Cansev M, Ilcol YO, Yilmaz MS, Hamurtekin E, Ulus IH: Peripheral administration of CDP-choline, phosphocholine or choline increases plasma adrenaline and noradrenaline concentrations. Auton Autacoid Pharmacol 28:41-58, 2008. https://doi.org/10.1111/j.1474-8673.2007.00416.x
12. Cansev M, Minbay Z, Goren B, Yaylagul EO, Cetinkaya M, Koksal N, Alkan T: Neuroprotective effects of uridine in a rat model of neonatal hypoxic-ischemic encephalopathy. Neurosci Lett 542:65-70, 2013. https://doi.org/10.1016/j.neulet.2013.02.035
13. Cansev M, Watkins CJ, Van Der Beek EM, Wurtman RJ: Oral uridine-5′-monophosphate (UMP) increases brain CDP-choline levels in gerbils. Brain Res 1058:101-108, 2005. https://doi.org/10.1016/j.brainres.2005.07.054
14. Cansev M, Yilmaz MS, Ilcol YO, Hamurtekin E, Ulus IH: Cardiovascular effects of CDP-choline and its metabolites: Involvement of peripheral autonomic nervous system. Eur J Pharmacol 577:129-142, 2007. https://doi.org/10.1016/j.ejphar.2007.08.029
15. Cavalcante Miranda de Assis D, Martins Lima Ê, Teixeira Goes B, Zugaib Cavalcanti J, Barbosa Paixão A, Vannier-Santos MA, Martinez AM, Baptista AF: The parameters of transcutaneous electrical nerve stimulation are critical to its regenerative effects when applied just after a sciatic crush lesion in mice. BioMed Res Int 2014:572949, 2014. https://doi.org/10.1155/2014/572949
16. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals: Guide for the Care and Use of Laboratory Animals. 8th ed. Washington (DC): National Academies Press (US), 2011.
17. Delibas B, Kaplan AA, Marangoz AH, Eltahir MI, Altun G, Kaplan S: The effect of dietary sesame oil and ginger oil as antioxidants in the adult rat dorsal root ganglia after peripheral nerve crush injury. Int J Neurosci 20:1-11, 2022. https://doi.org/10.1080/00207454.2022.2145475
18. Duman M, Martinez-Moreno M, Jacob C, Tapinos N: Functions of histone modifications and histone modifiers in Schwann cells. Glia 68:1584-1595, 2020. https://doi.org/10.1002/glia.23795
19. Faraco G, Pancani T, Formentini L, Mascagni P, Fossati G, Leoni F, Moroni F, Chiarugi A: Pharmacological inhibition of histone deacetylases by suberoylanilide hydroxamic acid specifically alters gene expression and reduces ischemic injury in the mouse brain. Mol Pharmacol 70:1876-1884, 2006. https://doi.org/10.1124/mol.106.027912
20. Ghnenis AB, Czaikowski RE, Zhang ZJ, Bushman JS: Toluidine blue staining of resin-embedded sections for evaluation of peripheral nerve morphology. J Vis Exp 137:58031, 2018. https://doi.org/10.3791/58031-v
21. Gocmen S, Sirin S, Oysul K, Ulas UH, Oztas E: The effects of low-dose radiation in the treatment of sciatic nerve injury in rats. Turk Neurosurg 22:167-173, 2012. https://doi.org/10.5137/1019-5149.JTN.5039-11.1
22. Gomez-Sanchez JA, Pilch KS, van der Lans M, Fazal SV, Benito C, Wagstaff LJ, Mirsky R, Jessen KR: After nerve injury, lineage tracing shows that myelin and remak schwann cells elongate extensively and branch to form repair schwann cells, which shorten radically on remyelination. J Neurosci 37:9086-9099, 2017. https://doi.org/10.1523/JNEUROSCI.1453-17.2017
23. Goren B, Cakir A, Ocalan B, Serter Kocoglu S, Alkan T, Cansev M, Kahveci N: Long-term cognitive effects of uridine treatment in a neonatal rat model of hypoxic-ischemic encephalopathy. Brain Res 1659:81-87, 2017. https://doi.org/10.1016/j.brainres.2017.01.026
24. Goren B, Cakir A, Sevinc C, Serter Kocoglu S, Ocalan B, Oy C, Minbay Z, Kahveci N, Alkan T, Cansev M: Uridine treatment protects against neonatal brain damage and long-term cognitive deficits caused by hyperoxia. Brain Res 1676:57-68, 2017. https://doi.org/10.1016/j.brainres.2017.09.010
25. Gorgulu A, Imer M, Simsek O, Sencer A, Kutlu K, Cobanoglu S: The effect of aprotinin on extraneural scarring in peripheral nerve surgery: An experimental study. Acta Neurochir 140:1303-1307, 1998. https://doi.org/10.1007/s007010050254
26. Gundogdu EB, Bekar A, Turkyilmaz M, Gumus A, Kafa IM, Cansev M: CDP-choline modulates matrix metalloproteinases in rat sciatic injury. J Surg Res 200:655-663, 2016. https://doi.org/10.1016/j.jss.2015.10.003
27. Gurunluoglu R, Gurunluoglu A: Paul of Aegina: Landmark in surgical progress. World J Surg 27:18-25, 2003. https://doi.org/10.1007/s00268-002-6464-8
28. He X, Zhang L, Queme LF, Liu X, Lu A, Waclaw RR, Dong X, Zhou W, Kidd G, Yoon SO, Buonanno A, Rubin JB, Xin M, Nave KA, Trapp BD, Jankowski MP, Lu QR: A histone deacetylase 3-dependent pathway delimits peripheral myelin growth and functional regeneration. Nat Med 24:338-351, 2018. https://doi.org/10.1038/nm.4483
29. Hughes PM, Wells GMA, Clements JM, Gearing AJ, Redford EJ, Davies M, Smith KJ, Hughes RA, Brown MC, Miller KM: Matrix metalloproteinase expression during experimental autoimmune neuritis. Brain 121:481-494, 1998. https://doi.org/10.1093/brain/121.3.481
30. Hussain G, Wang J, Rasul A, Anwar H, Qasim M, Zafar S, Aziz N, Razzaq A, Hussain R, de Aguilar JG, Sun T: Current status of therapeutic approaches against peripheral nerve injuries: A detailed story from injury to recovery. Int J Biol Sci 16:116-134, 2020. https://doi.org/10.7150/ijbs.35653
31. Ilcol YO, Cansev M, Yilmaz MS, Hamurtekin E, Ulus IH: Intraperitoneal administration of CDP-choline and its cholinergic and pyrimidinergic metabolites induce hyperglycemia in rats: Involvement of the sympathoadrenal system. Arch Physiol Biochem 113:186-201, 2007. https://doi.org/10.1080/13813450701531243
32. Ilcol YO, Cansev M, Yilmaz MS, Hamurtekin E, Ulus IH: Peripheral administration of CDP-choline and its cholinergic metabolites increases serum insulin: Muscarinic and nicotinic acetylcholine receptors are both involved in their actions. Neurosci Lett 431:71-76, 2008. https://doi.org/10.1016/j.neulet.2007.11.024

33. Kaplan T, Kafa IM, Cansev M, Bekar A, Karli N, Taskapilioglu MO, Kanar F: Investigation of the dose-dependency of citicoline effects on nerve regeneration and functional recovery in a rat model of sciatic nerve injury. Turk Neurosurg 24:54-62, 2014. https://doi.org/10.5137/1019-5149.JTN.8451-13.0
34. Kavlak E, Belge F, Unsal C, Uner AG, Cavlak U, Comlekci S: Effects of pulsed electromagnetic field and swimming exercise on rats with experimental sciatic nerve injury. J Phys Ther Sci 26:1355-1361, 2014. https://doi.org/10.1589/jpts.26.1355
35. Kayhan Kustepe E, Altunkaynak BZ, Alkan I, Kivrak EG, Yildiran A, Geuna S: Potential effects of stem cells derived from the peripheral nerve and adipose tissue after the nerve crush injury in control and obese rats. J Invest Surg 35:1021-1033, 2022. https://doi.org/10.1080/08941939.2021.1991530
36. Kazantsev AG, Thompson LM: Therapeutic application of histone deacetylase inhibitors for central nervous system disorders. Nat Rev Drug Discov 7:854-868, 2008. https://doi.org/10.1038/nrd2681
37. Kennedy EP, Weiss SB: The function of cytidine coenzymes in the biosynthesis of phospholipides. J Biol Chem 222:193-214, 1956. https://doi.org/10.1016/S0021-9258(19)50785-2
38. Khademi E, Mahabadi VP, Ahmadvand H, Akbari E, Khalatbary AR: Anti-inflammatory and anti-apoptotic effects of hyperbaric oxygen preconditioning in a rat model of cisplatin-induced peripheral neuropathy. Iran J Basic Med Sci 23:321-328, 2020.
39. Khezri MK, Turkkan A, Koc C, Salman B, Levent P, Cakir A, Kafa IM, Cansev M, Bekar A: Anti-apoptotic and anti-oxidant effects of systemic uridine treatment in an experimental model of sciatic nerve injury. Turk Neurosurg 31:373-378, 2021
40. Khezri MK, Turkkan A, Koc C, Salman B, Levent P, Cakir A, Kafa IM, Cansev M, Bekar A: Uridine treatment improves nerve regeneration and functional recovery in a rat model of sciatic nerve injury. Turk Neurosurg 32:935-943, 2022.
41. Kim M, Park C, Jung J, Yeo SG: The histone deacetylase class I, II inhibitor trichostatin A delays peripheral neurodegeneration. J Mol Histol 50:167-178, 2019. https://doi.org/10.1007/s10735-019-09815-1
42. Koyuncuoglu T, Turkyilmaz M, Goren B, Cetinkaya M, Cansev M, Alkan T: Uridine protects against hypoxic-ischemic brain injury by reducing histone deacetylase activity in neonatal rats. Restor Neurol Neurosci 33:777-784, 2015. https://doi.org/10.3233/RNN-150549
43. Lindsay RW, Heaton JT, Edwards C, Smitson C, Hadlock TA: Nimodipine and acceleration of functional recovery of the facial nerve after crush injury. Arch Facial Plast Surg 12:49-52, 2010. https://doi.org/10.1001/archfaci.2009.95
44. Liu Z, Li W, Geng L, Sun L, Wang Q, Yu Y, Yan P, Liang C, Ren J, Song M, Zhao Q, Lei J, Cai Y, Li J, Yan K, Wu Z, Chu Q, Li J, Wang S, Li C, Han JJ, Hernandez-Benitez R, Shyh-Chang N, Belmonte JCI, Zhang W, Qu J, Liu GH: Cross-species metabolomic analysis identifies uridine as a potent regeneration promoting factor. Cell Discov 8:6, 2022. https://doi.org/10.1038/s41421-021-00361-3
45. Mafi P, Hindocha S, Dhital M, Saleh M: Advances of peripheral nerve repair techniques to improve hand function: A systematic review of literature. Open Orthop J 6:60-68, 2012. https://doi.org/10.2174/1874325001206010060
46. McMorrow LA, Kosalko A, Robinson D, Saiani A, Reid AJ: Advancing our understanding of the chronically denervated schwann cell: A potential therapeutic target? Biomolecules 12(8):1128, 2022. https://doi.org/10.3390/biom12081128
47. Mohammadi R, Yadegarazadi MJ, Amini K: Peripheral nerve regeneration following transection injury to rat sciatic nerve by local application of adrenocorticotropic hormone. J Craniomaxillofac Surg 42:784-789, 2014. https://doi.org/10.1016/j.jcms.2013.11.012
48. Nadeau S, Filali M, Zhang J, Kerr BJ, Rivest S, Soulet D, Iwakura Y, de Rivero Vaccari JP, Keane RW, Lacroix S: Functional recovery after peripheral nerve injury is dependent on the pro-inflammatory cytokines IL-1β and TNF: Implications for neuropathic pain. J Neurosci 31:12533-12542, 2011. https://doi.org/10.1523/JNEUROSCI.2840-11.2011
49. Naik AK, Tandan SK, Dudhgaonkar SP, Jadhav SH, Kataria M, Prakash VR, Kumar D: Role of oxidative stress in pathophysiology of peripheral neuropathy and modulation by N-acetyl-l-cysteine in rats. Eur J Pain 10:573-579, 2006. https://doi.org/10.1016/j.ejpain.2005.08.006
50. Noble J, Munro CA, Prasad VS, Midha R: Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries. J Trauma 45:116-122, 1998. https://doi.org/10.1097/00005373-199807000-00025
51. Oktay MF, Askar I, Yildirim A, Gurlek A, Akkus M, Topcu I, Meric F: Effects of antineoplastic agents on the peripheral nerves under a surgical tissue expansion procedure: An experimental study. Microsurgery 26:473-479, 2006. https://doi.org/10.1002/micr.20273
52. Olivera ALR: Apoptosis of sensory neurons and satellite cells after sciatic nerve transection in C57BL/6J mice. Braz J Med Biol Res 34:375-380, 2001. https://doi.org/10.1590/S0100-879X2001000300012
53. Olke HC, Bicer OS, Mirioglu A, Saker D, Ocal I, Ozkan C: Clinical, electrophysiological, and histomorphological effects of local coenzyme Q10 and vitamin E use in a rat model of peripheral nerve injury. Acta Orthop Traumatol Turc 57:23-29, 2023.
54. Ozay R, Bekar A, Kocaeli H, Karli N, Filiz G, Ulus IH: Citicoline improves functional recovery, promotes nerve regeneration, and reduces postoperative scarring after peripheral nerve surgery in rats. Surg Neurol 68:615-622, 2007. https://doi.org/10.1016/j.surneu.2006.12.054
55. Ozgenel GY, Filiz G: Combined application of human amniotic membrane wrapping and hyaluronic acid injection in epineurectomized rat sciatic nerve. J Reconstr Microsurg 20:153-157, 2004. https://doi.org/10.1055/s-2004-820772
56. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen OH, Rawle F, Reynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T, Würbel H: The ARRIVE guidelines 2.0. Updated guidelines for reporting animal research. J Cereb Blood Flow Metab 40:1769-1777, 2020. https://doi.org/10.1177/0271678X20943823
57. Petersen J, Russell L, Andrus K, MacKinnon M, Silver J, Kliot M: Reduction of extraneural scarring by ADCON-T/N after surgical intervention. Neurosurgery 38:976-984, 1996. https://doi.org/10.1097/00006123-199605000-00025

58. Pooler AM, Guez DH, Benedictus R, Wurtman RJ: Uridine enhances neurite outgrowth in nerve growth factor-differentiated PC12 [corrected]. Neuroscience 134:207-214, 2005. https://doi.org/10.1016/j.neuroscience.2005.03.050
59. Rahimian R, Fakhfouri G, Rasouli MR, Nouri M, Nezami BG, Paydar MJ, Asadi-Amoli F, Dehpour AR: Effect of pioglitazone on sciatic nerve ischemia/reperfusion injury in rats. Pediatr Neurosurg 45:126-131, 2009. https://doi.org/10.1159/000209287
60. Richardson UI, Watkins CJ, Pierre C, Ulus IH, Wurtman RJ: Stimulation of CDP-choline synthesis by uridine or cytidine in PC12 rat pheochromocytoma cells. Brain Res 971:161-167, 2003. https://doi.org/10.1016/S0006-8993(03)02333-3
61. Rivieccio MA, Brochier C, Willis DE, Walker BA, D'Annibale MA, McLaughlin K, Siddiq A, Kozikowski AP, Jaffrey SR, Twiss JL, Ratan RR, Langley B: HDAC6 is a target for protection and regeneration following injury in the nervous system. Proc Natl Acad Sci U S A 106:19599-19604, 2009. https://doi.org/10.1073/pnas.0907935106
62. Sadri-Vakili G, Cha JH: Histone deacetylase inhibitors: A novel therapeutic approach to huntingtons disease (complex mechanism of neuronal death). Curr Alzheimer Res 3:403-408, 2006. https://doi.org/10.2174/156720506778249407
63. Sakar M, Korkusuz P, Demirbilek M, Cetinkaya DU, Arslan S, Denkbas EB, Temucin CM, Bilgic E, Hazer DB, Bozkurt G: The effect of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHx) and human mesenchymal stem cell (hMSC) on axonal regeneration in experimental sciatic nerve damage. Int J Neurosci 124:685-696, 2014. https://doi.org/10.3109/00207454.2013.876636
64. Sakloth F, Manouras L, Avrampou K, Mitsi V, Serafini RA, Pryce KD, Cogliani V, Berton O, Jarpe M, Zachariou V: HDAC6-selective inhibitors decrease nerve-injury and inflammation-associated mechanical hypersensitivity in mice. Psychopharmacology 237:2139-2149, 2020. https://doi.org/10.1007/s00213-020-05525-9
65. Savci V, Goktalay G, Cansev M, Cavun S, Yilmaz MS, Ulus IH: Intravenously injected CDP-choline increases blood pressure and reverses hypotension in haemorrhagic shock: effect is mediated by central cholinergic activation. Eur J Pharmacol 468:129-39, 2003. https://doi.org/10.1016/S0014-2999(03)01602-9
66. Secades JJ, Gareri P: Citicoline: Pharmacological and clinical review, 2022 update. Rev Neurol 75:1-89, 2022. https://doi.org/10.33588/rn.75S05.2022311
67. Shukla S, Tekwani BL: Histone deacetylases inhibitors in neurodegenerative diseases, neuroprotection and neuronal differentiation. Front Pharmacol 11:537, 2020. https://doi.org/10.3389/fphar.2020.00537
68. Thorell L, Sjöberg LB, Hernell O: Nucleotides in human milk: Sources and metabolism by the newborn infant. Pediatr Res 40:845-852, 1996. https://doi.org/10.1203/00006450-199612000-00012
69. Turedi S, Yulug E, Alver A, Bodur A, Ince I: A morphological and biochemical evaluation of the effects of quercetin on experimental sciatic nerve damage in rats. Exp Ther Med 15:3215-3224, 2018
70. Uo T, Veenstra TD, Morrison RS: Histone deacetylase inhibitors prevent p53-dependent and p53-independent Bax-mediated neuronal apoptosis through two distinct mechanisms. J Neurosci 29:2824-2832, 2009. https://doi.org/10.1523/JNEUROSCI.6186-08.2009
71. Wu F, Jiao C, Yang Y, Liu F, Sun Z: Nerve conduit based on HAP/PDLLA/PRGD for peripheral nerve regeneration with sustained release of valproic acid. Cell Biol Int 45:1733-1742, 2021. https://doi.org/10.1002/cbin.11613
72. Wurtman RJ, Regan M, Ulus I, Yu L: Effect of oral CDP-choline on plasma choline and uridine levels in humans. Biochem Pharmacol 60:989-992, 2000. https://doi.org/10.1016/S0006-2952(00)00436-6
73. Yadav A, Huang TC, Chen SH, Ramasamy TS, Hsueh YY, Lin SP, Lu FI, Liu YH, Wu CC: Sodium phenylbutyrate inhibits Schwann cell inflammation via HDAC and NFκB to promote axonal regeneration and remyelination. J Neuroinflammation 18:238, 2021. https://doi.org/10.1186/s12974-021-02273-1
74. Yarar E, Kuruoglu E, Kocabicak E, Altun A, Genc E, Ozyurek H, Kefeli M, Marangoz AH, Aydin K, Cokluk C: Electrophysiological and histopathological effects of mesenchymal stem cells in treatment of experimental rat model of sciatic nerve injury. Int J Clin Exp Med 8:8776-8784, 2015
75. Yuan B, Zheng X, Wu ML, Yang Y, Chen JW, Gao HC, Liu J: Platelet-rich plasma gel-loaded collagen/chitosan composite film accelerated rat sciatic nerve injury repair. ACS Omega 8:2931-2941, 2023. https://doi.org/10.1021/acsomega.2c05351
76. Yuksel TN, Halici Z, Demir R, Cakir M, Calikoglu C, Ozdemir G, Unal D: Investigation of the effect of telmisartan on experimentally induced peripheral nerve injury in rats. Int J Neurosci 125:464-473, 2015. https://doi.org/10.3109/00207454.2014.948115