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Effects of Trauma and Cisplatin on Survival in Drg Neurons

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ABSTRACT

Today, peripheral nerve injuries are one of the clinically critical pathological conditions with a high prevalence and significantly reduce the quality of life of individuals. In peripheral nerve injuries, structural and functional deteriorations of varying rates and reversible or permanent nature are observed in sensory, motor, and autonomic nerve fibers. In peripheral nerve injuries, although the source varies, practical treatment approaches and chemical agents are needed to reverse the pathological processes that develop in the acute and chronic periods. In this study, the effects of trauma and neurotoxicity at the cellular level in the acute period were examined, and the role of these factors in neuronal survival was evaluated. Balb/C strain mice with high regeneration capacity, 6–8 weeks old, were used in the study. In vitro experiments were performed on dorsal root ganglion (DRG) neurons isolated from these animals. Three groups were formed in the experimental design: The control group, the axotomy (trauma) group, and the cisplatin-treated neurotoxicity group. According to the obtained data, it was observed that the traumatic effect modeled with the axotomy method reduced neuronal survival more significantly compared to cisplatin-induced neurotoxicity ($p<0.05$). These findings indicate that preserving neuronal survival in the acute period is critical in managing nerve injury.

Keywords: DRG, axotomy, neurotoxicity, cisplatin, trauma

Introduction

Damages in peripheral nerves usually occur due to traumatic injuries or toxic factors. Traumatic nerve damage develops due to mechanical effects such as cutting, crushing, or stretching. In contrast, toxic nerve damage can usually be observed after metabolic diseases or the use of some pharmacological agents such as chemotherapy.

Peripheral nerve damage is a significant health problem that causes temporary or permanent disability and negatively affects the individual's quality of life (1). Peripheral nerve injuries due to trauma develop as a result of various physical factors such as motor vehicle accidents, penetrating injuries, cuts, gunshot wounds, falls, burns, and bone fractures (1). Such injuries are more common, especially in childhood, and create a severe economic burden on the health system and society. In traumatic nerve injuries, axons, myelin sheath, Schwann cells, blood vessels, as well as fat and connective tissue can be damaged (1)

On the other hand, toxicity-induced peripheral nerve damage usually occurs as a result of metabolic diseases or the use of pharmacological agents such as chemotherapy. Chemotherapy is one of the basic approaches in cancer treatment, and platinum-based compounds are especially widely used. Cisplatin is widely used in the treatment of various solid tumors, especially lung, ovarian, breast, bladder, testicular and brain tumors (2). Cisplatin is an important chemotherapeutic agent with proven efficacy in cancer treatment; however, its widespread use may be limited by serious side effects, especially neurotoxicity. It has been stated that platinum derivatives show their toxicity, especially in the cell bodies of sensory neurons, and that chronic peripheral neurotoxicity usually progresses in the form of sensory neuropathy (3). Cisplatin has been shown to cause deterioration in sensory, motor and autonomic nerve functions, especially at cumulative doses exceeding 400 mg/m^2. (4, 5). Studies have reported that the prevalence of peripheral neuropathy due to neurotoxicity after chemotherapy decreases to approximately 68% in the 1st month, 60% in the 3rd month, and 30% in

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the 6th month or longer following the end of treatment (6). Currently, there is no effective and specific treatment for cisplatin-induced neuropathy; therefore, neurotoxicity management is limited to approaches such as dose adjustment or complete discontinuation of the drug (7).

This study aimed to comparatively evaluate the effects of trauma and toxicity-induced peripheral nerve injuries on neuronal survival in vitro.

Material and Method

This study was conducted on DRG neurons obtained from Balb/C strain, 6–8 weeks old laboratory mice. Subjects were sacrificed under deep anesthesia. Then, DRGs were isolated from the tissues removed from the spine in RPMI medium. The obtained DRGs were enzymatically dissociated using collagenase and trypsin enzymes in Neurobasal-A (NBA) medium; mechanical dissociation was performed with pipette tips and an agitator. Following the dissociation process, fetal bovine serum and trypsin inhibitor were added, and then cell suspension was obtained by centrifugation. The isolated neurons were seeded in NBA medium on culture plates coated with poly-l lysine and laminin (8).

Experimental Groups: Control Group (n=115): In this group, no intervention was made to the cells; only their natural survival processes in the culture were observed.

Axotomy Group (n=121): After culture, neurons were cut using laser microdissection from a distance of 50 µm to create a trauma model (9).

Cisplatin Group (n=112): A toxic effect was created by applying cisplatin at a 5 µg/ml concentration after culture (8).

Axotomy and Imaging Method: Axotomy was performed using a special inverted microscope system with an ultraviolet (UV) laser unit. This system consists of PALM Microlaser Technology and an LPMC system. Live cell tracking imaging was performed with the Cell Observer-Zeiss time-lapse microscopy system; images were obtained using a 20x magnification objective. Imaging and microscope control were performed in a computer environment with Axiovision 3.1 software.

Images were imaged with phase-contrast and propidium iodide (PI-dead cell dye) at 0th and 24th hours, while fluorescence imaging was performed at 48th hours using phase-contrast, PI, and Calcein-AM (CAM-live cell dye) (10).

Statistical Analysis: An Independent two proportions Z test was used to the compare the groups in terms of survival rate. The statistical significance level was considered as 5%, and the MINITAB (ver: 14) statistical package program was used for calculations.

Results

At 24th hours, the Control group significantly preserved survival compared to both the Axotomy and Cisplatin groups (p=0.001). The Cisplatin group maintained survival at a significantly higher level than the Axotomy group (p=0.004).

At 48th hours, the Control group significantly preserved survival compared to the Axotomy and Cisplatin groups (p=0.001). The Cisplatin group maintained survival at a significantly higher level than the Axotomy group (p=0.016).

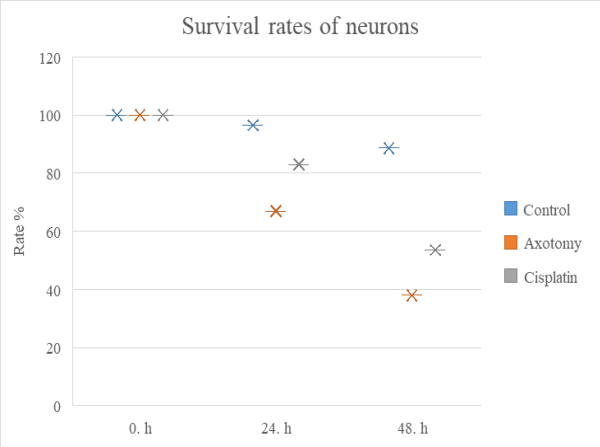
Discussion

Since neuronal losses usually lead to irreversible pathological consequences, preserving the viability of neurons exposed to trauma or toxicity is of great importance. This study evaluated the effects of both types of damage on neuronal survival by comparing trauma (axotomy) and toxicity (cisplatin application) models under in vitro conditions.

The findings were consistent with similar experimental studies. Significant cell death was observed in the axotomy group at 24th and 48th hours compared to the control group, indicating that neuronal trauma causes severe cellular losses (Table 1, Figures 1-3). In addition, the survival rate in the axotomy group was lower than in the cisplatin group. These findings are consistent with the increased apoptosis, decreased neuron number, and decreased NGF, BDNF, NT-3, and GDNF expression levels observed in experimental studies in which the L4 spinal nerve was cut (11). Similarly, it has been reported that axotomy to the L5 nerve causes changes in mechanical, heat, and cold sensitivity in the acute phase (12).

In another study in which sciatic nerve transection was performed, it was reported that histone H3 and H4 acetylation decreased, and HDAC1 expression increased. Apoptosis was triggered in DRG neurons (13), which shows that traumatic damage creates effects at the epigenetic level and results in cell death. The studies of Üstün et al., in which the lowest survival rate was reported in

Table 1: In Table 1, the survival rates at 24th hours are Control: 97%, Axotomy: 67%, Cisplatin: 84%. The survival rates at 48th hours are Control: 89%, Axotomy: 38%, Cisplatin: 54%. P values are given for comparisons between groups



Comparisons	24th hours p values	48th hours p values
Control-Axotomy	0.001	0.001
Control-Cisplatin	0.001	0.001
Axotomy-Cisplatin	0.004	0.016

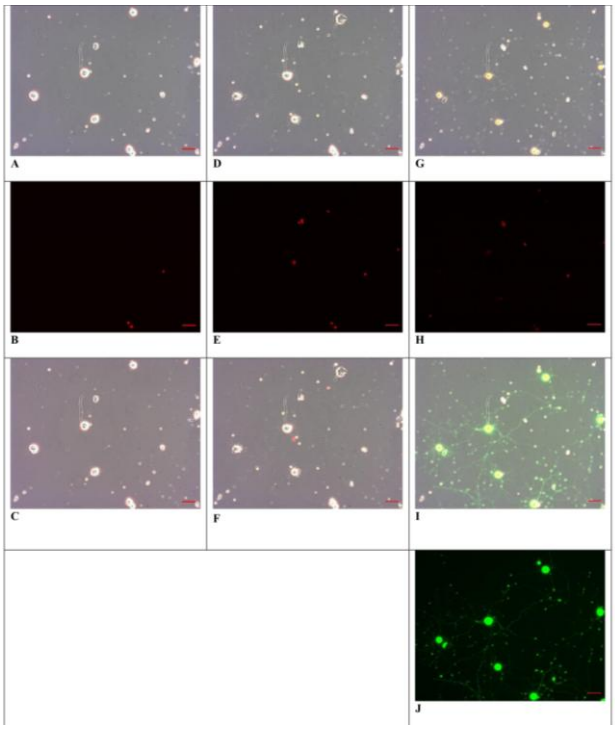


Fig. 1. Microscopic images of neurons from the control group. A: 0th hour phase. B: 0th hour PI. C: 0th hour merge. D: 24th hour phase. E: 24th hour PI. F: 24th hour merge. G: 48th hour phase. H: 48th hour PI. I: 48th hour merge. J: 48th hour CAM. (PI: Propidium iodide, CAM: Calcein AM. Objective magnification A-J: 20X, Scale bar: 50 µm)

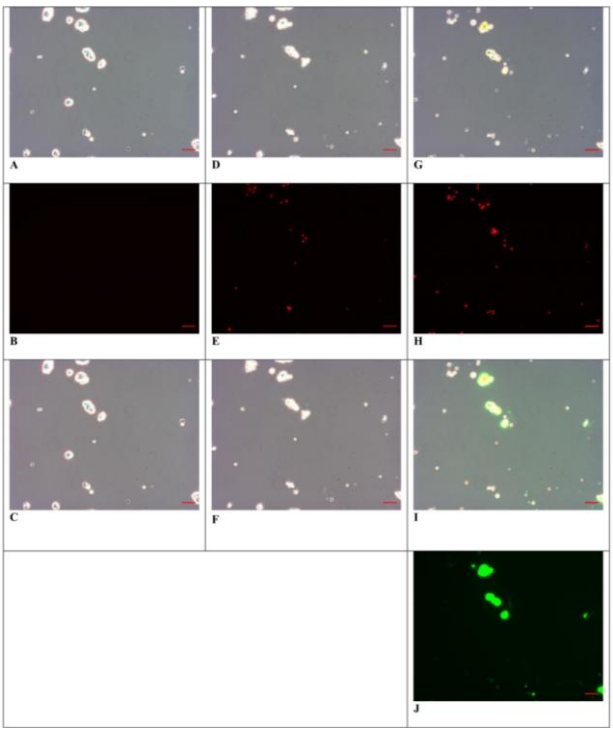


Fig. 2. Microscopic images of neurons from the cisplatin group. A: 0th hour phase. B: 0th hour PI. C: 0th hour merge. D: 24th hour phase. E: 24th hour PI. F: 24th hour merge. G: 48th hour phase. H: 48th hour PI. I: 48th hour merge. J: 48th hour CAM. (PI: Propidium iodide, CAM: Calcein AM. Objective magnification A-J: 20X, Scale bar: 50 µm)

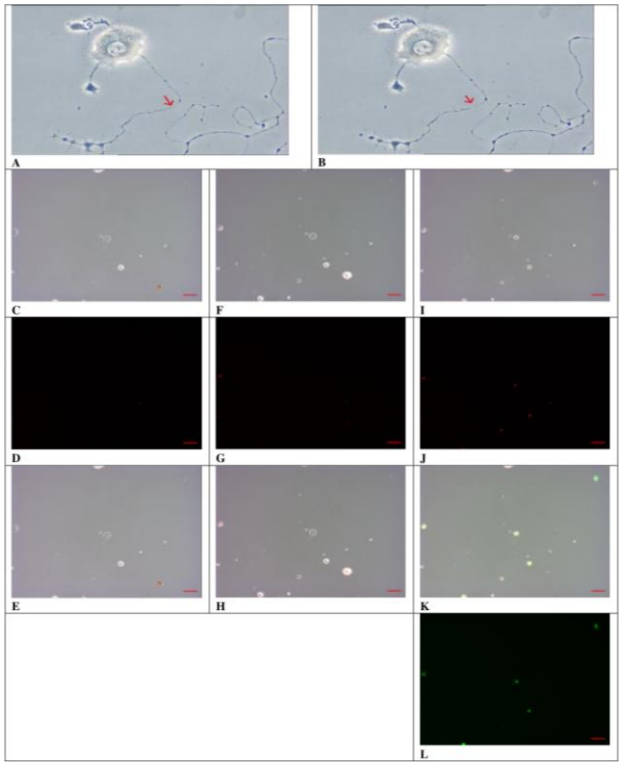


Fig. 3. Microscopic images of neurons from the axotomy group. A: Before axotomy. B: After axotomy. (PALM laser microscope). C: 0^{th} hour phase. D: 0^{th} hour PI. E: 0^{th} hour merge. F: 24^{th} hour phase. G: 24^{th} hour PI. H: 24^{th} hour merge. I: 48^{th} hour phase. J: 48^{th} hour PI. K: 48^{th} hour merge. L: 48^{th} hour CAM. (PI: Propidium iodide, CAM: Calcein AM. Objective magnification A-B: 63X; C-L: 20X, Scale bar: 50 µm)

DRG cultures subjected to axotomy, also parallel our findings (10).

Our study observed a significant decrease in survival rates in the cisplatin group at the 24^{th} and 48^{th} hours. However, this decrease was milder compared to the axotomy group, and it was shown that cisplatin application had a less destructive effect on neuronal viability. These findings are consistent with previous studies showing that cisplatin negatively affects both the viability and neurite extension capacity of neurons (8).

In addition, previous studies have reported that peripheral neuropathy can also develop due to reasons such as diabetes, toxin exposure, alcohol use, and nutritional deficiencies (14), that cisplatin causes more pronounced sensory symptoms in the hands, especially during long-term chemotherapy (3), that it causes thermal hyperalgesia and decreased grip strength (15), and that it triggers apoptosis by damaging both nuclear and mitochondrial DNA (7, 16). In these aspects, our study is consistent with the literature on the mechanisms of cisplatin-induced neurotoxicity.

In light of all these data, our study demonstrates that trauma has a more destructive effect on neuronal viability than cisplatin-induced toxicity. This finding is important for better understanding the impact of different pathological factors on neurons. Additionally, developing potential neuroprotective strategies may be essential in reducing nerve injury severity and improving clinical outcomes.

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