Research Article / Araştırma Makalesi
doi: 10.18678/dtfd.1517926

Duzce Med J, 2024;26(3):248-254
Düzce Tıp Fak Derg, 2024;26(3):248-254

Clinical and Histopathological Effects of Isotretinoin on Neuroregeneration in Experimental Spinal Cord Injury

Deneysel Omurilik Yaralanmasında İsotretinoinin Nörorejenerasyon Üzerine Klinik ve Histopatolojik Etkileri

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Received / Geliş Tarihi : 18.07.2024
Accepted / Kabul Tarihi : 09.12.2024
Available Online / Çevrimiçi Yayın Tarihi : 17.12.2024

ABSTRACT

Aim: Spinal cord injury is an important problem, and a fully effective treatment for it has not yet been developed. Isotretinoin is a retinoid known for its anti-inflammatory effect. The present study aimed to evaluate whether isotretinoin has a positive impact on neural tissue in post-injury damage.
Material and Methods: A total of 36 rats were randomly divided into 6 groups as control, sham, and injury with 14-day 7.5 mg/kg/day, 28-day 7.5 mg/kg/day, 14-day 15 mg/kg/day, and 28-day 15 mg/kg/day isotretinoin groups. Laminectomy was performed and spinal cord injury was produced by using the clip compression technique. Neurological examination was performed on days 1, 7, 14, and 28. After the treatment period, all rats were sacrificed, and their spinal cord samples were collected for histopathological assessment.
Results: Groups receiving 7.5 mg/kg/day ($p=0.048$) and 15 mg/kg/day ($p<0.001$) isotretinoin showed a significantly increased inclined plane angle on day 14 compared with the sham group. In hematoxylin and eosin, and Luxol fast blue staining, the 28-day isotretinoin of 15 mg/kg/day group and the control group had similar histopathological findings in motor neurons and glial cells. The cleaved caspase 3 expression was significantly diminished in the groups of 28-day isotretinoin 7.5 mg/kg/day, 14-day isotretinoin 15 mg/kg/day, and 28-day isotretinoin 15 mg/kg/day, compared to the sham group, along with the reduction in apoptosis.
Conclusion: Isotretinoin reduces neuronal apoptosis, diminishes pathological tissue damage, and improves functional recovery after injury. The observed prominent neuroprotective effects introduce isotretinoin as a promising therapy for spinal cord injury.
Keywords: Isotretinoin; neuroregeneration; spinal cord injury.

ÖZ

Amaç: Omurilik yaralanması önemli bir sorundur ve henüz tam olarak etkili bir tedavi geliştirilememiştir. İsotretinoin, anti-inflamatuar etkisiyle bilinen bir retinoiddir. Bu çalışmanın amacı, yaralanma sonrası sinir dokusu üzerinde isotretinoinin olumlu bir etkisinin olup olmadığını değerlendirmektir.
Gereç ve Yöntemler: Toplam 36 sıçan kontrol, sham ve travma ile 14 günlük 7,5 mg/kg/gün, 28 günlük 7,5 mg/kg/gün, 14 günlük 15 mg/kg/gün ve 28 günlük 15 mg/kg/gün isotretinoin grupları olmak üzere 6 gruba ayrıldı. Sıçanlara laminektomi yapıldı ve omurilik yaralanması klip kompresyon tekniği kullanılarak oluşturuldu. Nörolojik muayene 1, 7, 14 ve 28. günlerde yapıldı. Tedavi süresinden sonra tüm sıçanlar öldürüldü ve histopatolojik değerlendirme için omurilik örnekleri toplandı.
Bulgular: 7,5 mg/kg/gün ($p=0,048$) ve 15 mg/kg/gün ($p<0,001$) isotretinoin alan gruplar, sham grubuna kıyasla 14. günde önemli ölçüde artmış eğik düzlem açısı gösterdi. Hematoksilen ve eozin ile Luxol fast blue ile boyamada 15 mg/kg/gün isotretinoin 28 günlük grubu ve kontrol grubunun motor nöronlarında ve glial hücrelerinde benzer histopatolojik bulgular görüldü. Cleaved kaspaz 3 ekspresyonu, sham grubuna kıyasla 28 günlük isotretinoin 7,5 mg/kg/gün, 14 günlük isotretinoin 15 mg/kg/gün ve 28 günlük isotretinoin 15 mg/kg/gün gruplarında anlamlı bir şekilde azaldı ve apoptozisde azalma olduğu görüldü.
Sonuç: İsotretinoin, nöronal apoptozu azaltır, patolojik doku hasarını azaltır ve yaralanma sonrası fonksiyonel iyileşmeye etki eder. Gözlemlenen belirgin nöroprotektif etkiler, isotretinoini omurilik yaralanması için umut verici bir tedavi olarak tanıtmaktadır.
Anahtar kelimeler: İsotretinoin; nörorejenerasyon; omurilik yaralanması.

INTRODUCTION

Acute spinal cord injury (ASCI) remains a critical and uncontrollable health issue, leading to significant morbidity and mortality. The consequences extend beyond individual health, affecting psychiatric well-being and socioeconomic stability. Individuals with ASCI often require ongoing care, and in severe cases, they may experience tetraplegia and need respiratory support, posing substantial burdens on families and national economies due to high treatment and care costs and loss of productivity. Despite advancements, effective treatment for ASCI remains elusive, with current approaches limited to supportive measures (1,2).

ASCI typically results from high-energy incidents such as traffic accidents, falls, workplace injuries, and sports activities, predominantly affecting the cervical spinal cord and dorsolumbar junction (3,4). The injury process in ASCI is twofold: primary damage, an immediate and unavoidable consequence of trauma, and secondary damage, which results from endogenous cell death pathways activated by the initial injury. Clinical interventions focus on mitigating secondary damage, primarily responsible for neurological dysfunction (5). Factors such as free radicals, lipid peroxidation, neurotransmission system disruptions, inflammation, ion channel dysregulation, and apoptosis contribute significantly to secondary damage (6). Current clinical trials aim to prevent these secondary injury mechanisms.

Retinoids, known for their roles in cell growth, differentiation, and tumor suppression, also possess immunomodulating effects. Isotretinoin (13-cis-retinoic acid, $C_{20}H_{28}O_2$), a first-generation retinoid, has been widely used to treat severe acne since the 1980s due to its anti-inflammatory properties, which it exerts through its effects on neutrophils and T lymphocytes (7-13).

Isotretinoin, a vitamin A derivative, plays a crucial role in the development of vertebrate tissues by regulating gene expression involved in cell proliferation, differentiation, and apoptosis (14). This study aimed to investigate the potential benefits of isotretinoin on neural tissues that have been damaged by ASCI.

MATERIAL AND METHODS

The study was conducted at the Experimental Animal Laboratory at the Düzce University and received funding from the Düzce University Scientific Research Projects. Ethics approval was granted by the Düzce University Experimental Animal Ethics Committee, decision number 2020/11/03. The study adhered to the EU Directive 2010/63/EU regarding animal experiments, ensuring the minimum number of animals were utilized.

Animals

This study used 36 adult female Sprague-Dawley rats, aged 2-4 weeks and weighing 200-250 grams. The rats were housed in standard cages under controlled conditions, including a 12-hour light/dark cycle and a consistent room temperature of 22±2°C. They were acclimated for seven days before beginning the experimental procedures.

Experimental Groups

The 36 rats were randomly assigned to six experimental groups, each consisting of six rats: group I (Control), only underwent laminectomy at the T7-T9 level without further treatment; group II (Sham), underwent laminectomy at the T7-T9 level, followed by one minute of spinal cord compression using an aneurysm clip, with the dura kept intact; group III (Isotretinoin 14/7.5), received isotretinoin at 7.5 mg/kg/day by gavage for 14 days post-compression; group IV (Isotretinoin 28/7.5), received isotretinoin at 7.5 mg/kg/day by gavage for 28 days post-compression; group V (Isotretinoin 14/15) also received isotretinoin at 15 mg/kg/day by gavage for 14 days post-compression; and group VI (Isotretinoin 28/15), received isotretinoin at 15 mg/kg/day by gavage for 28 days post-compression. Each group was carefully monitored to assess the impact of isotretinoin on neural tissue recovery after spinal cord injury (SCI).

Spinal Cord Injury

Before anesthesia, all rats were evaluated to ensure normal motor function. Anesthesia was administered intramuscularly using 50 mg/kg ketamine hydrochloride (Ketas, Pfizer; Istanbul, Turkey) and 10 mg/kg xylazine (Rompun, Bayer; Istanbul, Turkey). The rats were positioned prone, and their backs were shaved and disinfected with polyvidone iodine (Batticon, Adeka; Samsun, Turkey). A midline vertical incision was made between T5 and T12, guided by the interscapular distance and ribs. Bilateral paravertebral muscles were dissected, and a mastoid retractor was used (Figure 1A). Laminectomy was performed at T7-T9. In the control group, the surgical wound was closed post-laminectomy (Figure 1B). For the standard SCI procedure, the thoracic vertebrae were exposed, and laminectomy was performed for 1 minute using a 70 g closing-force aneurysm clip (Yasargil FE 721 Aesculap; Istanbul, Turkey) (Figure 1C), as previously described (14,15). Hemostasis was ensured, and the paravertebral muscles and skin were sutured with 3/0 Vicryl, following anatomical layers. The rats were allowed to recover at room temperature.

Evaluation of Recovery

The recovery was assessed using the inclined plane test and motor examination based on the Drummond and Moore (D&M) criteria. The inclined plane method, developed by Rivlin and Tator (16) in 1977, records the highest angle at which the experimental animal can remain stationary without slipping for 5 seconds. The rat was placed parallel to the ground on the inclined plane, and the table was tilted at various angles. The angle at which the rat slipped was noted on days 1, 7, 14, and 28. To evaluate functional recovery in all rats, post-traumatic motor examinations were conducted on days 1, 7, 14, and 28 using the D&M scale (14,17).

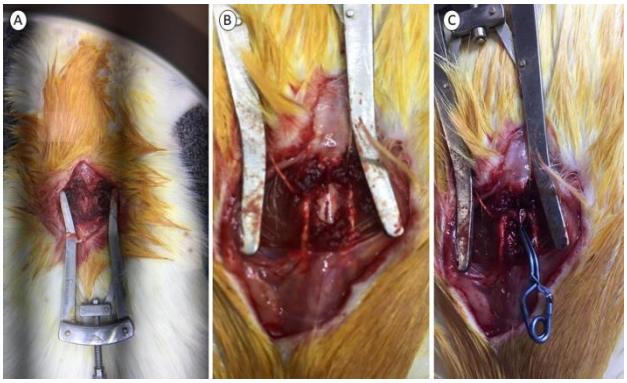

Figure 1. A) Retractor placed, **B)** Dura after laminectomy, **C)** Aneurysm clip application

Specimen Collection

At the conclusion of day 14, rats in groups I, II, III, and V were sacrificed. Similarly, rats in groups IV and VI were euthanized at the end of their 28-day follow-up period. The previously incised skin was reopened to access the site where the ASCI was induced using the clip-on dura after the laminectomy. The damaged section of the spinal cord was then carefully excised.

Histopathological Examination

Spinal cord specimens were preserved in 4% formaldehyde and embedded in paraffin. Thin sections, each 5 μm thick, were prepared from these paraffin blocks on silanized slides. After deparaffinization, some sections were stained with hematoxylin and eosin to assess tissue integrity and structure. Additional sections underwent Luxol fast blue staining to highlight and examine neuronal extensions. Immunohistochemical staining for cleaved caspase 3 was used to identify cells undergoing apoptosis. All stained histopathological samples were examined using light microscopy (Olympus Cx41-AxioCam Zeiss).

Statistical Analysis

The statistical analysis was done with the IBM SPSS v.22 package program. The normality assumption was assessed with the Shapiro-Wilk test. The Kruskal-Wallis test was used to compare the groups, followed by post hoc Bonferroni correction. The data were summarized with median, interquartile range, and minimum-maximum. Fisher-Freeman-Halton test was used to analyze categorical variables and given in number and percentage. A p-value of <0.05 indicated statistical significance.

RESULTS

Results from Motor Examination

A statistically significant difference was found between the experimental groups in terms of the inclined plane angles recorded on day 14 (p<0.001, Table 1). Compared to the control group, the inclined plane angles recorded on day 14 were statistically significantly lower in the sham, isotretinoin 7.5 mg/kg/day, and isotretinoin 15 mg/kg/day groups (p<0.001, p<0.001, and p=0.045, respectively). The groups of isotretinoin 7.5 mg/kg/day and isotretinoin 15 mg/kg/day had a significantly increased value of inclined plane angle, compared to the sham group (p=0.048, and p<0.001, respectively). There was no significant difference between the isotretinoin 7.5 mg/kg/day and isotretinoin 15 mg/kg/day groups in terms of the inclined plane angles measured on day 14 (p=0.180).

There was a statistically significant difference between the groups on day 14 in terms of the values measured in reference to the D&M criteria (p<0.001, Table 1). Again, compared to the control group, the D&M criteria scores of day 14 were statistically significantly lower in the sham, isotretinoin 7.5 mg/kg/day, and isotretinoin 15 mg/kg/day groups (p<0.001, p<0.001, and p=0.001, respectively). The D&M scores measured in the isotretinoin 7.5 mg/kg/day and isotretinoin 15 mg/kg/day groups were found to be similar to that of the sham group (p=0.291, and p=0.111, respectively). There was no significant difference between the isotretinoin 7.5 mg/kg/day and isotretinoin 15 mg/kg/day groups on day 14 either (p=0.510).

The inclined plane angles measured on day 28 were found to be different between the groups at a statistically significant level (p<0.001, Table 2). While the inclined plane angles of day 28 were statistically significantly lower in the sham and isotretinoin 15 mg/kg/day groups than in the control group (p<0.001, p=0.003, respectively), there was a significant difference between the sham group and the isotretinoin 7.5 mg/kg/day group (p=0.139). The inclined plane angle was found to be significantly higher in the isotretinoin 15 mg/kg/day group than in the sham group (p=0.003). There was no significant difference between the isotretinoin 7.5 mg/kg/day and isotretinoin 15 mg/kg/day groups in terms of inclined plane angle (p=0.834).

There was a statistically significant difference between the experimental groups also in D&M criteria scores measured on day 28 (p<0.002, Table 2). The D&M criteria scores of day 28 were statistically significantly lower in the sham, isotretinoin 7.5 mg/kg/day, and isotretinoin 15 mg/kg/day

Table 1. Inclined plane angles and Drummond and Moore criteria scores on day 14

	Control (n=6)	Sham (n=6)	Isotretinoin 14/7.5 (n=12)	Isotretinoin 14/15 (n=12)	p
Inclined plane angle	64.5 (1) [64-66]	44 (3) [42-46]	47 (1) [46-49]	48.5 (1) [48-50]	**<0.001**
D&M score	4 (0) [4-4]	0 (2) [0-4]	1 (0) [0-1]	1 (0) [1-1]	**<0.001**
D&M score, n (%)					
0	0 (0.0%)	4 (66.7%)	2 (16.7%)	0 (0.0%)	
1	0 (0.0%)	1 (16.7%)	10 (83.3%)	12 (100%)	**<0.001**
4	6 (100%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	

D&M: Drummond and Moore, Isotretinoin 14/7.5: received isotretinoin at 7.5 mg/kg/day for 14 days, Isotretinoin 14/15: received isotretinoin at 15 mg/kg/day for 14 days, descriptive statistics were as median (interquartile range) [minimum-maximum] for numerical data, and as frequency (percentage) for categorical data

Table 2. Inclined plane angles and Drummond and Moore criteria scores on day 28

	Control (n=6)	Sham (n=6)	Isotretinoin 28/7.5 (n=6)	Isotretinoin 28/15 (n=6)	p
Inclined plane angle	68.5 (2) [66-69]	46 (2) [45-48]	50 (1) [50-51]	52 (1) [52-54]	**<0.001**
D&M score	4 (0) [4-4]	0.5 (2) [0-4]	1 (0) [0-1]	1.5 (1) [1-2]	**0.002**
D&M score, n (%)					
0	0 (0.0%)	3 (50.0%)	1 (16.7%)	0 (0.0%)	
1	0 (0.0%)	2 (33.3%)	5 (83.3%)	3 (50.0%)	**<0.001**
2	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (50.0%)	
4	6 (100%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	

D&M: Drummond and Moore, Isotretinoin 28/7.5: received isotretinoin at 7.5 mg/kg/day for 28 days, Isotretinoin 28/15: received isotretinoin at 15 mg/kg/day for 28 days, descriptive statistics were as median (interquartile range) [minimum-maximum] for numerical data, and as frequency (percentage) for categorical data

groups than in the control group (p=0.001, p=0.001, and p=0.033, respectively). The D&M criteria scores in the isotretinoin 7.5 mg/kg/day and isotretinoin 15 mg/kg/day groups were similar to that of the sham group (p=0.914 and p=0.211, respectively), while there was no significant difference between the isotretinoin 7.5 mg/kg/day and isotretinoin 15 mg/kg/day groups (p=0.254).

Histopathological Findings

Hematoxylin and Eosin Staining

The hematoxylin and eosin staining did not reveal any histopathological changes in motor neurons and glial cells in the control group (Figure 2A). In the rats in the sham group, it was observed that the cytoplasm of the motor neuron turned pink and its nucleus turned purple, which indicated that their cell integrity was disrupted (Figure 2B). In the 14-day isotretinoin of 7.5 mg/kg/day group, cytoplasmic volumes of the motor neurons were decreased, the nuclei that were supposed to be visualized in purple were not definable and there were edematous areas between cell extensions. In glial cells, no histopathological changes were observed compared to the control group and the sham group (Figure 2C). In the 28-day isotretinoin of 7.5 mg/kg/day group, the cytoplasmic volumes of the motor neurons decreased and the cytoplasmic limits of some neurons could not be identified. In glial cells, no histopathological changes were observed compared to the control and sham groups (Figure 2D). In the 14-day isotretinoin of 15 mg/kg/day group, the cytoplasmic margins of the motor neurons, and the nuclei of purple color were all identifiable and there was no edema between the cell extensions. In glial cells, no histopathological changes were observed compared to the control group and the sham group (Figure 2E). On day 28, it was observed that the cytoplasmic margins and nucleus of motor neurons could be selected similarly to the control group. In glial cells, histopathological findings were found to be similar to those in the control group (Figure 2F).

Luxol Fast Blue Staining

There were no histopathological changes in motor neurons and glial cells observed in the Luxol fast blue staining of the control group (Figure 3A). In the Luxol fast staining of the sham group, dendrite extensions originating from motor neurons were observed (Figure 3B). In the 14-day isotretinoin of 7.5 mg/kg/day group, the motor neurons were seen to have diminished volume of cell bodies, and their extensions were not well identified (Figure 3C). In the 28-day isotretinoin of 7.5 mg/kg/day group, the volumes of the motor neurons were close to the control group and the cell extensions were identifiable (Figure 3D). In the 14-day isotretinoin of 15 mg/kg/day group, the cytoplasmic volumes and cell extensions of the motor neurons were found to be similar to the control group (Figure 3E). In the 14-day isotretinoin of 15 mg/kg/day group, the cytoplasmic volumes and cell extensions of the motor neurons were found to be the same as the control group (Figure 3F).

Immunohistochemical Staining of Cleaved Caspase 3

The immunohistochemical staining of cleaved caspase 3 did not reveal any immunohistochemical reaction in the motor neurons of the control group. Only the glial cells exhibited a reaction, interpreted as a physiological process (Figure 4A). It was observed that the expression of cleaved caspase 3 in motor neurons significantly increased in the sham group compared to the control group. The pronounced brown color, indicative of a dense expression pattern, was observed in some motor neurons. Additionally, the reactions in glial cells were higher than those in the control group (Figure 4B). In the 14-day isotretinoin treatment at 7.5 mg/kg/day group, the expression of cleaved caspase 3 in motor neurons and glial cells was higher than in the control group but lower than in the sham group (Figure 4C). Similarly, in the group treated with isotretinoin at 7.5 mg/kg/day for 28 days, the expression was similar to the control group and decreased compared to

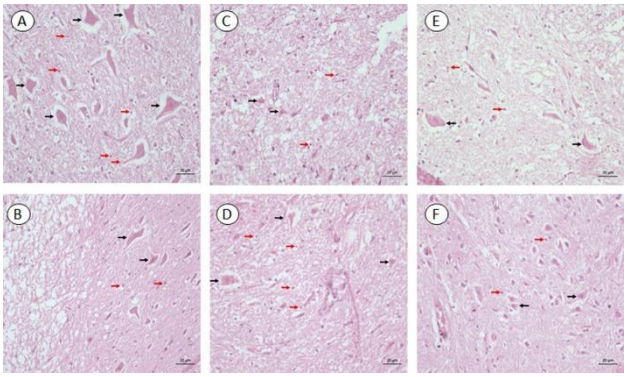

Figure 2. A) Control, **B)** sham, and **C)** 14-day 7.5 mg/kg/day, **D)** 28-day 7.5 mg/kg/day, **E)** 14-day 15 mg/kg/day, **F)** 28-day 15 mg/kg/day isotretinoin groups (black arrow points at motor neuron, red arrow at the glial cell, H&Ex400)

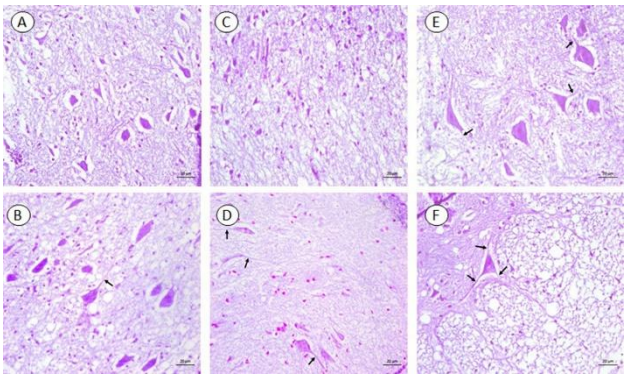

Figure 3. A) Control, **B)** sham, and **C)** 14-day 7.5 mg/kg/day, **D)** 28-day 7.5 mg/kg/day, **E)** 14-day 15 mg/kg/day, **F)** 28-day 15 mg/kg/day isotretinoin groups (black arrow points at dendrite extensions of the motor neuron) (Luxol fast bluex400)

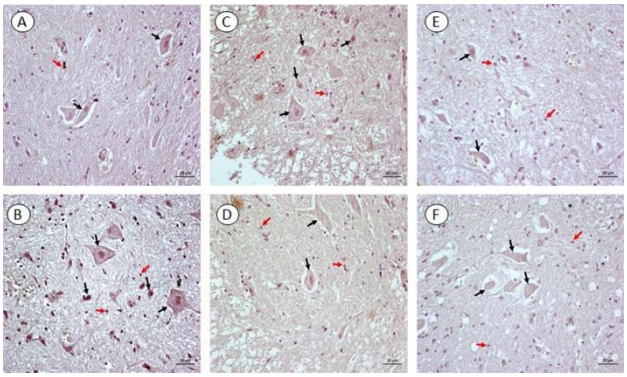

Figure 4. A) Control, **B)** sham, and **C)** 14-day 7.5 mg/kg/day, **D)** 28-day 7.5 mg/kg/day, **E)** 14-day 15 mg/kg/day, **F)** 28-day 15 mg/kg/day isotretinoin groups (black arrow points at motor neuron, red arrow at glial cell) (cleaved caspase 3x400)

the sham group (Figure 4D). The 14-day isotretinoin treatment at 15 mg/kg/day group showed cleaved caspase 3 expression levels similar to the control group and decreased compared to the sham group (Figure 4E). The 28-day isotretinoin treatment at 15 mg/kg/day group exhibited expression levels identical to those of the control group (Figure 4F).

Cleaved Caspase 3 Antibody

The cleaved caspase 3 expression was significantly increased in the sham group and the 14-day isotretinoin of 7.5 mg/kg/day group compared to the control group. The cleaved caspase 3 expression was statistically significantly decreased in the 28-day isotretinoin of 7.5 mg/kg/day, and 14-day and 28-day isotretinoin of 15 mg/kg/day groups, compared to the sham group. The cleaved caspase 3 expressions in the 28-day isotretinoin of 7.5 mg/kg/day, and 14-day and 28-day isotretinoin of 15 mg/kg/day groups were the same as in the control group. There was no statistically significant difference between the 14-day and the 28-day isotretinoin of 7.5 mg/kg/day and 14-day isotretinoin of 15 mg/kg/day groups.

DISCUSSION

Neurological dysfunction is usually because of secondary damage rather than the primary damage in SCI (5). The continuation of tissue damage due to the secondary damage mechanisms occurring after trauma has led to numerous experimental studies conducted to discover the treatment agents that are involved in this process (18-20). In the study, we investigate the therapeutic effect of isotretinoin on SCI.

Trauma itself causes the primary injury of sensitive spinal cord tissue that triggers secondary injury by enhanced inflammation, apoptosis, and free radical formation (21,22). Secondary damage mechanisms such as neurogenic shock, hemorrhages, ischemic reperfusion injury, secondary adverse effects of calcium, fluid-electrolyte imbalance, mitochondrial dysfunction, and apoptosis cause real damage to the spinal cord and healing mechanism (23). Caspase 3 is activated in the cells upon the resulting perfusion disorder, and apoptosis becomes dominant (21,22). As a result, SCI initiates a process that disrupts the blood-spinal cord barrier, leading to the infiltration of immune cells and the activation of inflammatory signaling pathways (24). Inflammation is responsible for secondary damage to the core and surrounding regions of the injury site (25). Vascular damage causes apoptosis, edema, and destruction of the white matter of the spinal cord (18,26).

Neutrophils, monocytes, microglia, and T-lymphocytes gather at the damaged area upon the inflammation response in ASCI. Neutrophils are immune cells that first arrive in the damaged area, activating other inflammatory cells and glial cells and secreting the cytokine, protease, and free radicals of the cascade that result in neuronal damage and death (27). Therefore, considered to be potentially effective in suppressing the inflammation; steroids, opioid antagonists, calcium channel blockers, volume expanders, tirilazad mesylate, and especially methylprednisolone due to its anti-inflammatory effect were widely used to suppress the inflammation (28). However, although intensive experimental and clinical studies have been carried out on this subject to date, such a molecule that has proven effective in this regard has not yet been described.

Isotretinoin is a molecule that acts as an anti-inflammatory agent by reducing the migration and movement of neutrophils to the tissue. It is also reported to stimulate the natural immune system and have an anti-inflammatory effect as it reduces T cell response (7,9). It has been shown to significantly diminish the levels of TNF-α, IL-4, IL-17, and IFN-γ released from T lymphocytes and have immunoregulatory effects (10-13). Isotretinoin also acts on matrix metalloproteinases (MMP). It is reported to reduce alleviate scar formation by reducing MMP-9 and MMP-13 (11,29).

In our study, we used isotretinoin, which has not been studied on a rat model before and whose anti-inflammatory effect is well known. Different groups of rats were formed considering that its effects may potentially vary according to the dose and term of its administration. To determine the dose of isotretinoin to be administered to rats, the rat dose of 7.5 mg/kg/day was taken as the base dose, after being calculated as the equivalent to the standard dose of isotretinoin (1 mg/kg/day) used for treating nodulocystic acne (30). The high dose was taken as twice this base dose.

Motor deficits were not found in the rats of the control group where only laminectomy was performed. It was planned to exclude any such rats that develop motor deficits. The inclined plane angles in the 7.5 mg/kg/day and 15 mg/kg/day isotretinoin groups were statistically lower than in the control group, while these inclined plane angles were increased significantly compared to the sham group. This finding indicated that isotretinoin rehabilitated walking on the inclined plane and resulted in improved motor examination. We found that this effect continued in the 28-day terms as well, but there was no further improvement as the dose increased. According to the evaluation based on the D&M criteria scores, we found that the low-high doses or short-long terms of isotretinoin administration did not make a difference when compared to the sham group. We believe that the reason why we were unable to detect the differences in the inclined plane angles in the D&M criteria scores is that we have not been able to correctly evaluate the traumatized subjects.

When cell integrity was evaluated by hematoxylin and eosin staining, it was observed that 7.5 mg/kg/day isotretinoin did not generate the expected changes in glial cells. In the 28-day isotretinoin of 7.5 mg/kg/day group, showed that the cytoplasmic volumes of motor neurons were found to diminish, and the cytoplasmic margins of some neurons were not identifiable. In glial cells, no histopathological changes were observed compared to the control and sham groups. In 14-day of increased treatment dose, it was seen that edema decreased in the motor neurons, the nuclei were identifiable, as well as that the cytoplasmic margins and nuclei of the motor neurons were identifiable similarly to the control group, while glial cells did not change reveal any changes as the term extended. Histopathological findings of the glial cells were found to be similar to that of the control group. These findings show that, as the dose and number of days of isotretinoin administration increase, isotretinoin significantly reduces necrosis and provides a level of improvement that makes a difference in histopathological terms.

In the examination by Luxol fast staining, it was observed that, in the 14-day isotretinoin of 7.5 mg/kg/day, the volume of the cell bodies of motor neurons diminished and

their extensions were not well identified, while the volumes of motor neurons were close to the control group and their cell extensions were identifiable in the 28-day term of the administration of the same dose. In the groups where the dose was 15 mg/kg/day, it was observed that the recovery was detected in the staining examinations on day 14. When the duration of treatment was increased to 28 days, the findings were found to be the same.

In the cleaved caspase 3 immunohistochemical staining where we intended to expose the cells' progress toward apoptosis, the 14-day isotretinoin of 7.5 mg/kg/day group had decreased staining of motor neurons and glial cells compared to the control group. It was determined that the staining decreased even more when the time was extended. It was also found that the cleaved caspase 3 expression of motor neurons and glial cells in the groups with increased dose was similar to the control group, while it was lower than the sham group. When the treatment period was 28 days, the cleaved caspase 3 expression continued to be the same as in the control group and its effect was maintained. These results show that isotretinoin corrects and restores the edema, ischemia, and membrane damage occurring as a result of the primary damage caused by trauma in ASCI. In addition, cleaved caspase 3 staining also demonstrated that it alleviates apoptosis, which is a very important step in secondary damage. Given the fact that these effects get more potent as the dose of isotretinoin and its duration of use increases, it makes sense to associate these effects with isotretinoin use. We believe that the motor recovery revealed in the inclined plane angle evaluation is thanks to the anti-inflammatory, antiapoptotic, and immunoregulatory effects of isotretinoin.

CONCLUSION

While the primary objective of this study is not to develop a standalone treatment agent for spinal cord trauma, it aims to identify a therapeutic agent that can complement both medical and surgical interventions. Based on the clinical and histopathological results obtained in the present study, it was understood that isotretinoin can be useful in ASCI.

Ethics Committee Approval: The study was approved by the Local Ethics Committee on Animal Experiments of Düzce University (16.11.2020, 11/03).

Conflict of Interest: None declared by the authors.

Financial Disclosure: This study was supported financially by the Düzce University Scientific Research Projects Unit (Grant/Award Number: 2021.04-1185).

Acknowledgments: None declared by the authors.

Author Contributions: Idea/Concept: DŞ, ÖP; Design: DŞ, ÖP; Data Collection/Processing: DŞ, ÖP, GK, MY, KK; Analysis/Interpretation: DŞ, ÖP, GK, MY, KK; Literature Review: DŞ, ÖP; Drafting/Writing: DŞ, ÖP, GK, MY, KK; Critical Review: ÖP.

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