ORIGINAL ARTICLE

DOI: 10.4274/nkmj.galenos.2022.62687
Nam Kem Med J 2022;10(3):302-307

The Protective Effects of Diclofenac Sodium and Montelukast Sodium on Acute Inflammation in Traumatic Spinal Cord Injury: An Experimental Study in Rats

Diklofenak Sodyum ve Montelukast Sodyumun Travmatik Omurilik Yaralanmalarında Akut Enflamasyon Üzerindeki Koruyucu Etkileri: Sıçanlarda Deneysel Bir Çalışma

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ABSTRACT

Aim: The aim of this study was to investigate the protective effects of diclofenac sodium (DF) and montelukast sodium (ML) on acute inflammation in traumatic spinal cord injury (T-SCI).

Materials and Methods: Forty Sprague-Dawley rats were randomly divided into five groups. While no intervention was made in the control group, spinal cord injury was applied to the trauma group. DF, ML and DF+ML were administered intraperitoneally to the remaining three groups after trauma. After rats were sacrificed, tissue samples containing both spinal cord and dura were subjected to histopathological examination and scored for edema, necrosis, inflammatory cells, apoptosis, neuron damage, and bleeding.

Results: There was a significant difference in the histopathological changes between the control and trauma groups ($p<0.05$). Histopathological scores of the trauma group and trauma+drug groups were similar ($p>0.05$). In the comparison of the control group and the other groups, no significant difference in edema was found in the tDF group ($p=0.059$). When the inflammatory cells were examined, it was seen that the cell amount was the least in the tDF group ($p=0.068$). It was observed that necrosis ($p=0.1$), apoptosis ($p=0.061$) and neural damage status ($p=0.139$) were the least in the tDF+ML combined group. There was no significant difference between the groups in terms of the amount of bleeding ($p>0.05$).

Conclusion: While the use of DF alone reduced the number of edema and inflammatory cells, the combined use of DF+ML reduced the development of necrosis, apoptosis and neural damage in T-SCI.

Keywords: Diclofenac sodium, inflammation, montelukast sodium, spinal cord injury, trauma

ÖZ

Amaç: Bu çalışmanın amacı travmatik omurilik yaralanmasında (T-SCI) diklofenak sodyum (DF) ve montelukast sodyumun (ML) akut enflamasyon üzerindeki koruyucu etkilerini araştırmaktır.

Gereç ve Yöntem: Kırk Sprague-Dawley sıçanı rastgele beş gruba ayrıldı. Kontrol grubuna herhangi bir müdahale yapılmazken, travma grubuna SCI uygulandı. Kalan üç gruba travma sonrası diklofenak sodyum (tDF), ML (tML) ve tDF+ML intraperitoneal yolla uygulandı. Sıçanlar sakrifiye edildikten sonra hem omurilik hem de dura içeren doku örnekleri histopatolojik incelemeye tabi tutuldu ve ödem, nekroz, enflamatuvar hücreler, apoptoz, nöron hasarı ve kanama açısından skorlandı.

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Received: 23.02.2022 **Accepted**: 11.06.2022

Bulgular: Kontrol ve travma grupları arasındaki histopatolojik değişikliklerde gruplar arasında anlamlı fark bulundu (p<0,05). Travma grubu ve travma+ilaç gruplarının histopatolojik skorları benzerdi (p>0,05). Kontrol grubu ile gruplar arasında ödem farkı olmayan grubun tDF grubu olduğu görüldü (p=0,059). Enflamatuvar hücreler incelendiğinde hücre miktarının en az tDF grubunda olduğu izlendi (p=0,068). Nekroz (p=0,1), apoptoz (p=0,061) ve nöral hasar durumunun (p=0,139) tDF+ML kombine grubunda en az olduğu görüldü. Kanama miktarı açısından gruplar arasında anlamlı bir fark yoktu (p>0,05).

Sonuç: DF'nin tek başına kullanımı ödem ve enflamatuvar hücre sayısını azaltırken, DF+ML'nin birlikte kullanımının T-SCI'da nekroz, apoptoz ve nöral hasar gelişimini azalttığı saptandı.

Anahtar Kelimeler: Diklofenak sodyum, enflamasyon, montelukast sodyum, omurilik yaralanması, travma

INTRODUCTION

Spinal cord injury (SCI) is a condition that can have temporary or permanent impact on spinal cord functions and that has physically, psychologically and socially devastating outcomes[1]. The incidence of SCI varies among geographical regions and over time. Annual incidence rate is reported to be 10.4-83 people per million[2].

Despite all therapeutic options, traumatic spinal cord injury (T-SCI)-related morbidity and mortality remain high[3]. Initially, primary injury occurs with the involvement of the spinal cord and surrounding tissues due to trauma-related mechanical reasons. Thereafter, secondary injury occurs by various mechanisms. Progressive edema, increasing ischemia and triggered pro-apoptotic pathways lead to additional tissue injury and the process results in cell death[4]. Primary injury usually cannot be prevented and its severity cannot be changed as it occurs due to unexpected reasons during this process. Thus, preventing or reducing and ameliorating secondary injury has been the main goal of the treatment. Nevertheless, there is yet no definite approach for the prevention or management of secondary injury, and numerous agents are under investigation[5]. Non-steroidal anti-inflammatory drugs (NSAIDs) as well are among the pharmacological agents used in the experimental and clinical studies.

Inflammation is a defense mechanism against injury. However, inflammatory processes may have harmful effects as well as benefits depending on various factors and time[6]. Anti-inflammatory drugs are used to reduce/ameliorate secondary injury by means of preventing/reducing inflammation. Montelukast sodium (ML) is a leukotriene receptor antagonist that reduces its effects by binding to cysteinyl leukotriene receptors to which leukotrienes, one of the arachidonic acid metabolites released from mast cells, eosinophils and other inflammatory cells that play a role in inflammation[7]. Diclofenac sodium (DF), on the other hand, has an anti-inflammatory effect by inhibiting cyclooxygenase 1 and 2 (COX-2) enzymes involved in arachidonic acid metabolism, preventing the synthesis of inflammatory mediators[8].

The present study aimed to investigate whether the inflammatory agents, DF and/or ML had protective effects on acute inflammation in T-SCI cases. For this purpose, an experimental rat model of T-SCI was created and histopathological examination was performed to investigate the effect of drugs on trauma-related injury.

MATERIALS AND METHODS

In the present study, 40 male Sprague-Dawley rats aged between 10 and 12 weeks and weighing 230-280 gr were used. This study was approved by the Local Ethics Committee for Animal Experiments at the meeting of the University of Health Sciences Turkey, Haydarpaşa Numune Training and Research Hospital (decision number: 2017-05/05, date: 01.11.2017). As anti-inflammatory drugs, DF (Voltaren, Novartis, İstanbul, Turkey) and ML (Singulair, Merck Sharp Dohme, İstanbul, Turkey) were used.

The rats were divided into 5 groups, each comprising 8 rats, as follows:

1. The control group (C): no SCI and no medication
2. The trauma group (T): only spinal cord injury, no medications.
3. The trauma+DF group (tDF): diclofenac administration after spinal cord injury.
4. The trauma+ML group (tML): montelukast administration after spinal cord injury.
5. The trauma+DF+ML group (tDF+ML): diclofenac+montelukast administration after spinal cord injury.

All the rats were kept in rooms provided with adequate air circulation and ambient temperature without food or water restriction. Following spinal cord injury, the subjects were placed in an appropriate environment for the maintenance of care, their daily dressing was done, and the subjects in the treatment groups received relevant medications.

Surgical Procedure

SCI was created in all rats excluding those in the control group. Prior to the surgical procedure, ketamine 60 mg/kg (Ketalar, Parke-Davis, Eczacıbaşı, İstanbul, Turkey) and Xylazine 10 mg/kg (Rompun, Bayer Pharmaceuticals, İstanbul, Turkey) were administered for general anesthesia. Surgical procedures

were performed on rats fixed in prone position onto the experimental boards. The surgical area was shaved, and regional antisepsis was provided using povidone iodine solution (Poviod 10% polyvinylpyrrolidone-iodine complex, Saba İlac, İstanbul, Turkey). After covering with sterile drapes, cutaneous and subcutaneous incisions were made at the level of T9-T12 vertebrae. Subperiosteal stripping of the paravertebral muscles was performed. Following T9-T10-T11 laminectomy and flavectomy, the dura was exposed. Extradural SCI was created by one minute compression of the spinal cord using Yaşargil Aneurysm Clip.

Drug Administration

Diclofenac sodium: The subjects in the relevant groups were administered 10 mg/kg DF twice a day by intraperitoneal route for 72 hours after spinal cord injury.

Montelukast sodium: The subjects in the relevant groups were administered 10 mg/kg ML twice a day by intraperitoneal route for 72 hours after spinal cord injury.

Follow-Up

After 72-hours of medical treatment and follow-up period, the subjects were sacrificed using 100 mg/kg pentothal sodium (Pental Sodyum, Ulagay İlac, İstanbul, Turkey). The operation site was opened under sterile conditions and the lesion area was exposed. The spinal cord, dura and the surrounding tissues including 5 mm of intact tissue from both proximal and distal segments were excised together. These tissue samples were fixed in 10% formalin solution and then were transferred to the laboratory for histopathological examination. During follow-up period, a total of two subjects, one from the trauma group and one from the trauma+DF group, died.

Histopathological Examination

The sections obtained from the tissue samples were stained with hematoxylin-eosin, and were examined under light microscope for the presence of edema, necrosis, inflammatory cells, apoptosis, neuron injury, and bleeding, and each finding was scored with the grading system which was summarized in Table 1. The histopathological examination findings can be seen in Figure 1.

Statistical Analysis

The Number Cruncher Statistical Software 2007 (NCSS, LLC, Kaysville, Utah, USA) was used for statistical analysis. Descriptive statistics were expressed as numbers and percentages for categorical variables and as median, 25th percentile, and 75th percentile for numerical variables. In multiple group comparisons with non-normal distribution, the Kruskal-Wallis test was used, whereas pairwise comparisons were performed using the Mann-Whitney U test with Bonferroni correction. The level of statistical significance was considered to be $p<0.05$.

RESULTS

The histopathological scores and statistical differences between the control group and other groups are summarized in Table 2. It was observed that the parameters between the control group and the trauma group were significantly different ($p<0.05$). Histopathological scores of the trauma group and trauma+drug groups were similar ($p>0.05$). The edema levels among the groups were examined and it was seen that the group with no difference was the tDF group compared to the control group ($p=0.059$) and the groups with a significant difference were the tDF+ML group ($p=0.034$) and the tML group ($p=0.007$), respectively. It was observed that the least edema was in the tDF group. In the evaluation of necrosis, the group with the least necrosis was tDF+ML ($p=0.1$), while the groups with more necrosis were tDF ($p=0.017$) and tML ($p=0.008$). Considering the number of inflammatory cells, the lowest number of cells was observed in the tDF group ($p=0.068$), while more cells were observed in the tDF+ML group ($p=0.035$) and in the tML group ($p=0.002$), respectively. While

Table 1. Grading system of histopathological changes

Grade	Histopathological changes
0	No change
1	Changes are present in less than 25% of the area
2	Changes are present in 25-50% of the area
3	Changes are present in 51-75% of the area
4	Changes are present in 76-99% of the area
5	Changes are present in the entire area

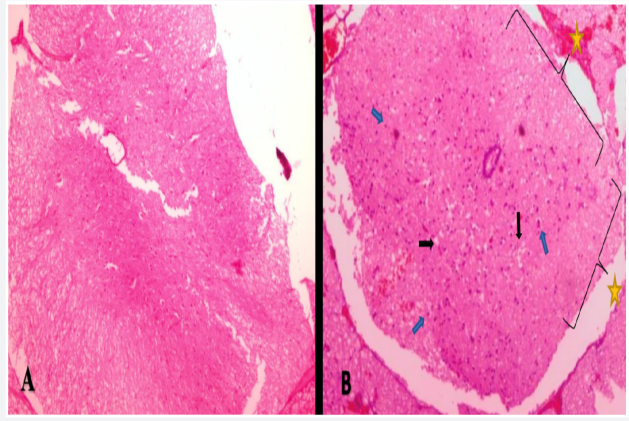

Figure 1. A) Histopathological section of the control group. B) Histopathological section of the trauma group. Numerous inflammatory cell images (blue arrows), areas of necrosis (black arrows) and prominent peripheral edema areas (areas marked with asterisks)

Table 2. Histopathological scores and statistical differences between the control group and other groups

	Edema		Necrosis		Inflammatory cell		Apoptosis		Neuron injury		Bleeding	
	Median	p	Median	p	Median	p	Median	p	Median	p	Median	p
Control	0 (0-0)		0 (0-0)		0 (0-0)		0 (0-0)		0 (0-0)		0 (0-0)	
Trauma	5 (4-5)	0.001	5 (4-5)	0.001	5 (3-5)	0.001	5 (4-5)	0.001	5 (5-5)	0.001	5 (3-5)	0.001
tDF	2 (2-3)	0.59	4 (3-4)	0.017	3 (2-3)	0.068	3 (2-4)	0.039	3 (3-4)	0.011	3 (3-3)	0.031
tML	3 (2-4)	0.007	4 (3-4)	0.008	3 (3-4)	0.002	3 (3-4)	0.004	4 (3-4)	0.005	3 (2-4)	0.014
tDF+ML	2 (2-4)	0.034	2 (2-4)	0.1	3 (2-3)	0.035	3 (2-3)	0.061	3 (2-3)	0.139	3 (2-4)	0.014

tDF: Trauma+diclofenac sodium, tML: Trauma+montelukast, tDF+ML: Trauma+diclofenac sodium+montelukast

the group with the least apoptosis was tDF+ML (p=0.061), it was found to be significantly higher in the tDF group (p=0.039) and tML group (p=0.004), respectively. While neuronal injury was seen at least in the tDF+ML group (p=0.139), significant neural damage was observed in the tDF group (p=0.011) and tML group (p=0.005), respectively. Considering the amount of bleeding in the preparations, it was observed that there was significant bleeding in all groups compared to the control group (p<0.05).

DISCUSSION

T-SCIs are clinical conditions that may lead to devastating physical, psychological and social consequences in the lives of patients. However, a definitive treatment method has not been developed despite all the researches and treatment modalities applied today[1]. The severity of primary injury is the most critical factor in determining the prognosis of T-SCI. Nevertheless, the mechanisms leading to secondary injury may intensify the injury or may affect healing processes and thus, may affect overall morbidity and mortality.

Inflammation has a critical role in secondary damage. The aim of inflammation is to stabilize and limit the existing damage, and to fulfill the necessary conditions for the repair of the organism, to clean the cell residues and to remove harmful substances in the environment. In case of high severity, neuronal cell damage may increase with the increase of damage, and as a result, functional results may worsen[9,10]. The mechanisms of secondary injury are complex and include various conditions such as neurogenic shock, bleeding, ischemia-reperfusion, excitotoxicity, calcium-mediated processes, fluid-electrolyte imbalance, immunological processes, apoptosis, and mitochondrial dysfunction[11]. So far, various medical agents have been investigated for the treatment of SCI. These agents include corticosteroids, vasopressors, minocycline, magnesium, riluzole, glyburide (glibenclamide), thyrotropin-releasing hormone, opioid receptor antagonists (naloxone), granulocyte colony stimulating factor, cethrin, gangliosides, antioxidants, calcium channel blockers, sodium channel blockers, and many others[12,13]. Corticosteroids are being used for a long time in many experimental and clinical trials due to their anti-inflammatory, edema-resolving and antioxidant characteristics. However, it is currently accepted that corticosteroids have no effect when compared to placebo at 6-12 months of follow-up and therefore have no long-term benefits[14].

Due to the strong anti-inflammatory effects of NSAIDs, it is expected that they may have anti-inflammatory effects in secondary damage. In experimental studies, inhibition of the RhoA pathway and neuroprotective effect by reducing apoptosis and providing histological improvement bring these drugs to a promising position in terms of treatment[15]. In our study, when the edema level among the groups was examined, it was seen that the group that did not differ from the control group was the tDF group. There was no significant difference in the ML group in terms of edema. In addition, when the tDF+tML group and other drug groups were compared, it was found that combined drug administration reduced the anti-edema effect compared to single applications. In the study of Saiwai et al.[16], the importance of leukotrienes in the injury mechanisms after SCI was emphasized. In the study conducted by Cavus et al.[17], the effects were compared with montelukast and methylprednisolone and it was reported to be neuroprotective. Genovese et al.[18] compared the effects of montelukast and zilueton and found the anti-edema effects to be significant.

Inflammation is the basic defense mechanism of the organism and begins to appear in the trauma area in the 3rd post-traumatic hour and can remain at the maximum level until the 3rd day[19]. Schwartz[20] reported that the presence of neutrophils was necessary for repair after axonal damage and that T lymphocytes in the environment were necessary for defensive and repair events. Schwab et al.[21] stated that the amount of LTC4 and TXA2, which are arachidonic acid metabolites in CSF, increased 5-9 times higher than normal after the injury. In our study, when the number of inflammatory cells in the groups was compared, it was found that the number of cells was the lowest in the tDF group. No significant difference was found in the other groups. Hains et al.[22] reported that COX-2 inhibitors improved histological and motor function results.

In the literature, studies in which indomethacin and ketorolac were applied intrathecally have been reported to have a protective effects[23,24]. Genovese et al.[18] also showed that zileuton and montelukast sodium, which are 5-lipoxygenase inhibitors, decreased the number of inflammatory cells and myeloperoxidase activity, which increased in the 24 hours after trauma.

As a result of the comparisons between the groups, it was seen that necrosis, apoptosis and neural injury were the least in the group, in which tDF+ML was used in combination, compared to the other groups. It has been shown that caspas-8 and 9 are activated in the lesion center at the 6th hour after trauma and this activity lasts for 7 days[25]. Inflammatory cytokines, especially TNF-α released from neurons, activate the RhoA pathway and cause apoptosis[24]. Mills et al.[26] showed that activated Rho pathway induced Bcl-2 dependent apoptosis. Xing et al.[27] stated that the inhibition of RhoA in rats, which they applied NSAID treatment for 5 post-traumatic days, especially protected oligodenrocytes from apoptosis, and increased axonal myelination in the rostral and caudal of the lesion. In the experimental study on rats, Wang et al.[28] reported that the treatment of ibuprofen given at a dose of 60-70 mg/kg for 1-28 days had a protective effect on the rostral axons of the spinal cord, but this effect was not observed in the caudal fibers. As a result, they stated that ibuprofen could be a potential agent. Sharp et al.[29] reported that 60 mg/kg ibuprofen treatment for 42 days after trauma resulted in improvement in Basso-Beattie-Bresnahan score and protection in motor functions in rats as a result of treatment.

Post-traumatic bleeding is a pathology that begins to occur after primary injury and continues with secondary injury. In their study, Noble and Wrathall[30] stated that the localization of bleeding after SCI was related to the severity and direction of the blow that caused the primary injury, and they reported that bleeding localizations could be seen simultaneously in the rostral and caudal regions of the spinal cord and in more than one region. Erşahin et al.[31] reported that ML reduced bleeding in the spinal cord white matter. Saiwai et al.[16] reported that neutrophils that migrated to the environment at the 12th hour after trauma increased the bleeding status and this situation decreased due to the decreased cell migration after the use of ONO-4057, which is an LTB_4 antagonist. In our study, we detected that there was no significant difference between the groups in terms of the amount of bleeding.

Study Limitations

The most important limitation of our study is that it is not supported by functional recovery and biochemical parameters such as apoptosis and inflammation markers, as well as investigating the patological changes.

CONCLUSION

In our study, it was determined that the application of DF alone in traumatic SCI had a reducing effect in the development of edema and the number of inflammatory cells, while the application of DF+ML had a reducing effect on the development of necrosis, apoptosis, and neural damage.

Acknowledgment

We thank Assoc. Prof. Meltem Öznur from Namık Kemal University, Department of Pathology for her supports during the writing of our article.

Ethics

Ethics Committee Approval: The study were approved by the Local Ethics Committee for Animal Experiments of University of Health Sciences Turkey, Haydarpaşa Numune Training and Research Hospital (decision number: 2017-05/05, date: 01.11.2017).

Informed Consent: Animal experiments.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: T.E., D.C., F.V.A., Concept: T.E., B.E., Design: T.E., M.İ., F.V.A., Data Collection or Processing: T.E., D.C., F.V.A., Analysis or Interpretation: T.E., F.V.A., T.T., T.Ç., Literature Search: T.E., M.İ., B.E., Writing: T.E., M.İ., B.E., T.T., T.Ç.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Ning GZ, Mu ZP, Shangguan L, Tang Y, Li CQ, Zhang ZF, et al. Epidemiological features of traumatic spinal cord injury in Chongqing, China. J Spinal Cord Med. 2016;39:455-60.
2. Dumont RJ, Okonkwo DO, Verma S, Hurlbert RJ, Boulos PT, Ellegala DB, Dumont AS. Acute spinal cord injury, part I: pathophysiologic mechanisms. Clin Neuropharmacol. 2001;24:254-64.
3. Karamehmetoğlu SS, Nas K, Karacan I, Sarac AJ, Koyuncu H, Ataoğlu S, et al. Traumatic spinal cord injuries in southeast Turkey: an epidemiological study. Spinal Cord. 1997;35:531-3.
4. Zileli M. Omurilik Yaralanmasında Epidemioloji ve Prognoz. Zileli M, Özer F. Eds. Omurilik ve Omurga Cerrahisi. İzmir:Meta Basım Evi. 2002;885-92.
5. Tator CH. Spine-spinal cord relationships in spinal cord trauma. Clin Neurosurg. 1983;30:479-94.
6. Bethea JR, Dietrich WD. Targeting the host inflammatory response in traumatic spinal cord injury. Curr Opin Neurol. 2002;15:355-60.
7. Tintinger GR, Feldman C, Theron AJ, Anderson R. Montelukast: more than a cysteinyl leukotriene receptor antagonist? ScientificWorldJournal. 2010;10:2403-13.
8. Gan TJ. Diclofenac: an update on its mechanism of action and safety profile. Curr Med Res Opin. 2010;26:1715-31.

9. Fehlings MG, Nguyen DH. Immunoglobulin G: a potential treatment to attenuate neuroinflammation following spinal cord injury. J Clin Immunol. 2010;30(Suppl 1):S109-12.

10. Profyris C, Cheema SS, Zang D, Azari MF, Boyle K, Petratos S. Degenerative and regenerative mechanisms governing spinal cord injury. Neurobiol Dis. 2004;15:415-36.

11. Dumont RJ, Okonkwo DO, Verma S, Hurlbert RJ, Boulos PT, Ellegala DB, et al. Acute spinal cord injury, part I: pathophysiologic mechanisms. Clin Neuropharmacol. 2001;24:254-64.

12. Dumont RJ, Verma S, Okonkwo DO, Hurlbert RJ, Boulos PT, Ellegala DB, et al. Acute spinal cord injury, part II: contemporary pharmacotherapy. Clin Neuropharmacol. 2001;24:265-79.

13. Karsy M, Hawryluk G. Pharmacologic Management of Acute Spinal Cord Injury. Neurosurg Clin N Am. 2017;28:49-62.

14. Bracken MB, Shepard MJ, Holford TR, Leo-Summers L, Aldrich EF, Fazl M, et al. Administration of methylprednisolone for 24 or 48 hours or tirilazad mesylate for 48 hours in the treatment of acute spinal cord injury. Results of the Third National Acute Spinal Cord Injury Randomized Controlled Trial. National Acute Spinal Cord Injury Study. JAMA. 1997;277:1597-604.

15. Hayta E, Elden H. Acute spinal cord injury: A review of pathophysiology and potential of non-steroidal anti-inflammatory drugs for pharmacological intervention. J Chem Neuroanat. 2018;87:25-31.

16. Saiwai H, Ohkawa Y, Yamada H, Kumamaru H, Harada A, Okano H, et al. The LTB4-BLT1 axis mediates neutrophil infiltration and secondary injury in experimental spinal cord injury. Am J Pathol. 2010;176:2352-66.

17. Cavus G, Altas M, Aras M, Ozgür T, Serarslan Y, Yilmaz N, et al. Effects of montelukast and methylprednisolone on experimental spinal cord injury in rats. Eur Rev Med Pharmacol Sci. 2014;18:1770-7.

18. Genovese T, Rossi A, Mazzon E, Di Paola R, Muià C, Caminiti R, et al. Effects of zileuton and montelukast in mouse experimental spinal cord injury. Br J Pharmacol. 2008;153:568-82.

19. Carlson SL, Parrish ME, Springer JE, Doty K, Dossett L. Acute inflammatory response in spinal cord following impact injury. Exp Neurol. 1998;151:77-88.

20. Schwartz M. Autoimmune involvement in CNS trauma is beneficial if well controlled. Prog Brain Res. 2000;128:259-63.

21. Schwab JM, Zhang Y, Kopp MA, Brommer B, Popovich PG. The paradox of chronic neuroinflammation, systemic immune suppression, autoimmunity after traumatic chronic spinal cord injury. Exp Neurol. 2014;258:121-9.

22. Hains BC, Yucra JA, Hulsebosch CE. Reduction of pathological and behavioral deficits following spinal cord contusion injury with the selective cyclooxygenase-2 inhibitor NS-398. J Neurotrauma. 2001;18:409-23.

23. Guth L. A reassessment of LPS/indomethacin/pregnenolone combination therapy after spinal cord injury in rats. Exp Neurol. 2012;233:686.

24. Hsieh YC, Liang WY, Tsai SK, Wong CS. Intrathecal ketorolac pretreatment reduced spinal cord ischemic injury in rats. Anesth Analg. 2005;100:1134-9.

25. Schnell L, Fearn S, Klassen H, Schwab ME, Perry VH. Acute inflammatory responses to mechanical lesions in the CNS: differences between brain and spinal cord. Eur J Neurosci. 1999;11:3648-58.

26. Mills JC, Stone NL, Erhardt J, Pittman RN. Apoptotic membrane blebbing is regulated by myosin light chain phosphorylation. J Cell Biol. 1998;140:627-36.

27. Xing B, Li H, Wang H, Mukhopadhyay D, Fisher D, Gilpin CJ, et al. RhoA-inhibiting NSAIDs promote axonal myelination after spinal cord injury. Exp Neurol. 2011;231:247-60.

28. Wang X, Budel S, Baughman K, Gould G, Song KH, Strittmatter SM. Ibuprofen enhances recovery from spinal cord injury by limiting tissue loss and stimulating axonal growth. J Neurotrauma. 2009;26:81-95.

29. Sharp KG, Yee KM, Stiles TL, Aguilar RM, Steward O. A re-assessment of the effects of treatment with a non-steroidal anti-inflammatory (ibuprofen) on promoting axon regeneration via RhoA inhibition after spinal cord injury. Exp Neurol. 2013;248:321-37.

30. Noble LJ, Wrathall JR. Spinal cord contusion in the rat: morphometric analyses of alterations in the spinal cord. Exp Neurol. 1985;88:135-49.

31. Erşahin M, Çevik Ö, Akakın D, Şener A, Özbay L, Yegen BC, et al. Montelukast inhibits caspase-3 activity and ameliorates oxidative damage in the spinal cord and urinary bladder of rats with spinal cord injury. Prostaglandins Other Lipid Mediat. 2012;99:131-9.