Turkish Neurosurgery 2010, Vol: 20, No: 2, 173-179

Original Investigation

Ultrastructural Changes in the Liver after Experimental Spinal Cord Injury in Rats: Effects of Methylprednisolone, Immunoglobulin G and Albumin

Sıçanlarda Spinal Kord Yaralanmasından Sonra Karaciğer İnce Yapısındaki Değişiklikler: Metilprednizolon, İmmünoglobulin G ve Albuminin Etkileri

Serap EREL[1]
Beril GOK[2]
Kemal KISMET[3]
Husamettin ERDAMAR[4]
Mustafa SARGON[5]
Mehmet Ali AKKUS[6]

[1,3,6] Ankara Training and Research Hospital, General Surgery, Ankara, Turkey
[2] Ankara Ataturk Training and Research Hospital, Department of Neurosurgery Ankara, Turkey
[4] Gazi University, Faculty of Medicine, Department of Biochemistry, Ankara, Turkey
[5] Hacettepe University, Faculty of Medicine, Department of Anatomy, Ankara, Turkey

ABSTRACT

AIM: Spinal Cord Injury (SCI) is routinely treated with standardized methyl prednisolone sodium succinate (MPSS) dose, so it is reassuring to find its effects on liver. We also evaluated the effects of albumin and immunoglobulin G (Ig G) therapies on liver if they are used in case of experimental SCI.

MATERIAL and METHODS: The rats were allocated into six groups as control, trauma, vehicle, MPSS, Ig G and albumin consisting 8 rats for each. The rats with SCI were assigned to 30mg/kg MPSS, 5 mg/kg albumin and 400 mg/kg Ig G treatments. Tissue samples from liver were obtained for light and electron microscopy examinations and determination of myeloperoxidase (MPO) activity.

RESULTS: Trauma increased MPO activity and caused cellular changes of liver tissue. Both albumin and Ig G treatments decreased MPO activity significantly. The light and electron microscopic evaluations showed remarkable preservation of liver ultra-structure with all treatments including MPSS.

CONCLUSIONS: SCI resulted in neutrophil infiltration and changes in ultra-structure of liver. It was revealed that MPSS has no detrimental effects on liver. Although all treatments preserved liver tissue structure, Although all treatments preserved liver tissue structure, Ig G and albumin treatments also prevented neutrophil infiltration. To provide protection from secondary liver injury after SCI, use of albumin and Ig G treatments may be beneficial.

KEYWORDS: Liver, Spinal cord injury, Methyl prednisolone sodium succinate, Myeloperoxidase, Albumin, Immunoglobulin G, Rats

ÖZ

AMAÇ: Spinal Kord Yaralanması (SKY) genellikle standardize metilprednizolon sodyum süksinat (MPSS) dozu ile tedavi edilir, bu nedenle karaciğere etkisini bulmak güven vericidir. Albümin ve İmmünoglobulin G (Ig G) tedavilerinin deneysel SKY'de kullanıldığında karaciğer üzerine etkileri de değerlendirildi.

YÖNTEM ve GEREÇ: Sıçanlar her biri 8 sıçandan oluşan kontrol, travma, taşıyıcı, MPSS, Ig G ve albümin olmak üzere 6 gruba ayrıldı. SKY'lı sıçanlar 30 mg/kg MPSS, 5 mg/kg albümin ve 400mg/kg Ig G tedavilerine atandı. Karaciğerden doku örnekleri ışık ve elektron mikroskopi incelemeleri ve myeloperoksidaz (MPO) aktivitelerinin değerlendirilmesi için alındı.

BULGULAR: Travma MPO aktivitesi artırmış ve karaciğer dokusunda hücresel değişikliğe neden olmuştur. Albümin ve Ig G tedavileri MPO aktivitelerini anlamlı olarak düşürmüştür. Işık ve elektron mikroskopik değerlendirmelerinde MPSS'yi de içeren tüm tedavilerle karaciğer ince yapısının anlamlı olarak korunduğu gösterilmiştir.

SONUÇ: SKY nötrofil infiltrasyonuna ve karaciğerin ince yapısının bozulmasına neden olur. MPSS' nin karaciğere zararlı etkileri olmadığı gösterilmiştir. Her ne kadar tüm tedaviler karaciğer yapısını korusa da Ig G ve albümin nötrofil infiltrasyonunu da engellemişlerdir. SKY'den sonra ikincil yaralanmalardan korumak için Ig G ve albümin tedavilerinin kullanımı yararlı olabilir.

ANAHTAR SÖZCÜKLER: Karaciğer, Spinal kord yaralanması, Metil prednizolon süksinat, Myeloperoksidaz, Albümin, İmmünoglobulin G, Sıçanlar

Received : 31.07.2009
Accepted : 13.11.2009

Correspondence address:
Serap EREL
Phone : +90 312 595 34 00
E-mail : erelserap@yahoo.com

INTRODUCTION

Spinal injury involves all organ systems of the body depending on the level of lesion. The concept of primary and secondary injury has laid more stress on prevention and treatment of secondary injury. Methyl prednisolone is the only drug approved in the treatment of acute spinal cord trauma and still remains the drug of choice for prevention of secondary injury. Although the first National Acute Spinal Cord Injury Study (NASCIS) I, II and III intended to demonstrate the effective role of high-dose steroids in the treatment of acute spinal cord trauma, controversy still exists regarding the efficacy and complications of this treatment (4-6).

NASCIS II/III has demonstrated the end organ effects of high dose methyl prednisolone treatment, including pulmonary insufficiency, rate of infection and death. There is only one study analyzing the histological changes of methyl prednisolone sodium succinate (MPSS) on the end organs (16). But they only studied the microscopic appearances by histological staining techniques to analyze these effects.

This study attempts to characterize histological and functional response of liver to human dose equivalent (HDE) intravenous methyl prednisolone administration in a rodent model of acute spinal cord injury. The second objective of the present study was to evaluate the effects of human serum albumin and, human immunoglobulin G (Ig G) therapy on liver when they are used in SCI. Human albumin is unique multifunctional natural protein with antioxidant properties after systemic injury. Immunomodulator treatment that prevents the destructive effects of inflammatory cascades may have protective properties on inflammatory responses after spinal cord injury in rat. Therefore, we investigated the effects of a well-known anti-inflammatory immunomodulator, Ig G on liver of rats that underwent SCI. The results of albumin and Ig G treatments were also compared with that of MPSS since it has been widely used in patients with SCI.

MATERIAL and METHODS

Animals:

The procedures in this experimental study were performed in accordance with the National Guidelines for the Use and Care of Laboratory Animals and approved by the Animal Ethics Committee of Ankara Training and Research Hospital.

Forty-eight Wistar- Albino adult female rats weighing 210-250 g were included in this study. Animals were deprived of food, but had free access to water 2 h before anesthesia. The rats were housed under constant temperature individually in wire cages with 12 h light-dark cycle.

Surgical procedure

All the rats were anesthetized with xylazine, 10 mg/kg (Bayer, Istanbul, Turkey) and ketamine hydrochloride, 60 mg/kg (Parke Davis, Istanbul, Turkey). Rats were placed in prone position. With T_5-T_{12} midline skin incision, paravertebral muscles were dissected and T_7-T_{10} laminectomy was performed. In the control group, trauma was not introduced and normal spinal cord samples were obtained. In trauma, vehicle, MPSS, albumin and Ig G groups, the dura left intact and spinal cord contusion injury was produced by the weight drop method of Allen as 50 g-cm (1). Following the surgical and traumatic interventions, surgical wound was closed in layers with silk sutures. Anesthesia was reintroduced twenty-four hours after trauma induction. All animals were sacrificed under deep anesthesia at the end of the experiment. Tissue samples from right lobe of liver were kept in –196°C (liquid nitrogen) until biochemical evaluation. Blood samples were obtained for liver function tests including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyltransferase (GGT).

Treatments

The rats were randomly and blindly allocated into six groups as control, trauma, vehicle, MPSS, Ig G and albumin consisting 8 rats for each. The dose of MPSS was human dose equivalent of MPSS according to NASCIS II. Control and trauma groups received no medication. In the vehicle group, laminectomy was performed; animals received a single dose of 1 mL of vehicle (saline) intraperitoneally, immediately after trauma. In the MPSS group, animals received a single dose of 30 mg/kg MPSS (Prednol L®, Mustafa Nevzat, Istanbul, Turkey) intraperitoneal, immediately after trauma. In the Ig G group, intraperitoneal 400 mg/kg Ig G (IG Vena®, Onko, Istanbul, Turkey) was given immediately after trauma. In the albumin group,

intraperitoneal 5 mg/kg albumin (Plasbumin®, Biem, Istanbul, Turkey) was given immediately after trauma. The dose of Ig G delivered was chosen based on the weight-based dose for human patients (0.2–2.5 g/kg).

Sample preparation and determination of myeloperoxidase activity

The myeloperoxidase (MPO) activity levels were measured with the method described by Hamada and Taoka (15,22). 10% (w/v) the liver homogenate was suspended in 20-mM phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethyl ammonium bromide (HETAB) and sonicated for 30 s. After centrifugation (4500^{x}g at 4°C for 20 min), 0.1 ml of supernatant was added to 0.6 ml of 0.1 M phosphate buffer (pH 6.0) with 0.05% H_2O_2 containing 1.25 mg ml^{-1} of o-dianisidine. The assay was begun by adding 0.1 ml of sample and was stopped 5 min later adding 0.1 ml of NaN_3 (1%). Absorbance at 460 nm after 5 min was measured in a spectrophotometer, and MPO activity was calculated using a standard curve prepared with purified MPO. Tissue MPO activity was expressed as units (U) per gram of wet tissue (U/g).

Sample preparation for microscopic examination

The specimens of liver were fixed in 2.5% gluteraldehyde for 6 h. The tissues were then post-fixed in 1 % osmium tetroxide (OsO_4) and dehydrated in increasing concentrations of alcohol. Following this procedure, the samples were washed with propylene oxide and embedded in epoxy resin embedding media.

For light microscopic examination:

The semi-thin sections about 2 μm in thickness were cut with glass knife on LKB Nova ultratome (Bromma, Sweden). Then, these sections were stained with methylene blue and examined by Nikon Optiphod (Japan) light microscope.

The liver specimens were evaluated to assess the morphology of the hepatocytes, portal areas, sinusoidal lesions, cellular infiltration in the lobule or portal spaces and parenchymal lesions. The investigator evaluating the sections was blinded to the group information.

For transmission electron microscopic examination:

The ultrathin sections about 60 Ëm in thickness were cut with a glass knife on the LKB Nova ultramicrotome (Bromma, Sweden) and taken over copper grids. These sections were then stained with uranyl acetate and lead citrate and examined with Jeol JEM 1200 EX transmission electron microscope (Tokyo, Japan) and photographed. The investigator evaluating the sections was blinded to the group information.

Biochemical analysis:

The biochemical analyses were made by an autoanalyzer (Olympus AU 640, Japan) using commercial kits.

Statistical analysis

All the data collected from the experiment was coded, recorded and analyzed by using SPSS 13.0.1 for Windows (SPSS Inc., Chicago, USA). In each test, the data was expressed as the mean value ± SD and considered statistically significant at $p<0.05$. For comparing differences among three or more groups, the differences in parametric data were analyzed with the one-way analysis of variance (ANOVA). The differences in non-parametric data were analyzed with Kruskal-Wallis variance test. When analysis of variance showed a significant difference, the post-hoc multiple comparison test was applied to demonstrate the differences.

RESULTS

Biochemical results:

Liver function tests were in normal ranges in all groups except from that of trauma group. There was slight elevation level of liver enzymes in trauma group but the difference was not statistically significant when compared with other groups.

Myeloperoxidase activity

MPO activity was used as an indicator of polymorphonuclear (PMN) cell infiltration. The mean values of MPO activities were given in Table I. There was statistically significant difference between control and trauma groups in the means of MPO activity ($p=0.004$), but no significant difference was observed when MPO activities of trauma and MPSS groups were compared ($p>0.05$).

Table I: The Myeloperoxidase (MPO) Levels of Liver Tissue and Comparisons of MPO Activities (Mean ± SD).

Groups	MPO (U/gr wet tissue)	P values
Control-(I)	1.91 ± 0.23	I vs VI: p>0.05; I with other groups p<0.05
Trauma-(II)	3.33 ± 0.43	II vs III: p>0.05 II vs V: p=0.004; II vs VI: p=0.004
Vehicle-(III)	2.67 ± 0.63	III vs V: p=0.002; III vs VI: p=0.002
MPSS-(IV)	2.69 ± 0.24	IV vs III: p>0.05; IV vs II: p>0.05
Ig G-(V)	1.23 ± 0.17	V vs III: p=0.002; V vs IV: p=0.002
Albumin-(VI)	2.07 ± 0.16	VI vs IV: p=0.002

(**IgG:** Immunoglobulin G, **MPO:** Myeloperoxidase, **MPSS:** methyl prednisolone sodium succinate)

Albumin and Ig G treatments decreased MPO activity significantly (p=0.004) when compared with the effect of MPSS treatment on MPO activity. Albumin and Ig G treatment also significantly decreased MPO activity when compared with all groups (p=0.004). There was no significant difference in MPO activities between control and albumin treated group (p>0.05).

Light microscopic findings

The control group demonstrated normal histological structure and trauma produced minimal damage with evidences of neutrophil infiltration. In vehicle group, the histological findings were similar to trauma group. In MPSS group, evidences of neutrophil infiltration were prominent. In albumin and Ig G groups, histological structure was very similar to the control group.

Transmission electron microscopic findings

The control group demonstrated normal rat liver ultra structure and trauma produced mild damage. In trauma liver group, a dilatation was observed in the perinuclear cisterna (Figure 1A). Nucleus was found to be normal in all groups. Remarkable preservation of tissue structure with all treatments was demonstrated including MPSS treatment (Figure 1B,C,D).

DISCUSSION

After trauma, both primary and secondary injury pathways were observed in the spinal cord (8, 10). Understanding the events causing both of these injury pathways is vital for determination of appropriate intervention.

The only generally accepted medical treatment in an acute spinal cord injury is the intravenous administration of high doses of methyl prednisolone. There are many complications observed with the use of high dose steroids. High dose MPSS after an acute spinal cord injury might result in immune suppression during the vital early resuscitation phase. The eosinophilic infiltrates were demonstrated in pulmonary tissue of rats from 0 to 48 hours. This may explain association of complications with use of high dose MPSS. The NASCIS I, II and II have tried to establish the benefits of this treatment regarding its dose, timing and duration (4-6).

NASCIS II and III have demonstrated the end organ effects of high dose methyl prednisolone treatment, including pulmonary compromise, fulminant sepsis, increase rate of infection and death (5, 6). Kubeck et al (16) evaluated end organ effects of high dose human equivalent methyl prednisolone in a spinal cord injury in rat model. They have demonstrated that of all the end organs analyzed the spleens were the most affected. Lymphocyte depletion was observed as little as 4 hours after methyl prednisolone infusion and continued until 48 hr. Kidney and hepatic tissue was not different significantly from controls. They have just demonstrated the light microscopic changes. In our study, in MPSS group, evidences of neutrophil infiltration were prominent. In albumin and Ig G groups, ultrastructure of liver was very similar to that of control group demonstrating the beneficial effects of these treatments.

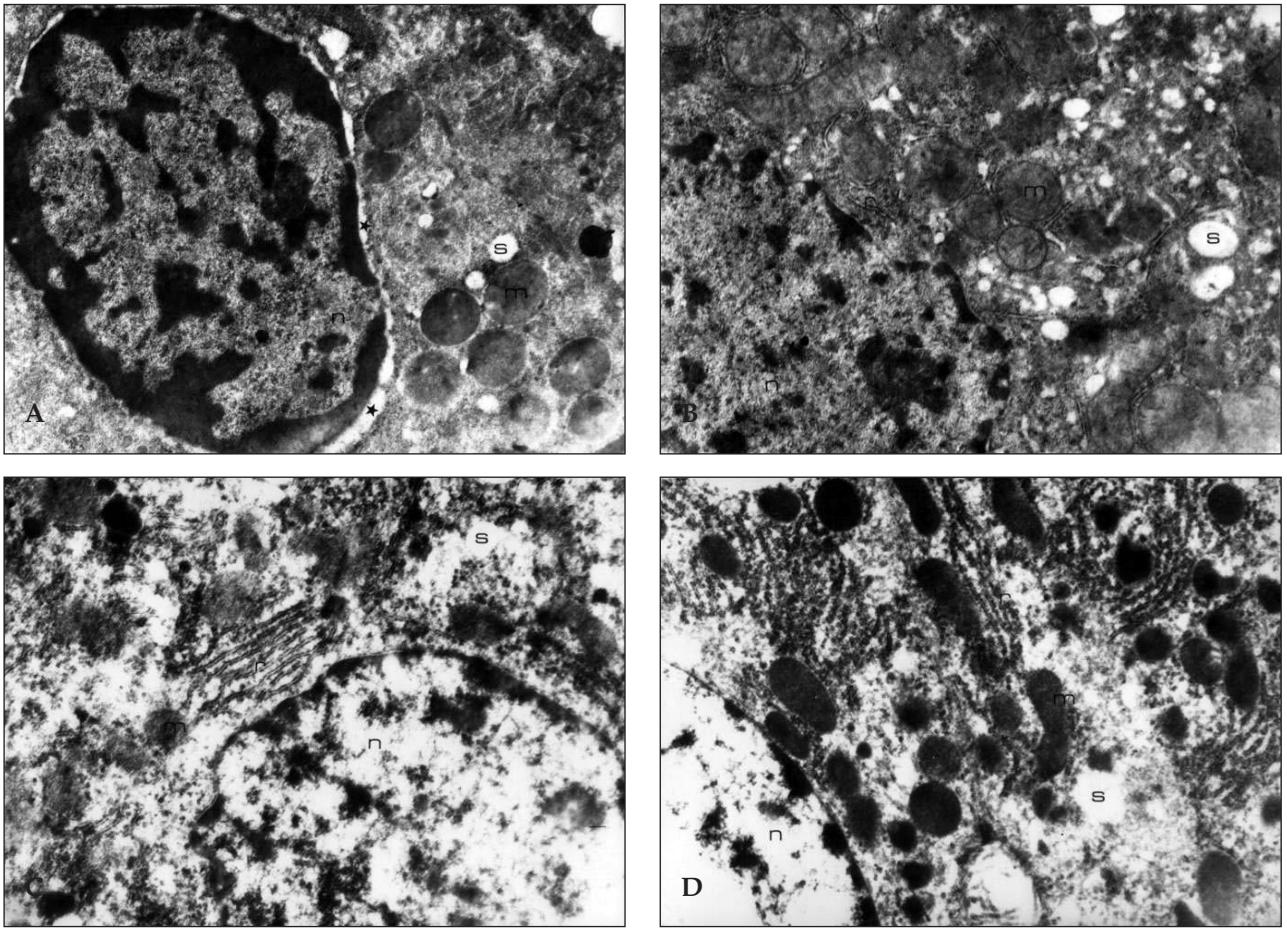

Figure 1: *Transmission electron micrographs of liver. No significant pathological findings in B; C and D meaning protective effects of MPSS; albumin and Ig G treatments.* ***(A)*** *Trauma group: dilated perinuclear cisterna of hepatocyte was observed,* ***(B)*** *MPSS-treated group: preserved ultrastructure of the hepatocyte.* ***(C)*** *Albumin-treated group: preserved ultrastructure of the hepatocyte.* ***(D)*** *Ig G-treated group: preserved ultrastructure of the hepatocyte (m, mitochondria; n, nucleus; s, smooth endoplasmic reticulum; r, rough endoplasmic reticulum; *dilated perinuclear cisterna; 10,000x magnification).*

MPSS has still been the only agent used by many clinic for the human SCI (19). Therefore, it is reassuring to find no evidence of compromised liver function from this steroid protocol. In order to determine the impact of extremely large doses of methyl prednisolone, naloxone, or of spinal cord injury itself on liver enzymes, the results of SGOT, SGPT, alkaline phosphatase and total bilirubin tests were obtained 24 hours, 3 and 10 days after the end of the study drug infusions in spinal cord injured patients entered in the NASCIS. The variations in enzyme levels appeared to be the result of the spinal cord injury, not the study drugs (21). In another study, the mean and median days of elevations of liver enzymes after trauma were 22 and 18, respectively. The elevations were thought to be related to liver injury but their mechanisms were not classified (3). In our study, although there was slight elevation in liver enzyme levels of trauma group, we could not find any significant difference when all groups were compared.

Multiple alterations in inflammatory and immunologic functions have been demonstrated in both clinical and experimental studies after trauma. Many activators may induce local and systemic inflammatory responses. SCI induces acute, local inflammation leading to secondary injury at the lesion site. There is also an activation of circulating inflammatory cells that may cause damage outside the spinal cord. Gris et al (13) studied systemic inflammation after SCI and its effects on lungs, kidney. The number of circulating neutrophils significantly increased by 3-10 folds from 2-24 hr after SCI. MPO and matrix metalloproteinase-9

activities in lung and kidney homogenates increased (12h-7d after SCI). The spinal cord injury may result in pathological T cell responses. In an experimental spinal cord injury, marked dysregulation of B cell function (autoimmunity) with pathological potential was demonstrated (2). The new mechanism of action of Ig G through interference of 4-integrin-dependent leucocyte recruitment in both an animal model and human multiple sclerosis was reported (17). Moreover, new agents were also studying as an immunomodulator treatment such as interferon-beta and were demonstrated to have obvious neuroprotection after acute contusion injury to the rat spinal cord (12). Therefore, it is essential to know their effects on liver, the major drug extraction center.

Clinically available pharmacological agents for treatment of acute SCI do not inhibit neutrophil activation. The effect of antithrombin III (AT-III) on neutrophil activation was studied in rats. The results demonstrate that AT-III treatment may reduce secondary structural changes in damaged rat spinal cord tissue by inhibiting leukocyte activation (11). In the present study, we used the MPO activity as an indicator of neutrophil accumulation in the liver. We found that the MPO activity in the liver tissue had increased in trauma group. MPSS treatment resulted in similar neutrophil accumulation with trauma group. Because of their decreasing effects on the MPO levels, albumin and/or Ig G may be used as an adjunct to MPSS for control of neutrophil accumulation in liver.

It is well known that SCI produces hemodynamic alterations, including a reduction in liver blood flow. Guizar-Sahagun et al. (14) demonstrated that SCI was associated with significant decreases in mean vascular blood flow (MVBF) in liver, spleen, muscle and fore footpad skin. Changes in MVBF in hind footpad skin and kidney were not significant. Changes were more pronounced at 1 h and 1 day post-SCI. Significant differences between MVBF after T-2 and T-9 SCI occurred only in liver. Acute SCI significantly alters the pharmacokinetics of high extraction drugs. It was reported that pharmacokinetic alterations might be reversed by increasing liver perfusion. L-arginine was studied to reverse alterations in drug dispositions by increasing hepatic blood flow (23). The preserving of liver ultra-structure is important for elimination of many drugs given during acute phase of spinal cord injury. Salas et al (20) studied liver ultra-structure during acute stress with different acute stressors including spinal cord transection. After 48 hours of exposure to stress, fragmentation and dilatation of rough endoplasmic reticulum, glycogen depletion and mitochondrial enlargement were observed under electron microscopy. The most striking change was reported as an increase in the number and size of autophagic vacuoles. In our study, since the rats were exposed to stress of spinal cord injury just for 24 h, we have only observed a dilatation in the perinuclear cisterna in trauma group. The preservation of the liver ultra-structure was demonstrated in all treatment groups.

The patients with SCI have a decreased immune function, especially succeeding the SCI, and an impaired nutritional status which may contribute to delay of wound healing (18). The indicators of nutritional function, prealbumin have been shown to be low in SCI patients with pressure ulcers (9). Cain et al (7) demonstrated in their study that post injury injection of albumin IV or into the site of injury immediately after injury both may result in improvement of locomotor function. They concluded that albumin might be a potentially useful agent for treatment of neurotraumas. In our study we demonstrated its benefits on liver tissue. Therefore, its use in spinal cord injury may gain importance with further studies.

CONCLUSIONS

Our results demonstrated remarkable preservation of tissue structure with MPSS treatment. When albumin and Ig G was applied as an adjunct to MPSS treatment after spinal cord trauma in rats for their anti-inflammatory and immunomodulator protective effects, decrease in neutrophil infiltration and preserving of normal liver tissue architecture was observed. The albumin and Ig G may be used as an adjunct treatment to MPSS in order to protect the organs from secondary injury of spinal traumas. Additional research is necessary to further evaluate the potential benefits of Ig G and albumin treatments when used together with MPSS for the management of SCI in humans.

REFERENCES

1. Allen AR: Surgery of experimental lesion of spinal cord equivalent to crush injury of fracture dislocation of spinal column: a preliminary report. JAMA 57: 878–880, 1991

2. Ankeny DP, Lucin KM, Sanders VM, McGaughy VM, Popovich PG: Spinal cord injury triggers systemic autoimmunity: evidence for chronic B lymphocyte activation and lupus-like autoantibody synthesis. J Neurochem 99(4):1073-1087, 2006
3. Bloom KK, Freed MM: Liver enzyme abnormalities in spinal cord injury. J Am Paraplegia Soc 12(1):11-13, 1989
4. Bracken MB, Collins WF, Freeman DF, Shepard MJ, Wagner FW, Silten RM, Hellenbrand KG, Ransohoff J, Hunt WE, Perot PL Jr, et al: Efficacy of methylprednisolone in acute spinal cord injury. JAMA 251:45–52, 1984
5. Bracken MB, Shepard MJ, Collins WF, Holford TR, Young W, Baskin DS, Eisenberg HM, Flamm E, Leo-Summers L, Maroon J, et al: A randomized controlled trial of methylprednisolone or naloxone in the treatment of acute spinal-cord injury. Results of the Second National Acute Spinal Cord Injury Study. N Engl J Med 322:1405–1411, 1990
6. Bracken MB, Shepard MJ, Holford TR, Leo-Summers L, Aldrich EF, Fazl M, Fehlings M, Herr DL, Hitchon PW, Marshall LF, Nockels RP, Pascale V, Perot PL Jr, Piepmeier J, Sonntag VK, Wagner F, Wilberger JE, Winn HR, Young W: 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 277(20): 1597–1604, 1997
7. Cain LD, Nie L, Hughes MG, Johnson K, Echetebu C, Xu GY, Hulsebosch CE, Mc Adoo DJ: Serum albumin improves recovery from spinal cord injury. J Neurosci Res 15, 85(7):1558-1567, 2007
8. Carlson GD, Gorden CD, Nakazawa S, Wada E, Smith JS, LaManna JC: Sustained spinal cord compression: Part II: Effect of methylprednisolone on regional blood flow and recovery of somatosensory evoked potentials. J Bone Joint Surg Am 85-A(1):95–101, 2003
9. Cruse JM, Lewis RE, Dilioglou S, Roe DL, Wallace WF, Chen RS: Review of immune function, healing of pressure ulcers, and nutritional status in patients with spinal cord injury. J Spinal Cord Med 23(2):129-35, 2000
10. Drescher WR, Weigert KP, Bunger MH, Hansen ES, Bünger CE: Spinal blood flow in 24-hour megadose glucocorticoid treatment in awake pigs. J Neurosurg 99 (3 Suppl):286-290, 2003
11. Erman T, Yildiz MS, Gocer AI, Zorludemir S, Demirhindi H, Tuna M: Effects of antithrombin III on myeloperoxidase activity, superoxide dismutase activity, and malondialdehyde levels and histopathological findings after spinal cord injury in the rat. Neurosurgery 56 (4), 828-835, 2005
12. Gok B, Okutan O, Beskonakli E, Palaoglu S, Erdamar H, Sargon MF: Effect of immunomodulation with human interferon-beta on early functional recovery from experimental spinal cord injury. Spine (Phila Pa 1976) 32(8):873-880, 2007
13. Gris D, Hamilton EF, Weaver LC: The systemic inflammatory response after spinal cord injury damages lungs and kidneys. Exp Neurol 211(1):259-270, 2008
14. Guízar-Sahagún G, Velasco-Hernández L, Martínez-Cruz A, Castaneda-Hernandez G, Bravo G, Rojas G, Hong E : Systemic microcirculation after complete high and low thoracic spinal cord section in rats. J Neurotrauma 21(11):1614-1623, 2004
15. Hamada Y, Ikata T, Katoh S, Nakauchi K, Niwa M, Kawai Y, Fukuzawa K: Involvement of an intracellular adhesion molecule-dependent pathway in the pathogenesis of secondary changes after spinal cord injury in rats. J Neurochem 66(4):1525-1531,1996
16. Kubeck JP, Merola A, Mathur S, Brkaric M, Majid K, Shanti N, Caruso S, Yuan S, Lowe T, Dwyer A, Haher T, O'Brien M: End organ effects of high-dose human equivalent methylprednisolone in a spinal cord injury rat model. Spine 31(3):257-261, 2006
17. Lapointe BM, Herx LM, Gill V, Metz LM, Kubes P: IVIg therapy in brain inflammation: Etiology-dependent differential effects on leucocyte recruitment, Brain 127(Pt 12):2649-2656, 2004
18. Lynch AC, Palmer C, Lynch AC, Anthony A, Roake JA, Frye J, Frizelle FA: Nutritional and immune status following spinal cord injury: A case controlled study. Spinal Cord 40(12):627-630, 2002
19. Profyris C, Cheema SS, Zang D, Azari MF, Boyle K, Petratos S: Degenerative end regenerative mechanisms governing spinal cord injury. Neurobiol Dis 15(3):415–436, 2004
20. Salas M, Tuchweber B, Kourounakis P: Liver ultrastructure during acute stress. Pathol Res Pract 167(2-4):217-233, 1980
21. Shepard MJ, Bracken MB: The effect of methylprednisolone, naloxone, and spinal cord trauma on four liver enzymes: Observations from NASCIS 2. National Acute Spinal Cord Injury Study. Paraplegia 32(4):236-245, 1994
22. Taoka Y, Okajima K, Uchiba M, Murakami K, Kushimoto S, Johno M, Naruo M, Okabe H, Takatsuki K: Role of neutrophils in spinal cord injury in rats. Neuroscience 79:1177–1182, 1997
23. Vertiz-Hernandez A, Castaneda-Hernandez G, Martinez-Cruz A, Cruz-Antonio L, Grijalva I, Guizar-Sahagun G: L-arginine reverses alterations in drug disposition induced by spinal cord injury by increasing hepatic blood flow. J Neurotrauma 24(12):1855-1862, 2007