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ORIGINAL: Evaluation of Leukocyte Response due to Implant of a Controlled Released Drug Delivery System of Chitosan Hydrogel Loaded with Selenium Nanoparticle in Rats with Experimental Spinal Cord Injury

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ABSTRACT

Introduction: Traumatic spinal cord injury (SCI) is one of the main injuries of the central nervous system. The aim of this study was to evaluate leukocyte changes following implantation of a controlled drug delivery system of chitosan hydrogel loaded with selenium nanoparticles in rats with spinal cord injury.

Material and Methods: For this purpose, 60 adult female rats with experimental thoracic spinal cord compression were divided into three equal groups: control group (did not receive any medication), chitosan group (received chitosan hydrogels), and nanoselnium group (received chitosan hydrogels containing selenium nanoparticles). Total and differential white blood cell count and neutrophil to lymphocyte ratio were measured on days 3, 7, 21 and 28 after induction of spinal cord injury.

Results: The results showed that the total white blood cells and lymphocytes in the control group was significantly higher than the chitosan and nanoselnium groups in the various times. In addition, it was found that although in the chitosan group, a decrease in neutrophil population was observed, but in the nanoselnium group, the decrease in neutrophil population was significantly more than the other groups. Significant reduction of neutrophils to lymphocytes ratio on the third day of the study was also observed in the nanoselenium group compared to the other two groups.

Conclusion: Implantation of chitosan hydrogel loaded with selenium nanoparticles controls the leukocyte response after spinal cord injury and thus potentially has a neuroprotective effect on spinal cord injury by controlling the secretion of inflammatory cytokines from the leukocytes.

Introduction

Spinal cord injuries (SCIs) affect many people in the world every year and cause permanent disability and reduced quality of life. The main tenets of spinal cord injury studies in rodents, primates, and humans are about the inflammatory response, tissue damage, and neurodegeneration after SCI (1). SCI leads to systemic inflammatory responses and is characterized by an increase in the number of

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immune cells and inflammatory mediators in the bloodstream, which leads to the infiltration of inflammatory cells into other organs and impairs the body's function. SCI also causes immune system failure through disruption of the immune system, which ultimately leads to a weak response to pathogens (2). Traumatic injury due to spinal cord compression has two phases in terms of pathology, which are known as primary and secondary injuries (3).

Primary injury can be caused by physical compression of the spine, stretching of nerve tissue, or impaired local blood supply. This damage causes deformity of the spine and narrowing of the spinal canal, which leads to significant changes in the volume and size of the spinal cord. Pathologically, primary damage occurs over a short period of time in a limited area, such as direct damage to nerve cells, glial or endothelial due to mechanical damage characterized by bleeding, edema, and ischemia. Secondary injuries are a set of complex processes that occur after a primary injury and last from a few minutes to a few weeks after SCI. Several mechanisms underlie the pathogenesis of secondary injuries; including nerve degeneration, gliosis and inflammation. Following SCI, the inflammatory microenvironment is activated by activated microglia cells and astrocytes, and macrophage infiltration greatly contributes to the progression of secondary damage (4-6).

Localized inflammatory microenvironment within the damaged spinal cord is a collection of degenerative neurons, damaged myelin sheaths, damaged endothelial cells and active glial cells and infiltrating cells, and this microenvironment itself produces various types of inflammatory mediators (7). In addition to the occurrence of inflammation in the spinal tissue, SCI can cause systemic inflammatory response syndrome (SIRS), which is a life-threatening condition and can affect various organs (8-10). Epidemiological analysis has shown a direct link between systemic inflammation and the pathogenesis of post-injury complications. Thus, patients with SCI with SIRS positive have higher severity of injury and higher incidence of complications than patients with SIRS negative (11). Macrophage migration inhibitory factor (MIF) is mainly involved in systemic inflammation, indicating that SCI-induced neuroendocrine changes promote systemic inflammation. Activation of microglia (neurons-immune cells of the central nervous system) that occur chronically in the hippocampus and cortex after SCI indicates that neuroimmune disorders are involved in systemic inflammation after SCI (12).

Neutrophils are the first inflammatory cells to reach the lesion site in neural and non-neural tissues. With their phagocytic properties and other properties, they are able to remove tissue debris and restore vital balance (homeostasis). Neutrophil accumulation after traumatic spinal cord injury, as measured by myeloperoxidase / MPO activity, increases significantly within 3 hours and remains elevated for up to 3 days after SCI (13).

Typically, neutrophils accumulate in the vascular endothelium. Although neutrophils also accumulate intra-parenchymally in the vicinity of hemorrhagic necrosis sites, there is little histological evidence to suggest that neutrophils have penetrated the spinal cord (13). The use of higher levels of neutrophils in the spinal cord may be due to stronger transregulation of the intercellular adhesion molecule 1 (ICAM-1) and the endothelial-platelet cell adhesion molecule (PECAM). Both molecules are expressed in endothelial cells; but higher levels of them are found in the spinal cord than in the cerebral cortex (14). Neutrophils play a role in modulating secondary damage by releasing neutrophil proteases and reactive oxygen species. Neutrophilic elastase is an enzyme that is able to damage endothelial cells and thus increase vascular permeability. Because bleeding after spinal cord injury is significantly reduced by a neutrophilic elastase inhibitor, bleeding may be a result of endothelial cell damage due to neutrophilic elastase (15). The phagocytic response of mononuclear cells to SCI is significantly greater than that seen as a result of a lesion similar to the anterior part of the brain (16).

The long-term presence of microglia or active

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macrophages in the CNS tissue, unlike the peripheral nervous system, has effects that can be harmful or beneficial (17). For example, long-term release of inflammatory-promoting cytokines by microglia or macrophages may cause further degradation. On the other hand, activation of microglia as well as astrocytes may lead to the production of growth factors necessary for nerve survival and tissue repair. In addition, the results show that the displacement of peripherally activated macrophages has beneficial consequences in restoring spinal function. It is clear that the environment determines the response of macrophages (18).

T lymphocytes are scattered in healthy spinal cord and gradually increase in the first week, mainly in the center of the lesion, after damage along with microglia activation and peripheral macrophage infiltration (19). Under normal circumstances, activated T cells can cross the Blood Brain Barrier (BBB) and enter the CNS parenchyma. Compared to inflammatory cells involved in SCI, the number of lymphocytes is low (16). Nevertheless, T lymphocytes play an important role in the CNS immune system; because once activated, T lymphocytes may kill target cells and produce cytokines (20). Schwartz et al. noted that both intrinsic and acquired autoimmune responses may be required for recovery after CNS axonal injury that macrophages are needed for repair, and that T cells activated against CNS antigens are needed for defense and protection (17). An injection of autoimmune T cells into the CNS myelin-based protein has been shown to reduce the spread of injury and increase recovery after spinal cord injury due to spinal cord compression in rats (21).

Neutrophil to lymphocyte ratio (NLR) is used as a strong prognostic indicator for patients with various diseases. It is also a simple and inexpensive indicator for evaluating the inflammatory response. Neutrophil activation activates a number of different cell types that are involved in acute and chronic inflammation and are associated with cancer treatment outcomes. In addition, NLR has been suggested as a sensitive indicator for measuring the state of the immune system in various conditions, such as malignancy (22), cardiovascular disease (23), and stroke.

Another important point is the design and construction of drug control systems that can be very useful in the management of treatment methods by drugs. The use of hydrogel-based drug delivery systems has been developed as a means of delivering more effective treatment by increasing the effectiveness of the tumor against material with minimal damage to the host immune system. Chitosan hydrogel has a high biocompatibility and causes controlled and slow release of the drug, depending on different environmental conditions (24). Unlike many other cells in the human body, neurons have relatively less capacity to divide. In fact, nerve tissue cannot be rebuilt after injury. One study found that selenium nanoparticles (SeNPs) downregulated the mRNA expression of proinflammatory cytokines, including iNOS, IL-1 and TNF-α, and reduced inflammation (25). It has been shown that the combination of SeNPs with water-soluble polysaccharides derived from Ganoderma lucidum (SPS) can also reduce inflammation by inhibiting NF-κB, JNK 1/2 and activation of P38 MAPKs. SeNPs can increase the activity of selenozymes with equal efficiency and less toxicity than selenite, selenium-methyl selenocysteine and selenomethionine (26). Selenium nanoparticles can be used instead of selenomethionine because of their up-regulatory effects on glutathione peroxidase and thioredoxin reductase compared to those with much lower toxicity (27). A 2015 study of selenium nanoparticles at a concentration of 250 mg/kg body weight showed that selenium nanoparticles act as a strong anti-inflammatory and significantly reduce the parameters of arthritis (28).

Due to the antioxidant and anti-inflammatory effects of selenium nanoparticles, if these nanoparticles have the capacity to control the leukocyte response, they can somehow control inflammation and its side effects (secondary consequences) in SCI. Therefore, the present study investigates the leukocyte

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response following implantation of a chitosan hydrogel loaded with selenium nanoparticles as its drug delivery system in rats with experimental spinal cord injury.

Methods

Synthesis of chitosan hydrogels loaded with selenium nanoparticles

Chitosan hydrogel was prepared by adding sodium hydroxide solution to acetic acid solution containing chitosan (29). Selenium nanoparticles were synthesized by chemical reduction of sodium selenite through the reduced form of glutathione (30). In order to prepare the chitosan hydrogel loaded with selenium nanoparticles, after preparing the hydrogel, water-soluble selenium nanoparticles were added to it and the final solution was prepared after gradual addition of beta-glycerophosphate to the chitosan.

Animals and studied groups

The laboratory animal selected for the present study was a two-month-old female Wistar rat (200-250 g) that was kept in a standard environment for two weeks before the study. Sixty rats that were randomly divided into three equal groups; (A) control group with spinal cord compression who did not receive any pharmacological intervention; (B) the chitosan group that received chitosan hydrogels at the site of spinal cord injury; (C) the nanoselenium group receiving chitosan hydrogel loaded with selenium nanoparticles at the site of spinal cord injury.

Surgical procedures and medication

To induce of experimental spinal cord injury, the animals were placed in a chest position and surgery was performed under complete asepsis. Rats were first anesthetized by intraperitoneal injection of a combination of ketamine (80 mg/kg) and xylazine (5 mg/kg). The skin of the animals was disinfected and prepared for surgery at the site of surgery. At the surgical site, subcutaneous injection of 1% lidocaine solution in the amount of 0.05 ml was performed; the rats were then subjected to inhalation anesthesia with isoflurane gas using a mask. The muscle structure adjacent to the vertebrae was isolated from the spinous process of thoracic vertebrae to expose of spine through a 2 cm incision and dorsal laminectomy eighth and ninth thoracic vertebrae was performed (31). After observing the spinal cord, aneurysm clips were placed on either side of the spinal cord for 1 minute (31).

Then, in the nanoselenium group, before closing the surgical site, a therapeutic hydrogel (chitosan hydrogel loaded with selenium nanoparticles) with a volume of 0.5 cc (at a dose of 0.25 mg/kg per day) was placed in the surgical site. In the chitosan group, only chitosan hydrogels with the same volume as the previous group were implanted at the site of injury, and in the control group, no material was implanted at the site of the experimental injury. Finally, after ensuring that there was no bleeding, in all groups, the muscles and skin of the surgical site were sutured with 0-4 monocryl and 0-4 nylon sutures, respectively, in a simple continuous pattern. To prevent possible postoperative infections, intramuscular injection of the antibiotic ampicillin (20 mg/kg) was performed daily for 5 days after surgery. The animal was also monitored for behavior, food intake, weight, surgical wound, bladder size, and sutures at least twice daily. In the first week after surgery, the bladder was manipulated digitally twice a day and in subsequent weeks once a day with caution to prevent possible urinary tract infections. Days 3, 7, 21 and 28 after surgery were considered as sampling times in each group. Therefore, blood samples were collected from the hearts of 5 rats at any time following induction of injectable anesthesia. The blood sample was poured into test tubes with anticoagulant (EDTA). Therefore, in order to count the total number of white blood cells, the cell counter device was used and for the differential count of white blood cells, blood smears was prepared on the slide (32). The slides were then stained with Wright Giemsa staining and the white blood cells were counted manually using a light microscope (32).

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Statistical analysis

The mean (± SD) of the measured parameters was compared between different groups using SPSS software version 23 and ANOVA statistical analysis and Tukey post hoc test at a significance level of P<0.05.

Results

Total white blood cell count

On days 3 and 21 after surgery, the mean of this parameter in the two groups of chitosan and nanoselnium compared to the control group showed a significant decrease (P<0.05). On day 7 after surgery, the mean of this parameter was significantly lower in the nanoselenium group than in the other two groups (P<0.05). On day 28 of the study, no significant differences were observed between any of the study groups (***Figure 1***).

Lymphocyte count

At the time of 7, a significant decrease in the mean lymphocyte population was observed in the nanoselenium group compared to the control and chitosan groups (P<0.05). Also, in times 3 and 21 of the study, a significant decrease in the mean of this parameter was observed in the nanoselenium and chitosan groups compared to the control group (P<0.05). At the time of 28, no significant difference was observed between the mean lymphocyte counts in the study groups (***Figure 2***).

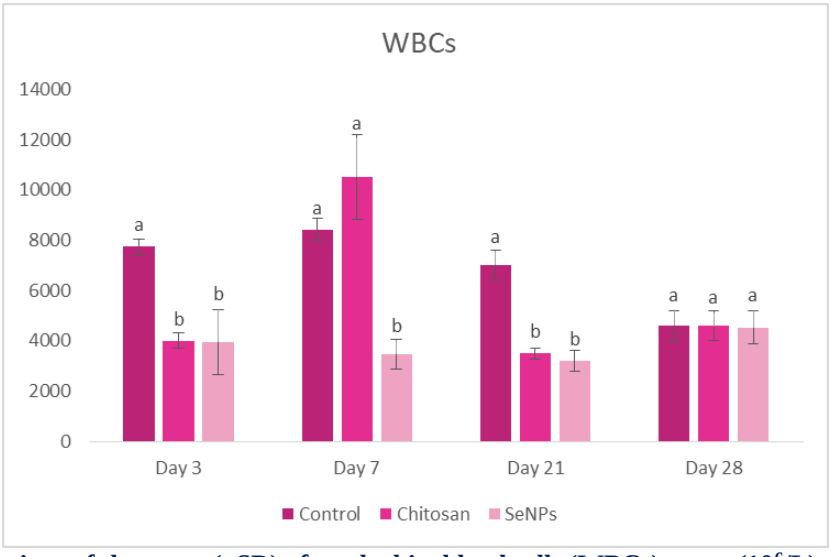

Figure 1. Comparison of the mean (±SD) of total white blood cells (WBCs) count (10^6/L) at different times in the studied groups. The presence of different letters in each column indicates a significant difference.

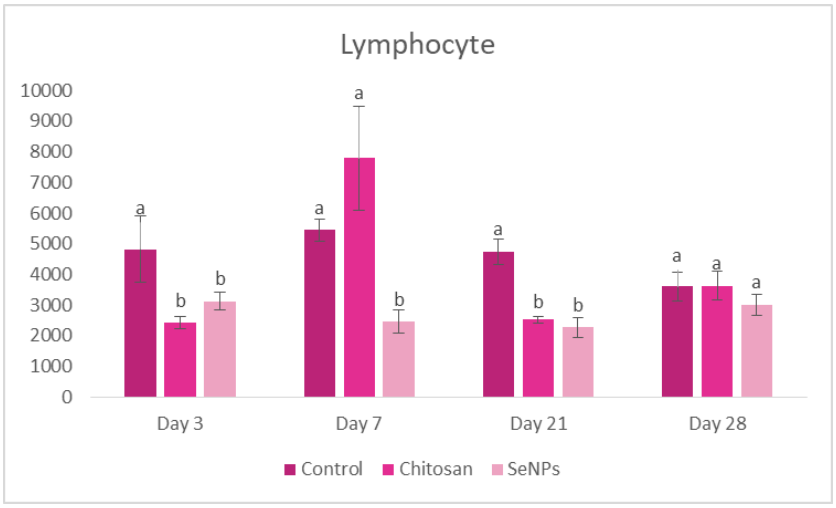

Figure 2. Comparison of mean (± SD) lymphocyte counts (10^6/L) at different times in the studied groups. The presence of different letters in each column indicates a significant difference.

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Neutrophil count

On days 3 and 21 after experimental SCI, a significant decrease in the mean of this parameter was observed in the nanoselenium and chitosan groups compared to the control group (P<0.05). On the seventh day of the present study, a significant decrease in neutrophil population was observed in the nanoselnium group compared to the control and chitosan groups (P<0.05). But on day 28 of the study, no significant difference was observed between the mean of this parameter in the study groups (***Figure 3***).

Neutrophils to lymphocytes ratio (NLR)

On the third day of the present study, a significant decrease in the mean of this parameter was observed in the nanoselenium group compared to the control and chitosan groups (P<0.05). On days 7, 21 and 28 of the study, no significant differences were observed in the study groups (***Figure 4***).

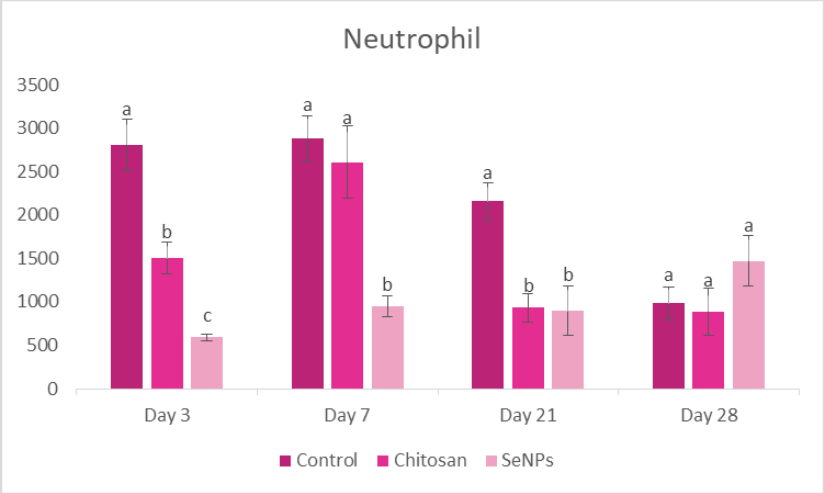

Figure 3. Comparison of mean (± SD) count of neutrophils (10^6/L) at different times in the various groups. The presence of different letters in each column indicates a significant difference.

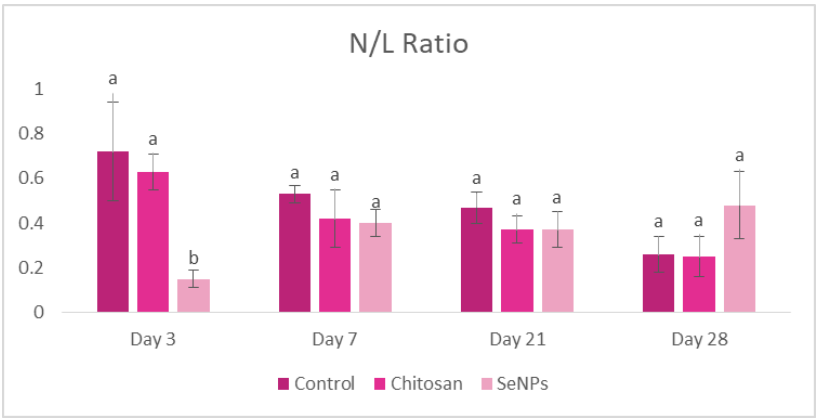

Figure 4. Comparison of mean (±SD) NLR at different times in the studied groups. The presence of different letters in each column indicates a significant difference.

Monocyte count

In the days 3, 7, and 28 of the present study, no significant difference was observed in the mean monocyte population between the studied groups. However, on day 21 of the study in the control group, the mean of this parameter was significantly higher than the chitosan and nanoselnium groups (***Figure 5***).

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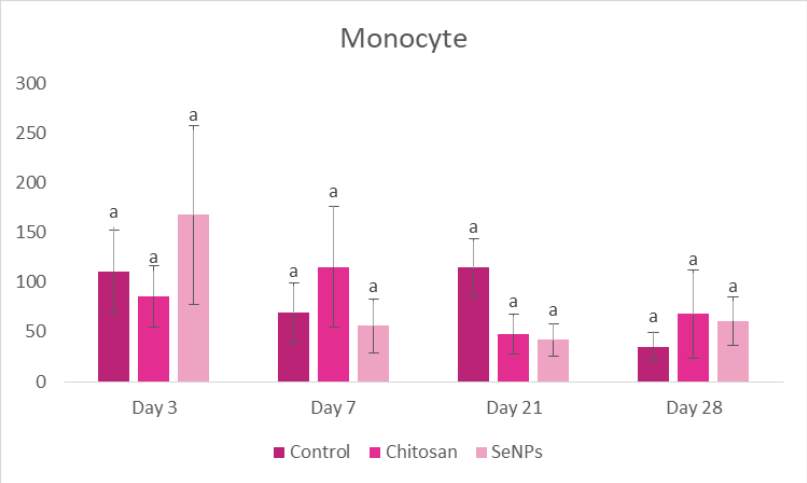

Figure 5. Comparison of mean (±SD) of monocytes (10^6/L) at different times in the various groups. The presence of different letters in each column indicates a significant difference.

Discussion

In the present study, the leukocyte response was investigated following the application of a controlled delivery system of chitosan hydrogel loaded with selenium nanoparticles in rats with experimental SCI. Traumatic spinal cord injury results in an acute inflammatory response that ultimately causes secondary damage and destruction in the area of the spinal cord injury. Many intrathecal events have been reported in the literature due to SCI, but it should be noted that following the activation of inflammatory cells through the bloodstream, a systemic response is induced that may extend to external tissues other than the central nervous system. In fact, SCI can trigger an inflammatory response that affects other organs in the body (70), and reciprocally, systemic inflammatory responses can affect the destruction and improving of CNS tissue (33). Leukocytes are the main responders to SCI. Obviously, the presence of these cells leads to the production of various (pro-inflammatory or anti-inflammatory) cytokines. The result of recruitment of these cells to the damaged site is an inflammatory response. Therefore, by evaluating the leukocytes as a simple and inexpensive test, important information about the patient's inflammatory response can be obtained. Injuries to the CNS appear to lead to significant changes in the total number of leukocytes or white blood cells (WBCs). For example, a leukocyte peak after delayed cerebral ischemia is indicated as a sign of a risk factor for cerebrovascular spasm following subarachnoid aneurysm bleeding (34).

It was also observed that the total number of leukocytes in patients in 3.5±1 hours after SCI was higher than normal and this increase in leukocytes was due to an increase in the number of neutrophils (35). It has been observed that the recruitment and uptake of neutrophils following a traumatic injury to the spinal cord is greater than when an experimental traumatic injury occurs in the brain (16). The use of higher levels of neutrophils in the spinal cord may be due to stronger upregulation of the intercellular adhesion molecule 1 (ICAM-1) and the endothelial-platelet cell adhesion molecule (PECAM). Both molecules are expressed in endothelial cells; but higher amounts of them are found in the spinal cord compared to the cerebral cortex (14). Dusart and Schwab (1993) showed that neutrophils and macrophages enter the spinal cord after SCI at a coordinated time interval. Neutrophils accumulate within 1 hour, are most abundant within 24 hours, and begin to decline within 48 hours (36).

In fact, neutrophils cross the bloodstream and enter the spinal cord within the first hour after injury (13, 37). Their population increases sharply in the injured spinal cord tissue and peaks within 24 hours after injury. The

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presence of neutrophils is more limited to the acute stage of SCI, as they are rarely seen in the subacute stage of spinal cord injury (38). In the study of Javdani et al. In 2018, a significant increase in the number of circulating neutrophils was observed 3 days after SCI and a decreasing trend of these cells was recorded from day 7 after spinal cord injury (39). The role of neutrophils in the pathophysiology of SCI is controversial. Evidence suggests that neutrophils are involved in phagocytosis and the clearance of tissue debris (37). They activate inflammatory cytokines, proteases, and free radicals that destroy the extracellular matrix, activate astrocytes, and microglia, and cause neuroinflammation (37). Although neutrophils are commonly associated with tissue damage, killing them jeopardizes the healing process and prevents performance improvement (40). Stimulated neutrophils have been shown to release IL-1 receptor antagonists that can exert neuroprotective effects following SCI (41).

In addition, removal of neutrophils alters the expression of cytokines and chemokines and the downregulation of growth factors, including fibroblast growth factors (FGFs), vascular endothelial growth factors (VEGFs), and morphogenic bone proteins (BMPs) that impair SCI. It is in the process of natural healing (40). In general, neutrophils play an important role in regulating neuroinflammation in the early stages of SCI, which forms the immune phase and improve the immune system in later stages. The results of the present study showed that the neutrophil population on days 3, 7 and 21 after SCI was lower in the nanoselenium group than in the control group. In addition, a significant decrease in the number of neutrophils was observed in days 3 and 7 after SCI in the chitosan group compared to the control group. These results well demonstrate the effectiveness of selenium nanoparticles as well as chitosan hydrogels in controlling neutrophil populations.

T and B lymphocytes play an important role in the acquired immune response after SCI (42). In the first week of injury, lymphocytes enter the severe spinal cord of injured mice and rats and remain chronically in the SCI state (42, 43). In fact, spinal cord injury triggers a specific autoimmune response of the central nervous system in T and B cells that remains chronically active (43). Self-reactive T cells can have direct toxic effects on neurons and glial cells (42).In addition, T cells can indirectly affect neuronal function and survival through the production of cytokines and proinflammatory chemokines (including IL-1β, TNF-α, IL-12, CCL2, CCL5, and CXCL10) (404). Genetic removal of T cells (in hairless rats without thymus) or pharmacological inhibition of T cells (using cyclosporine A and tacrolimus) improves tissue preservation and function after SCI (404), indicating the effect of T cells on pathophysiology and repair of SCI (42). Under normal circumstances, activated T helper cells CD4+ (Teff) are suppressed by FoxP3 CD4+ regulatory T (Treg) cells. This inhibition is regulated by various mechanisms such as the release of anti-inflammatory cytokines IL-10 and TGF-β by Treg cells. In addition, inhibition of Treg cells by dendritic cells by providing antigen has been shown to inhibit Teff cell activation (42).

Following SCI, the Treg-Te setting is disrupted. Increased activity of self-activating Teff cells is involved in tissue damage by producing proinflammatory cytokines and chemokines, promoting M1-like macrophage phenotype and induction of neuronal and oligodendroglial apoptosis by FAS (44). In addition, Teff self-activating cells increase the activation and differentiation of antigen-specific B cells into antibody-producing plasma cells, leading to tissue damage after SCI. In patients with SCI and MS, myelin-specific proteins such as myelin-based protein (MBP) significantly increase the circulation of circulating T cells (45). Serological evaluation of patients with SCI shows high levels of CNS reactive IgM and IgG isotypes that confirm the SCI-induced autoimmune activity of T and B cells (43). In SCI animal models, serum IgM levels increased sharply with increasing levels of IgG1 and IgG2a in later times (37). T

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lymphocytes are scattered in healthy spinal cord and gradually increase in the first week, mainly in the center of the lesion, after damage along with microglia activation and peripheral macrophage infiltration (19). The decrease in T lymphocytes detected following SCI is consistent with previous reports showing a decrease in T lymphocyte responses compared with controls (healthy individuals without CNS injury) (46). An experimental study was performed by Riegger et al. (2009), including 16 patients with SCI and ten healthy controls; Decreases in monocytes, T lymphocytes, and B lymphocytes in peripheral blood were observed within 24 hours after SCI (47). In another study by Riegger et al. (2007), without administration of methylprednisolone sodium succinate, a significant reduction in lymphocyte count was observed after SCI (48). In the study of Javdani et al., on the seventh day after experimental SCI, a decrease in lymphocytes was reported in rats with SCI (39).

The results of the present study showed that on days 3, 7 and 21 after SCI, the lymphocyte population in the group treated with chitosan hydrogel loaded with selenium nanoparticles was lower than the control group. In addition, it was found that implantation of chitosan hydrogel at the site of spinal cord injury was able to reduce the lymphocyte population in the peripheral blood compared to the control group. Monocytes / macrophages are seen in the injured spinal cord in the first week after injury (49) and macrophage activation is maximal within 7 to 14 days after injury (50). Macrophages with phagocytic function partially support wound healing events. However, they are also a source of proinflammatory cytokines and neurotoxins, including reactive oxygen species and induced nitric oxide synthesis, and are involved in cell damage (45). Like other inflammatory cells, their relative contribution to injury or repair is determined by the collective impact of these opposite processes (45, 51).

In the study by Riegger et al. (2007), after SCI, ED9+ monocytes decreased dramatically in the first 24 hours compared to the control group. The decreasing trend continued until the beginning of the subacute stage reached its minimum level on the third day, which indicates <65% compared to the control group. Then, at the end of the subacute phase on day 7 and further entry into the chronic phase on day 14, ED9+ monocytes were recovered to approximately the size of the control group. With the exception of a significant decrease in HIS48+ 3 days after SCI, HIS48+ granulocytes remained at the same levels as the control group (48). The evidence gathered to date shows that inflammatory type 1 monocytes are adsorbed to the site of inflammation / injury via the MCP-1 CCR2 receptor and are primarily involved in inflammation, proteolysis, and phagocytosis. Another subset of monocytes that have recently received significant attention are the so-called type 2 resident monocytes, which appear to play a role in safety monitoring and the healing process (52). In a study by Javdani et al., Monocytes showed a significant increase compared to the control group on day 7 after SCI following oral administration of selenium nanoparticles in rats (39).

Another important anti-inflammatory mechanism of selenium is regulated by its effect on monocyte adhesion to endothelial cells and their penetration into tissues. Monocytes attach to the endothelium and become macrophages as major factors influencing innate immunity to inflammation (53). The adhesion of monocytes to the endothelium is mediated by L-selectin, which facilitates the migration of neutrophils during the inflammatory response and is mediated by various ligands. Oral selenium administration impairs the binding of monocytes (54). Therefore, Javdani et al. suggested that an increase in the number of monocytes following oral administration of selenium nanoparticles during days 7 and 14 after SCI may be the result (39). However, in the present study, no significant difference was observed in the population of peripheral blood monocytes in the groups treated with selenium nanoparticles, chitosan hydrogel and control.

Recent studies have also shown that NLR is a

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useful indicator for predicting clinical outcomes in TBI patients. High NLR is associated with poor outcomes (55). In addition, it is well established that the type and severity of TBI play an important role in activating the inflammatory response (56). Similarly, Chen et al. (2018) showed that high NLR values in TBI patients are associated with adverse outcomes (57). AL-Mufti et al. (2019) found that NLR values above 5.9 when admitting patients carry a twice as high risk of delayed cerebral ischemia after subarachnoid aneurysm bleeding (58). In the study of Javdani et al. (2018), it was found that spinal cord amputation leads to a significant increase in the total number of white blood cells, neutrophils as well as NLR, 3 days after SCI (39).

The results of the present study showed that NLR 3 days after SCI and after implantation of chitosan hydrogel loaded with selenium nanoparticles significantly reduced compared to the control and chitosan groups, which well shows the useful role of selenium nanoparticles in reducing this index. It has been previously stated that selenium plays a very important role in maintaining the physiological activity of the nervous system, such as signal transmission and development (59).

It also acts as a cofactor for the enzyme glutathione peroxidase and is present in selenoproteins in antioxidant defense. Selenium is known as a neuroprotective agent in a number of neurological diseases including epilepsy (59) as well as pain. The neuroprotective effects of selenium are attributed to its ability to inhibit apoptosis (60) and modulate Ca^{2+} uptake through ion channels (61). Selenium nanoparticles, as a bioactive compound of antioxidants, reduce the activity of enzymes that deplete reactive oxygen species such as glutathione peroxidase, superoxide dismutase and catalase in the brains of rats and mice and induce neuroprotective effects (39). It has been reported that selenium deficiency leads to weakened immunity and following immune-suppression of low doses of selenium, improvement in immunological function is seen (62). The most important activity of selenium is considered to be related to its antioxidant effects because this element leads to the formation of selenocysteine (a major component of GPX) (63). It should be noted that the toxicity of selenium in its nanoparticle form is much lower than the form of selenate and selenite ions. Therefore, selenium nanoparticles can be used in clinical interventions (64). Selenium deficiency has been shown to play an important risk factor in reducing lymphocyte reptoductive potency (65-68).

A study by Yazdi et al. (2013) found that administration of selenium nanoparticles for 30 days in mice exposed to x-rays with bone marrow suppression reduced the number of lymphocytes and neutrophils (69). Selenium nanoparticles prevent the differentiation of monocytes into macrophages and also prevent the migration of neutrophils and the adhesion of lymphocytes to endothelial cells by reducing the expression of L-selectin. It is stated that the antioxidant potential of selenium nanoparticles is due to the direct reduction of the level of different oxidant species and also the increase of GPX level (70, 71). These nanoparticles exert their antioxidant effect by purifying H2O2 and phospholipid and lipid hyperoxides and converting them to alcohol and water (70).

Conclusion

It seems that the modulation of the leukocyte response in SCI following implant of chitosan hydrogel loaded with selenium nanoparticles is the result of the anti-inflammatory and antioxidant properties of selenium nanoparticles. So, Secondary injury is controlled after SCI by controlling the leukocyte response by restricting the secretion of pro-inflammatory cytokines. It can be concluded that chitosan hydrogel loaded with selenium nanoparticles can potentially have a neuroprotective effect after SCI.

Ethical standards statement

All the investigation procedures used in the

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current study were reviewed and approved by the Research Council of the Faculty of Veterinary Medicine of Shahrekord University.

Conflicts of interest

The authors declare no conflict of interest.

Authors' contributions

Each author has made an important scientific contribution to the study and has assisted with the drafting or revising of the manuscript.

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