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Original Article

The effect of sinularia on spinal cord trauma

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Abstract

Aim: Spinal cord traumas have become a serious health problem with the increase in the ageing population in society. The present study aimed to show the anti-inflammatory activity of methylprednisolone and sinularia administered following spinal cord trauma.
Materials and Methods: Sea corals are widely found on earth. Sinularia is one of the 17 known active substances of sinularia flexibilis, and several studies have been conducted on its anti-inflammatory, anti-oedema and cytotoxic effects. Despite the recent scientific studies, discussions continue as there are no effective treatment methods in use apart from methylprednisolone; however, the test animals received methylprednisolone treatment to compareits effects with those of sinularia, a secondary active ingredient of s. flexibilis.
Results: Paired comparisons for serum IL-1β and tissue TNF-α were made with Dunn's test. When serum IL-1β and tissue TNF-α levels were evaluated, a significant difference was detected in the group taking sinularia treatment compared to the trauma group Sinularia treatment was more efficient on different cytokines and compared to methylprednisolone treatment used routinely, it significantly affected both IL-1β level and TNF-α levels in serum
Conclusion: Anti-edematous, cytotoxic and anti-inflammatory effects of sinularia have been shown in our study. Sinularia, which has no known harmful effect on the human body according to the literature, may be clinically used in the future; however, more detailed and supportive experimental studies should be conducted on the subject.

Keywords: Cytokines; methylprednisolone; sinularia; spinal cord trauma

INTRODUCTION

Spinal cord trauma is a condition negatively affecting the life quality of the individuals and the families taking care of them due to the post-traumatic neurological deficit (1). Trauma causes a severe labor and economic loss due to early treatment and rehabilitation costs (2).

Soft corals are organisms which exist in different types in different areas of the world and have useful active metabolites (3). When considered in terms of chemical metabolites and biological activities, marine organisms including soft corals are similar to gold mine in scientific terms (4). Soft corals have sub-types such as sinularia querciformis, sinularia flexibilis or sinularia maxima (5). Originally isolated from the soft coral sinularia flexibilis collected from Hayman Island on the Great Barrier Reef of Australia, sinularia is the most common active substance among these (3, 5). Sinularia is a derivative of soft coral with a cytotoxic, anti-edematous, anti-carcinogenic and analgesic efficiency (4-6). Sinularia flexibilis is a well-known soft coral derivative with anticarcinogenic efficiency first studied by Weinheimer et al in 1977 (5).

There are studies showing the positive (motor and sensual functions) effects of methylprednisolone in 6 weeks, 6 months or a year if it is applied within the first eight hours of injury after spinal cord trauma (7,8).

Despite its negative effects on the life quality, an effective treatment method has not yet been found for the post-spinal cord trauma damage. A partial advantage was acquired from the medical treatment but a complete recovery was not provided in post-spinal cord trauma neurological functions even according to the latest studies in literature. After post-traumatic spinal cord injury, using neuroprotective effects of sinularia which has strong anti-inflammatory and anti-edematous effect in addition to methylprednisolone which is a drug known for its efficiency, our aim was to provide the improvement of new treatment

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methods to prevent the formation of neurological deficits occurring due to secondary damage and thus decrease disease-related mortality and morbidity.

MATERIALS and METHODS

Our study was approved by the Local Ethics Committee for Animal Experiments. Forty adult sprague dawley type female rats weighing 200–250 g was used in the study. The rats were randomly separated into four groups with 10 rats in each: control group with no surgical operation (Group I), trauma group with no medical treatment (Group II), trauma + sinularia (80 mg/kg) group (Group III), and trauma + methylprednisolone group (Group IV) (Table 1).

Table 1. Study groups and administered medical treatments

	Trauma	Treatment	Medication Dose
Group I (SHAM)	-	-	-
Group II (TRAUMA)	+	-	-
Group III	+	Sinularia	20 mg/kg
Group IV	+	Methylprednisolone	15 mg/kg

Many experimental models were developed to generate spinal cord trauma. The current models consist of dropping a weight from a height and spinal cord compression injury via clipping (9). In our study, aneurysm clipping method was performed to generate spinal cord trauma. Yaşargil aneurysm clip with 24 g force was kept closed for 1 min for extradural compression of the spinal cord following laminectomy and trauma was generated.

Sinularia flexibilis is cosmopolitan in its distribution and occurs in different seas. Chemical examination of several collections of this species led to the earliest isolation of a range of cembranoid diterpenes with potential anti-cancer activity. The secondary metabolites of s. flexibilis, their biological and pharmacological significance and various means of biomass supply for drug development (10).

Sinularia isolated from s. flexibilis was dissolved in dimethyl sulfoxide (Sigma–Aldrich Co. H157, Steinheim, Germany) diluted at a ratio of 0.06/1 with physiological saline solution (0.9% NaCl, Eczacıbaşı-Baxter, Istanbul, Turkey). The marine natural compound, sinularia, was isolated from the soft coral S. flexibilis as described and kindly provided by Dr. Jui-Hsin Su (National Museum of Marine Biology & Aquarium, Pingtung, Taiwan). Sinularia was intraperitoneally injected at a dose of 80 mg/kg (11).

Levels of Tumor necrosis factor-alfa (TNF-α) (Rat serum TNF-α ELISA Kit, Sandwich ELISA, LifeSpan BioSciences, USA, Rat tissue TNF-α ELISA Kit, Sandwich ELISA, LifeSpan BioSciences, USA) and Interleukin 1 beta (IL-1β) (Rat IL-1β ELISA Kit, Sandwich ELISA, LifeSpan BioSciences, USA, Rat tissue IL-1β ELISA Kit, Sandwich ELISA, LifeSpan BioSciences, USA) extracted from the medulla spinalis tissue and blood samples were measured using ELISA to evaluate the inflammatory response after spinal cord trauma and the effects of sinularia on this response.

Preparation of tissue samples

Tissue samples taken for biochemical analysis from subjects after spinal cord damage were homogenized in 0.9% NaCl with Janke-Kunke brand ultraturrax T-25 model tissue homogenizator device (IKA®-Werke GmbH & Co. KG., Staufen, Breisgau, Germany). Homogenized samples were centrifuged for 30 minutes at 5000 RPM AND +4 degrees using Hettick- Universal 320-R brand centrifuge device (Hettich Lab Technology, Tuttlingen, Germany). IL-1β (rat tissue IL-1β ELISA kit, Sandwich ELISA, LifeSpan BioSciences, USA) and TNF-α (rat tissue TNF-α ELISA kit, Sandwich ELISA, LifeSpan BioSciences, USA) levels which are acute phase reactants in the acquired supernatants were measured through ELISA method. Measured activities were calculated by proportioning per tissue gram protein with Lowry method.

Statistical analysis

Data analysis was made with IBM SPSS 21 package program. Values for quantative variables were shown as mean±standard deviation or median (Q1-Q3). Suitability of quantative variables to normal distribution was analyzed with Shapiro Wilk test. Groups with normal distribution were analyzed with One-way analysis of variance and the others were analyzed with Kruskal Wallis test. For variables with a significant difference in one way analysis of variance, Tukey test was used for the paired comparison of the variances which were homogenous and Tamhane test for those which were not. Paired comparisons for the variables with a significant difference for Kruskal Wallis test were made with Dunn's test. Values with $p<0.05$ were accepted as significant.

RESULTS

Serum TNF-α, tissue IL-1β variables were analyzed with one way analysis of variance as they had a normal distribution according to the groups. Values were given as mean±standard deviation (Figure 1). Group comparison was made with Kruskal Wallis analysis as serum IL-1β and tissue TNF-α was not suitable. Values were given as median (Q1-Q3) (Table 2).

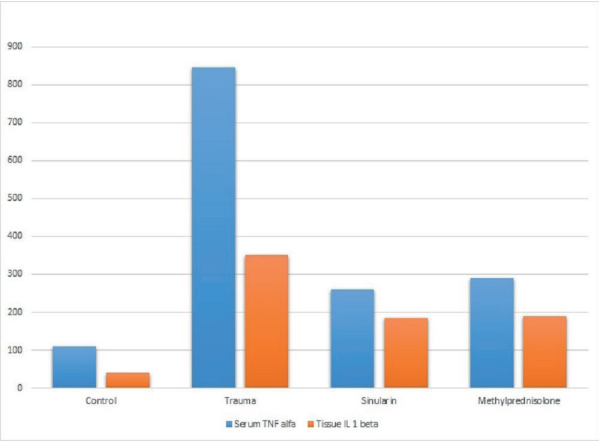

Figure 1. Serum TNF alfa, Tissue IL 1 beta variables were analyzed with One way analysis. Values were given as mean±standard deviation

Table 2. Serum TNF-α, tissue IL-1β variables analyz

Variables	Groups				P
	Control	Trauma	Sinularin	Methylprednisolone	
Serum IL-1β	35 (25-46.25)	360 (239.75-375)	72,5 (53.75-90)	117.5 (98.75-130)	0.001
Serum TNF-α	126.5±30.28	835±63.94	216.5±48.99	286.5±58.60	0.001
Tissue IL-1β	39.5±14.03	363.5±21.09	133.5±19.44	172±20.17	0.001
Tissue TNF-α	122.5 (115-136.25)	807.5 (766.25-878.75)	310 (241.25-345)	377.5 (321.25-413.75)	0.001

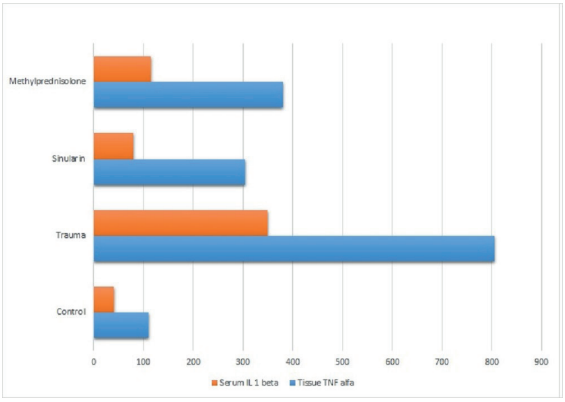

Figure 2. Comparisons for Serum IL 1 beta and Tissue TNF alfa were made with the percentiles (%50)

Paired comparisons for serum IL-1β and tissue TNF-α were made with Dunn's test. When serum IL-1β and tissue TNF-α levels were evaluated, a significant difference was detected in the group taking sinularia treatment compared to the trauma group (Table 3).

Since there was a homogenous variance in the groups for serum TNF-α and tissue IL-1β variables, the paired comparisons were made with Tukey test (Figure 2). A significant difference was observed in serum TNF-α levels in the group taking methylprednisolone and sinularia treatment compared to the trauma group. It was observed that both treatment groups were efficient but didn't have any superiority.

Sinularia treatment was more efficient on different cytokines and compared to methylprednisolone treatment used routinely, it significantly affected both IL-1β level and TNF-α levels in serum.

Table 3. Paired comparisons for serum IL-1β and tissue TNF-α with Dunn's test

Markers	Groups	Test Statistic	Standard Error	Significance
Serum IL-1β	Sinularia&Trauma	20.500	5.22	0.001
Tissue TNF-α	Sinularia&Trauma	18.100	5.226	0.003

DISCUSSION

The aim of the treatment is to prevent secondary damage to occur after spinal cord trauma. Use of neuroprotective agents protecting traumatized spinal cord both through surgical and medical treatment are the treatment methods known. After spinal cord trauma, drugs were used in experimental studies alone or in a combined form in order to determine whether they potentialize their effects or not (12). Especially in recent years, new studies are being continued to improve this treatment method although mesenchymal and neural crest sourced stem cells are used frequently in experimental studies (13-15).

In the first two hours following spinal cord trauma, histopathological findings such as axonal traumatic injury, gray substance hemorrhage, hemorrhagic necrosis and microglial activation are observed and acute phase reactants such as IL-1β, TNFα and IL-6 are released biochemically (16-19). In a study made especially on IL-1β, IL-1β release was shown to be induced in astrocytes around central channel after spinal cord injury (20). In a different research, it was shown that sea corals effected IL-12, TNFα and IL-1β levels (21,22). Starting from published materials available, we analyzed the influence of cytokines such as IL-1β and TNFα after spinal cord trauma and examined that the values of these cytokines increased accordingly in trauma group in our study.

The most important debate on methylprednisolone treatment is due to its side effects. Severe side effects such as gastrointestinal irritation or hepatotoxicity lead researchers to develop new drugs effective on this issue. Starting from this point, sinularia which has no known negative effect on human body and cells was used in our study. When its positive effects on hepatocellular carcinoma are considered, when used with methylprednisolone, sinularia can decrease the side effects of methylprednisolone (2,6).

Although it's not clear yet, there are studies showing the anti-inflammatory, antineoplastic and cytotoxic effect of soft corals through different active materials. In a study showing the anti-inflammatory efficiency of sinularia, it was stated that sinularia maxima type demonstrated anti-inflammatory efficiency with an inhibitory effect on IL-6 and IL-12 with the active molecules such as 12-hydroxy-

scabrolide A and 13-epi-scabrolide C (4). In our study we detected the positive effects on sinularia on IL-1β and TNFα levels in addition to cytokines studied in the related article. Also due to its efficiency on edema, we think that sinularin has an effect similar to methylprednisolone and would provide a more efficient treatment due to its anti-inflammatory and cytotoxic effects.

When the effects of sea products such as sinularia on the development of cancer and inflammatory diseases are examined, it was observed that they affect transcription factors such as nuclear factor kappa b. It was demonstrated that it regulates nuclear factor kappa b release causing many diseases when released in an abnormal amount and thus decreases the development of inflammatory diseases (23).

In a study showing the analgesic efficiency of sinularia, it was shown that it also suppressed the production of proinflammatory proteins, inducible nitric oxide synthase (iNOS) enzyme and cyclooxygenase 2 (COX-2) enzyme and increased Transforming growth factor beta (TGF β) production. It was shown that with its dose-related efficiency, sinularia decreased spinal neuroinflammation by decreasing leukocyte infiltration in lumbar spinal cord dorsal horn and by increasing TGF β production (5). Parallel to the findings, we detected in our study that sinularia suppressed cytokines such as TNFα and IL-1β when used with a dose of 80 mg/kg after spinal cord trauma and had an anti-inflammatory effect.

CONCLUSION

In our study, we analyzed sinularia efficiency in spinal traumas. Our results show that, with its known anti-edematous, cytotoxic and anti-inflammatory effects and no significant side effect in literature, sinularia prevented the damage occurring after spinal cord trauma by decreasing inflammation. With its low side effect profile and anti-inflammatory and anti-edematous efficiency in low doses, sinularia can be used as an alternative to the treatment methods used today and its edema-decreasing effect may have severe effects on preventing post-traumatic damage and we are planning to continue our studies in this direction.

Conflict of interest: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical approval: The study was approved by the Kutahya Saglık Bilimleri University Scientific Research and Publication Ethics Committee (2017.06.06 no decree dated 08.06.2017).

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