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The difference in motor improvements related to combination of omega-3 polyunsaturated fatty acid and alpha-tocopherol supplementations diet of weight-dropped induced spinal cord injury in rats

Mahadewa Tjokorda GB,* Wisnu A. Wardana, Wisnu Wardhana

ABSTRACT

Background: Spinal cord injury (SCI) is the cause of severe neurological disability, and no satisfactory treatment is currently available. Evidence suggests that omega-3 polyunsaturated fatty acid (PUFA) could target some of the pathological mechanisms that underlie damage after SCI. In parallel, promising effect of alpha-tocopherol (vitamin E) also shown as a potent antioxidant to prevent lipid peroxidation on SCI. Therefore, we aim to investigate the combined effect of omega-3 PUFA and vitamin E supplementation in improving motor function of weight dropped induced SCI in rats.

Methods: Twenty-five rats randomly divided into the five groups. SCI was induced in the rats by using a weight dropped model around T9-T11 vertebra level. Omega-3 PUFA and vitamin E was administered orally on each day for two weeks of treatment. The sham group underwent laminectomy without spinal cord compression and treatment. Motor function was measured at the 1st, 7th and 14th day.

Results: We found the significant difference of motoric function between omega-3 PUFA group and combination treatment group compared to the control ($p<0,05$). The highest BBB score on the first week was found in combination treatment group (mean 16±2.3), but no significant difference was found compared to the omega-3 PUFA group. On the subsequent week, combination treatment was showing marked improvement compared to the omega-3 PUFA group ($p<0,05$).

Conclusion: These finding had demonstrated the effect of Omega-3 PUFA as a neuroprotective agent to prevent the secondary injury in SCI-induced rats. The combination of omega-3 PUFA with vitamin E improves its efficacy to become a promising novel therapy for SCI.

Keywords: Omega-3 PUFA, vitamin E, improves motor function.
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Neurosurgery Department Udayana University-Sanglah Hospital, Bali, Indonesia

*Corresponding author: Tjokorda GB Mahadewa, MD.Ph.D., Associate Professor of Neurosurgery, University of Udayana, Division of Neurospine, Sanglah General Hospital, Kesehatan Street 1, Denpasar, Bali, Indonesia 80000.
(62361) 249988,
(62361) 244322,
tjokmahadewa@hotmail.com;
tjokmahadewa@unud.ac.id

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INTRODUCTION

Traumatic Spinal Cord Injury (TSCI) also known as spinal cord injury are catastrophic events resulted in disabilities on motor, sensory and autonomic functions.[1] American Spinal Cord Injury Association (ASIA) classified TSCI grading into categories based on severity of motor and sensory loss regarding involved level of spinal cord.[2] Significant functional defect after TSCI caused decreased of patient`s survival rate and quality of lives.[3]

Epidemic global study on 2011 reported prevalence of TSCI ranged 236 until 4187 cases for 1 million world's population. In general, TSCI incidence in developed countries is a little bit higher than developing countries. This condition may be caused by minimal standardized reports in developing countries.[1,4,5] Four wheel vehicles accident is responsible for the majority of TSCI in developed countries. On the other hand, two wheel vehicles accident, fall from rooftop or trees are dominating etiologies of TSCI in most of developing countries.[6]

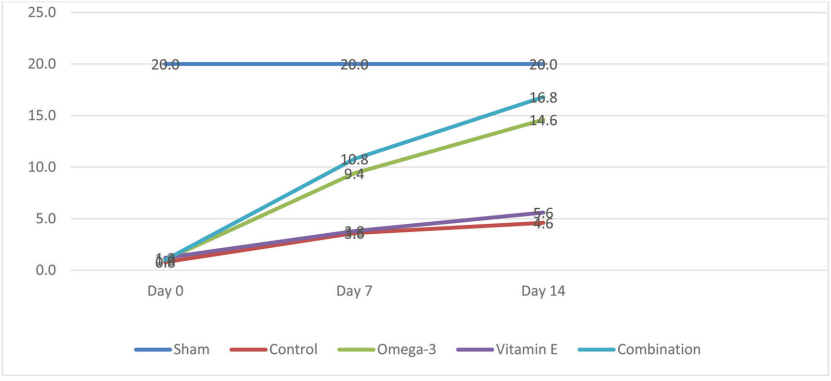
Based on age and gender, the highest incidence of TSCI occurred in young adults male. The range of age on onset of injuries in North America, Europe, and Asia are between 32-55.4 years old, 37-47.9 years old, and 26.8-56.6 years old, respectively.[1] Higher incidence of TSCI in younger age had resulted in severe economic loss for family, community and country.[3,7]

Human central nervous system (CNS) have limited regeneration response, making total recovery post trauma impossible.[8] Increasing understanding of pathological change after TSCI makes research of agents that accelerated healing process is possible. On acute phase of TSCI, there are two important mechanisms: the primary mechanism that involved mechanical destruction and spinal cord compression continued with biochemistry cascade and cellular responses also known as the subsequent degenerating process that affected glial cell and neuron.

Figure 1 Intraoperative view of Laminectomy procedure around the level of T9-T11 and the dural opening of the rats

Table 1 Characteristic of the Wistar rat pre and post-SCI induction

Groups	Weight (gram)	BBB score	
		Pre-SCI Induction	Post-SCI Induction
Control	180,2	20	0,8
Omega-3	185,4	20	1
Vitamin E	184	20	1,2
Combination	186	20	1
Sham	184,2	20	20 (only laminectomy)



In general, secondary degeneration process includes two important events: spreading of inflammation and membrane hydrolysis with lipid peroxidation. Both of those processes become an important target for TCSI therapy because both inhibitions are proven to increase TCSI healing responses. In vivo and in-vitro research revealed omega-3 polyunsaturated fatty acid (PUFA) and vitamin E have neuro protective potential that may inhibit secondary degeneration process on TSCI.[9,10] Omega-3 PUFA contents are α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[11] No research focused on combined effect of omega-3 PUFA and vitamin E on TSCI. Therefore, we evaluated the combination therapy of omega-3 PUFA and vitamin E for motor function restoration in rats with TSCI.

MATERIALS AND METHODS

This research is an experimental study applying randomized post test control group. Twenty-five male Wistar rats were randomly divided into five groups such as omega-3 PUFA group, vitamin E group, combination group, the control group, and sham group. The rats were placed in cages with the capacity of 2-3 rats/cage, under controlled conditions (light and dark cycles every 12 hours, at room temperature 26 degrees Celsius), with easy access to food and water. All of the rats were given drug by intramuscular injection of ketamine (50 mg/kg). Spinal cord injury induced by dropping a load of 20 grams weight from within a height of 10 mm on T9-11 spinal cord after the laminectomy was performed using the procedure described by Vibhor, et al. (2013). Mice placed on a proper operating table, and a linear incision made around thoracic 9-11 and sharp dissection in order lamina exposed.[12] Laminectomy was performed, the dura was opened about 3 cm, and a weight of 20 grams was dropped. After the procedure, the wound was closed layer by layer with threads vicryl 3.0.

Groups 1 and 2 received supplementation diet with Omega-3 PUFA and vitamin E that given orally for two weeks with the dosage 5ml/kg BW and 30mg/kg BW, respectively.Group 3 received combination therapy of both supplementations diet while the control group received placebo supplementation diet. Meanwhile, Sham group did not receive any supplementation diet.

Motor function was measured using BBB locomotive scale (range 0-20) on the end of the first and second week and observed by two researchers.[12] The result was analyzed by using one-way ANOVA for each week of measurement and considered significant at $p<0.05$.

Table I The Lower Portion of the BBB Locomotor Scale

Score	Operational Definition
0	No observable hindlimb (HL) movement
1	Slight movement of one or two joints (ankle, hip or knee) of the HL
2	Extenseive movement of one joint OR extensive movement in one joint and slight movement in on other joint
3	Extensive movement in two joints
4	Slight movement of all three joints
5	Slight movement of all two joints and extensive movement of the third
6	Extensive movement of two joints and slight movement of the third
7	Extensive movement of all three joints
8	Sweeping with no weight support OR plantar placement of the paw with no weight support
9	Plantar placement of the paw with weight support in stance only (i.e., when stationary) OR occasional, frequent, or consistent weight supported dorsal stepping and no plantar stepping
10	Occasional weight supported plantar steps, no forelimb (FL)-HL coordination
11	Frequent to consistent weight supported planter steps and no FL-HL coordination
12	Frequent to consistent weight supported planter steps and occasional FL-HL coordination
13	Frequent to consistent weight supported planter steps and frequent FL-HL coordination

Definitions are as follows:
Slight: partial joint movement through less than half of the range of joint motion.
Extensive: movement through more than half of the range of joint motion
Sweeping: rhythmic movement of HL in which all three joints are extended, then fully flex and extend again
Weight Support: contraction of the extensor muscles of the HL during plantar placement of the paw, or elevation of hindquarter
Plantar Stepping: the paw is in plantar contact with weight support and then the HL is advanced forward and plantar contact with weight support is reestablished
Dorsal Stepping: weight is supported through the dorsal surface of the paw at some point in the step cycle.
FL-HL Coordination: for every FL step, an HL step is taken and the HLs alternate
Occasional: ≤50%
Frequent: 51-94%
Consistent: 95-100%
This table is adapted from [4].

RESULTS

Spinal Cord Injury Induction

20 male Wistar rats from control, omega-3, vitamin E and combination therapy group have a low score of BBB after SCI induction using the weight dropped method. Meanwhile, there is no difference between pre and post-SCI induction on the sham groups. Characteristics of the Wistar rats shown in Table 1.

Improvement of Motoric Function After Treatment

The motoric function was measured on the day 7 and day 14 after SCI induction. Data of the BBB mean score on each week were presented in graph 1. All of the group showed improvement of the motoric function after day seven dan 14. The highest of BBB mean score on the day seven was shown by the combination treatment group (10.8) followed by Omega 3 group treatment (9.4). There was no difference between Vitamin E group and Control group (3.6). On the day 14, the average BBB scores were 16.8 and 14.8 in the combination treatment group and omega-3 group respectively. The little increment of BBB score was still found in the vitamin E group and control group. Overall, combination treatment group was showing the best improvement of measured BBB score on each week.

DISCUSSION

Spinal Cord Injury Induction

Samples from all the treatment groups were showing the similar result of SCI induction. The range of BBB score after induction of SCI is between 0 and 2. Low score of BBB is proving that weight dropped method as a reliable technique to induce SCI in rats. Besides, no defect was found in the sham group after laminectomy. Laminectomy had been done carefully without causing any harm to the spinal cord function. Therefore, we consider the defect on treatment group was only caused by the weight dropped.

Spinal axon injury allegedly occurred through a tear axons of the direct suppression of self-harm associated with traumatic. Early breakdown of axons results in the plasma membrane ion channels and the loss of calcium homeostasis dysregulation. Furthermore, a series of cascades activated calcium-dependent, resulting in damage to mitochondria and release of cytochrome c. In the end, cytochrome c release apoptosis cascade activates caspase-3-mediated proteolytic cleavage of the substrate axonal cytoskeletal produces the characteristic termination traumatic axonal injury.[13]

Improvement of Motoric Function After Treatment

Omega -3FA treatment has been proven to reduce oxidative stress, which causes mitochondrial dysfunction and cell death.[14] Research on hippocampal cells in the culture medium showed that DHA increases the activity of antioxidant enzymes, including glutathione peroxidase and glutathione reductase.[11] O3FA decrease the activity of COX, modulate the inflammatory process, inhibiting the formation of pro-inflammatory eicosanoids and cytokines.[15] O3FA play a protective role neural excitotoxicity including suppression, modulation of calcium and potassium channels, activation of gene transcription, the formation neuroprotectin-1

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and Resolvins.[16] Sarsilmaz (2003) research showed omega-3 PUFA might modulating few secondary CNS degeneration process by antioxidant and anti-inflammation effects by inhibiting proinflammation cytokines production.[11]

Meanwhile, vitamin E (alpha-tocopherol) is lipid soluble antioxidant that inhibits free radical development. Vitamin E also has a protective effect on lipid peroxidation that is critical for keeping functional for all biological cell membrane.[9] Monotherapy effectively with only omega-3 PUFA of vitamin E are already proven based on some experimental study in TSCI.

Vitamin E participates in the reduction reaction aims to remove free radicals in the body. Vitamin E also improves local microcirculation and protection against lipid peroxidase is essential to membrane integrity.[9] After one week of treatment, we found a significant difference of motoric function between omega-3 PUFA group and combination supplementation group compared to the control ($p<0,05$). The highest BBB score on the first week was found in combination supplementation group (mean 10,8±2,2), but no significant difference was found compared to the omega-3 PUFA group. On the subsequent week, combination treatment was showing marked improvement compared to the omega-3 PUFA group ($p<0,05$).

CONCLUSION

Based on the results of this study, it is concluded that the difference in motor improvements was related to the combination of Omega-3 PUFA and vitamin E supplementation diet of weight dropped induced spinal cord injury in rats. This is the first study that shows that different levels of motor recovery of rat's lower limb in experimental groups of supplemental diet. However, these results cannot be extrapolated to humans, and therefore, further studies should be conducted in the clinical setting to observe if the differences in motor improvements were related to the combination of Omega-3 PUFA and vitamin E supplementations diet in traumatic spinal cord injury of human as well. Our results may explain that the combination of dietary supplementations of omega-3 PUFA and vitamin E is better than giving separately.

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