FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 6 No. 1, March, 2022, pp 13 - 20
DOI: https://doi.org/10.33003/fjs-2022-0601-889

EFFECTS OF EXTRACELLULAR VESICLES ISOLATED FROM SUBVENTRICULAR ZONE ON MOLECULAR CHANGES FOLLOWING SPINAL CORD INJURY ON WISTAR RATS

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ABSTRACT

Spinal cord injury (SCI) may occur as a result of traumatic crash to the spine that dislocates one or many vertebrae. SCI has two events to bring about the injury in the spinal cord that result at the end it will lead to bad consequences and loss of neurons; SCI complications are general loss of motor and sensory function. Extracellular vesicles (EVs) are very small vesicles produced by cells that contribute to cell to cell communication, transferring bioactive lipids, proteins, RNA. The aim of this research is to determine the effect of EVs on inflammasomes complex after SCI on rats. EVs were harvested characterized and cultured as exosomes, the animals are divided to 5 groups: control, sham, vehicle, SCI + treatment, SCI + EVs group. Spinal cord injury was induced using weight compression. 10 µl of EVs were injected intrathecally into lumber cistern in a space between L4-L5 laminae slowly. Gene expression of Caspase-1, ASC and NLRP3 were determined by RT-PCR. Western blot for determining the protein content of the inflammasomes were carried out. SCI up regulated the level of inflammasome complex both mRNA and proteins. Inthrathecal injection of EVs down regulated the inflammasome complex and make neuronal recovery. SCI can up regulate the level of inflammasome complex both mRNA and proteins. EVs are good therapeutic agent for SCI and increase neuronal recovery, inthrathecal injection is the best method of injection MSCs for treatment of SCI.

Keywords: Trauma, exosome, inflammasome, Neuroinflammatory

INTRODUCTION

Spinal cord injury (SCI) is a deadly central nervous system (CNS) destruction due to incomplete or complete events that brings about accelerating state of destruction that involves biochemical, molecular, physiological as well as structural post injury tissue reorganization as well as many changes (Thuret *et al.,* 2006). SCI pathophysiology is known by two main events, a primary injury aa well as secondary injury that composed of many pathophysiology processes such as inflammation, excitotoxicity, apoptosis as well as demyelination Neuroinflammatory response is a response as a result of activation of the pro-inflammatory interleukin (IL) family like IL-1β and IL-18 that brings about cell apoptosis. These ILs has the same characteristics, has the same three-dimensional structure, as well, are formed as inactive cytoplasmic precursor forms (Schroder and Tschopp, 2010). Processing as well as activation of pro- IL-1β and IL-18 needs the activation of a multiprotein caspase-1 complex known as inflammasome (Schroder and Tschopp, 2010) this is the major signaling platform that determines pathogenic stressors. Many studies have shown the role of TNF-α in mechanism of SCI, due to SCI the level of TNF-α rises rapidly as well leads to initiation of neurons apoptosis in vitro (Sloka and Stefanelli, 2005).

SCI brings about molecular and cellular complex changes around the lesion area of CNS, which leads to loss of behavioral deficits as well as neuronal connectivity and functions. Many improvements were achieved in the early spinal cord surgery as well as successful therapy (Agostinello *et al.*, 2017), the treatment of cell destruction as well as loss of nerve fiber either occurring during the chronic or acute level of the disease are still in its infancy. Inflammatory events that is neuroinflammatory probably have an influence on the secondary injury of SCI, it was documented that controlling neuroinflammatory responses may increase functional CNS recovery in SCI rats model (Allison *et al.,* 2016).

Inflammasomes complexes are group of cytosolic protein that exist to initiate host immune responses to microbial infection as well as other cellular damage (Franchi *et al.,* 2012). The mechanism of neuroinflammatory pathways involves the initiation of the pro-inflammatory interleukin 1 (IL1) groups like IL-1β and IL-18 that initiate apoptosis. Activation as well as production of pro- IL-1β, IL-18 need the stimulation of a multiprotein caspase-1 complex known as inflammasome (Schroder and Tschopp, 2010), which is the most important signaling pathway that determines pathogenic stressors. Inflammasome complex activation in brain innate immune cells basically is among the key primary as well as a critical stage of neuroinflammation consecutively activated by disease stimuli as well as traumatic changes by pathogen-associated molecular pattern (PAMP) as well as damaged-associated molecular pattern (DAMP) molecules. The NACHT, LRR, and PYD domains consist a protein (NLRP) inflammasomes NLRP1 and NLRP3 containing three major constituents: that is a nucleotide oligomerization domain-like receptor (NLR), caspase-1 as well as adaptor known as apoptosis-associated speck-like protein (ASC) containing a caspase activation domain (Schroder and Tschopp, 2010). When the NLRPs are triggered it results to the recruitment of ASC that has caspase activation and recruitment domains (CARD). ASC then associates with the CARD of pro-caspase-1. This association will results to its transformation to active caspase-1 thereby convert pro forms of IL-1β and IL-18 in to very active forms, these mechanisms will initiate an inflammatory cascade (Petrilli *et al.*, 2007). From previous researches it shows that only few data documented that inflammasome pathway to be differentially regulated following spinal cord injury. Study conducted by De Rivero et. al., shows that NLRP1 is activated as a result of spinal cord injury, also the neutralization of ASC as a result of SCI decreases caspase-1 activation as well reduces processing IL-1β and IL-18 (de Rivero Vaccari *et al.,* 2008). Nevertheless, the introducing of anti-ASC neutralizing antibodies to injured

rats' leads to meaningful and successful tissue sparing and functional improvement (de Rivero Vaccari *et al.*, 2008). Some evidences revealed that, the expression level of caspase-1, NALP and ASC are elevated following spinal cord injury in rats as well hyperbaric oxygen treatment alleviated this effect (Liang *et al.*, 2015).

Subventricular Zone (SVZ) is the largest neurogenic region, where stem cells produced the immature neuroblast that can be transported to the olfactory bulb and multiply into different neurons (Lois and Alvarez-Buylla, 1994) as well as some oligodendrocytes (Menn *et al.*, 2006). In the human CNS, neuronal stem cell exists in the subventricular zone of the ventricles, as well as dentate gyrus (DG) of the hippocampus. Experimentally, neural/precausor cells (NPCs) proliferate and produce neurospheres, that are self-renewing and multipotent, as well can be multiply into neurons, oligodendrocytes as well as astrocytes (Anderson, 2001). Extracellular vesicles (EVs) are very smaller lipid membrane bilayer vesicles produced by different type of cell either normal or diseased. There are 3 different forms of EVs: Apoptotic bodies, exosomes as well as microvesicles and; these can only be differentiated based on their size and biogenesis (Mohankumar and Patel, 2015). Exosomes are 40-100 nm in diameter and secreted by multivesicular bodies. Microvesicles are 50-1000 nm in size and are produced through punch off directly from plasma membrane. Apoptotic bodies 800-5000 nm in size, produced by apoptotic cells (Mohankumar and Patel, 2015; Nishida-Aoki and Ochiya, 2015). This research is aimed to investigate the possibility of using SVZ derived EVs as therapeutic agent for SCI through intrathecal injection on SCI injured rats.

MATERIALS AND METHODS

Preparation and Isolation of Extracellular Vesicles

Neuronal stem cells were harvested from SVZ according to Aligholi *et al.,* (2014). Adult rats were used, intraperitoneally xylazine (10 mg/kg) and ketamine (80 mg/kg) were deeply anesthetized the rats, SVZ were dissected under sterile condition. Using surgical blade, mechanically the cells were dissociated to smaller fragment, 500 µL of 0.02% Trypsin/EDTA for 10 min at 37ºC as an enzyme was added, 500 µL of trypsin was then added as an inhibitor. The cells that was dissociated was grown in Dulbecco's modified Eagle's medium/F12 containing 1% N2 supplement, 3% B27 supplement, 2 µg/ml heparin, 1% penicillin/streptomycin, 1% glutamax, 10 µg/ml basic fibroblast growth factor and 20 µg/ml epidermal growth factor were incubated at 37^{O}C at 5% CO2. Every three consecutive day the medium was changed for period of three days. The cells were plated as primary culture (free-floating neurospheres). The Neurospheres was dissociated enzymatically in every five days, it was re-cultured in a fresh medium. According to Azari et al., the total number of the viable cells was determined using trypan blue exclusion each passage (Azari *et al.*, 2010). After passage 3 (85 % confluence) conditions medium was harvested every 72hrs. Briefly four centrifugation steps were carried out, the cells debris were collected through 3000 g for 10min and 20,000 g for 30min. The cells were centrifuged using ultracentrifugation at 110,000 g for 120min, using PBS the cells were washed and re-ultra-centrifuged again. Finally, the cells were re-suspended in PBS.

Animal housing and groups

Forty Wistar rats (Males, 12 weeks old) were used for this study, weighing 250 to 300 g (Pasteur, Iran). The rats were housed in cages with ad libitum accessed to food and water, acclimatized for the period of 3 days on controlled temperature condition with the light as well as dark cycle (12h for each) according to Azari et. al., (Azari *et al.*, 2010). The rats were handled in accordance with the rules and regulations of Iranian animal ethics society, Tehran University of Medical Sciences guidlines (Farahabadi *et al.*, 2016). The rats were allocated to five groups: Control group: No surgery (8 rats), Sham group: Laminectomy without SCI (8 rats), SCI without treatment group: With SCI but no any treatment (8 rats), Vehicle group: SCI treatedted with PBS medium only (8 rats), SCI + EVS group: SCI injected extracellular vesicles derived from SVZ (8 rats).

Spinal Cord Injury (SCI)

SCI was formed by weight compression method; the animals were anesthetized through intraperitoneal injection of xylazine 10 mg/kg and ketamine 80 mg/kg, to confirm the narcosis, an unconscious reflex test was carried out through severe punch. A Rongeur was used to make the laminectomy at vertebra (T10) by shaving the surgical area and positioned the rat on stereotactic frame, during the laminectomy the Dura mater of the spinal cord was maintained undisturbed. Using 50 g of weight for the period of 5 minutes, the spinal cord injury was made. The body temperature of rats was checked and maintained within the range of 36- 37℃ throughout the surgery period. After the surgery, the incised area was sutured using black silk (3-0) according to Farahabadi *et. al.* (2016). Intraperitoneally 1 ml ringer solution was injected to each rat after the injury in order avoid dehydration. After the rats woke up, neurologically the rats were monitored and evaluated for food, water and urination for 72hrs.

Injection of EVs

Intraperitoneally the rat was anesthetized with xylazine 10 mg/kg and ketamine 80 mg/kg, it was then placed in a flex position at the end part of small board. Around L4 to L5 vertebra the skin was shaved incised longitudinally, using Hamilton syringe 10 µl of EVs was injected intrathecally into lumber cistern in a space between L4-L5 laminae, also PBS was injected same method as vehicle control, in order to confirm truly the syringe entered the lumber cistern, a tail flicked was observed.

RNA Extractions and Real-Time PCR

Determination of gene expression was carried out on spinal cord tissues corresponding to the area of the injury. Extraction of total RNA was conducted using peqGold RNA TriFast (PeqLab, Germany). The purity was determined by 260/280 ratios of optical density from every sample (Nanodrop 1000, PeqLap, Germany). Complementary DNA was produced by reverse transcription (RT) -kit as well as random hexanucleotide primers. Determination of expression levels of genes targeted was conducted by a mixture of 2 µL reversed-transcribed cDNA, 2 µL RNAse-free water (Invitrogen, Germany), 5 µL 2× SensiMix SYBR as well as Fluorescein (Bioline, Germany), and 0.5 µL primers (10 pmol/µL). Quantitative real-time PCR (qrtPCR) was conducted by MyIQ detection system (Biorad, Germany). Relative quantification was determined using ΔΔCt-technique by qbase+software (Biogazelle, Belgium). The data generated was presented as relative amount of the target gene to the amount of a reference gene ß-actin. The results of sham group were set to 100 %. Data of interest was given as relative expression (Zendedel et al., 2016). The expression genes of Caspase-1, ASC and NLRP3 were determined and expressed. Table 1 shows the list of primers used for this experiment.

Table 1: Primer sequences and length; (bp) =base pair

Primer	Sequence	Length (bp)
Caspase-1		157
Forward	GTGGAGAGAAAGAAGGAGTGGT	
Reverse	GATGAGTGACTGAATGAAGAGG	
NLRP3		129
Forward	GGAGTGGATAGGTTTGCTGG	
Reverse	GGTGTAGGGTCTGTTGAGGT	
ASC		150
Forward	TCTGGAGGGGTATGGCTTGG	
Reverse	GAGTGCTTGCCTGTGTTGGT	
β –ACTIN		302
Forward	TCAGAGCAAGAGAGGCATCC	
Reverse	GGTCATCTTCTCACGGTTGG	

Western Blot Analysis

To determine the protein contents of the inflammasomes, a western blot technique was conducted with injured spinal cord corresponding to the area of injury, the tissue was removed freshly from the rats and kept at −80 °C. To determine the total protein, the tissue was lysed and centrifuged. The amount of total protein was identified using Total Protein Kit, Micro (Sigma, USA). 5 µg of protein content was pipetted, immunoblot was conducted using antibodies (1:1000 dilution) to NLRP3, Caspase-1and ASC (Biological USA). The protein was resolved in 14-20% TGX Criterion precasted gels, then transferred to polyvinylidene difluoride (PVDF) transfer membranes and was placed in blocking buffer (PBS, 0.1% Tween-20, 0.4% I-Block) using the primary antibodies it was then incubated for 1hr. It was again incubated for another 1hr using membranes together with anti-rabbit horseradish peroxidase (HRP)-linked antibodies (abcam Germany). Signal visualization technique was conducted using enhanced chemilluminescence (Sigma USA). The images of western blot analysis were determined with the same film exposure of 7min for all samples in order to maintain consistency for comparison (de Rivero Vaccari *et al.*, 2008). For positive control GAPDH (Thermoscientific USA) was used.

Statistical Analysis

The data generated was analyzed statistically using SPSS version 22 (International Business Machines [IBM] corporation, Armonk, NY, USA). One way- ANOVA test followed by a post-hoc test using SPSS statistic software was carried out to compare the mean data in groups of the study. $P \leq 0.05$ was considered significant.

RESULTS

Effect of Extracellular vesicles on the ASC Gene Expression

SCI was induced on the rats; this significantly increased the level of ASC transcription in spinal cord injury group rats when compared with control and laminectomy groups ($p<0.001$). It was observed that EVs had a great inhibitory effect on the level of ASC gene expression; it shows a significant decrease in treated rats compared to SCI and PBS groups ($p<0.001$). There is no significant difference observed between control and laminectomy ($p<0.963$), because it didn't show any expression of ASC gene expression, a significant difference was observed between control group and SCI group, control and PBS group, laminectomy group and SCI group, laminectomy and PBS group. Also no significance difference between SCI and PBS ($p<0.823$) (Fig. 1A), because in SCI and PBS group, SCI was induced but no injection of EVs, this shows no any effect when compared with treatment groups that is EVs.

Effect of Extracellular vesicles on the Caspase-1 Gene Expression

After inducing of SCI on the rats, the SCI significantly increased the level of Caspase-1 gene expression in rats of SCI group when compared with control and laminectomy groups ($p<0.001$). It was observed that injection of EVs had a great inhibitory effect on the level of Caspase-1 gene expression; it down regulated the expression of Caspase-1 gene expression, it shows a significant decrease in rats injected with Evs group (treated rats) when compared with SCI and PBS groups ($p<0.001$), this presented a great significant difference between EVs group and SCI, PBS groups. There was no significant difference observed between control and laminectomy animal groups ($p<0.999$), because both groups no SCI. But a great significant difference was observed between control animal group and SCI animal group; there is also significance difference between control group animals and PBS group animals. The results show a great change between laminectomy group and SCI group, a change was also observed between laminectomy group and PBS group. No any significance difference observed between SCI and PBS ($p<0.977$) (Fig. 1B), because no injection of EVs in these groups.

Effect of Extracellular vesicles on the NLRP3 Gene Expression

The SCI significantly increased the level of NLRP3 transcription in spinal cord injury animal group when compared with control and laminectomy groups ($p<0.001$). SCI up regulated the level of inflammasomes complex in which NLRP3 was among, it was observed in this result that NLRP3 gene expression was up regulated as a result of SCI (Fig. 1C). But after intrathecal injection of EVs it was observed that EVs had a great inhibitory effect on the level of NLRP3 gene expression in which it down regulated the level of NLRP3 gene expression; it shows a significant decrease in treated rats compared to SCI and PBS groups ($p<0.001$), this shows a significant difference between EVs (treatment group) and SCI, PBS groups. There were no any changes observed between control and laminectomy ($p<0.987$), because both groups no SCI, but there was a great difference observed between control group animal and SCI group animals, also, a great difference was observed between control group of rats and PBS group. There is also a significant change between laminectomy group and SCI group; there is also a great difference between laminectomy group and PBS group. The results shows no significance difference between SCI and PBS ($p<0.474$) (Fig. 1C), because no injection of EVs in SCI and PBS groups.

Effect of Extracellular vesicles on the ASC protein synthesis

The effect of EVs injection on ASC protein synthesis was

identified using western blot technique. The results show that ASC protein level shows an increase in SCI and PBS groups compared with the control and laminectomy groups ($p<0.001$), this shows that SCI raised the level of ASC protein synthesis. The protein expression was significantly decreased after intrathecal injection of EVs in that group when compared with SCI and PBS groups ($p<0.001$), this shows a great change between EVs group and SCI, PBS group. The results show no any change between control group and laminectomy group ($p<0.902$), because no SCI in such groups. But a change was observed between control group of rats and SCI group of rats; there is also significant difference between control group and PBS group. A change was also observed between laminectomy group and SCI group, likewise a change was seen between laminectomy group and PBS group. No any change seen between SCI and PBS group ($p<0.648$) (Fig. 2A), because no injection of EVs in such groups.

Effect of Extracellular vesicles on the Caspase-1 protein synthesis

The effect of intrathecal injection of EVs on Caspase-1 protein synthesis was identified using western blot technique. The results show that Caspase-1 protein synthesis level shows an increase in SCI and PBS rats

compared with the control rats and laminectomy rats ($p<0.001$), this shows a great difference in protein synthesis between control rats and SCI rats, remarkable change was also seen in protein synthesis between control rats and PBS rats. It was also observed in the results, a remarkable difference in protein synthesis of laminectomy group compared with SCI group, a remarkable difference was also seen in protein synthesis of laminectomy group compared with PBS group. Protein synthesis was significantly decreased following injection of EVs intrathecally in the rats compared with SCI and PBS groups ($p<0.001$), this presented a remarkable difference in protein synthesis between EVs and SCI, PBS groups. No any significance difference observed in protein synthesis between control and laminectomy groups ($p<0.716$), because no SCI in these groups. Also no any significance difference observed in protein synthesis between SCI and PBS groups ($p<0.8248$) (Fig. 2B), because no injection of EVs in both groups.

Effect of Extracellular vesicles on the NLRP3 protein synthesis

The effect of EVs injection on NLRP3 protein synthesis was identified using western blot technique. From the results it was seen that NLRP3 protein synthesis shows an increase in SCI and PBS groups compared to control and laminectomy groups ($p<0.001$), this shows a remarkable change in protein synthesis between EVs group and SCI, PBS groups. The protein synthesis was significantly decreased following injection EVs intrathecally in to the rats in EVs group compared with SCI and PBS rats ($p<0.001$), a remarkable different was seen in protein synthesis between EVs (treatment) group and SCI, PBS groups. No any remarkable difference seen in protein synthesis between control rats and laminectomy rats' groups ($p<0.6281$), because no SCI in these groups. But a significance difference was observed between control group and SCI group, a remarkable change was seen in protein synthesis between control rats and PBS rats. A remarkable difference was also seen in protein synthesis between laminectomy group and SCI, a change was also seen in protein synthesis between laminectomy group and PBS group. There is no any significance difference in protein synthesis between SCI and PBS groups ($p<0.4203$) (Fig. 2C) because no injection of EVs in these groups.

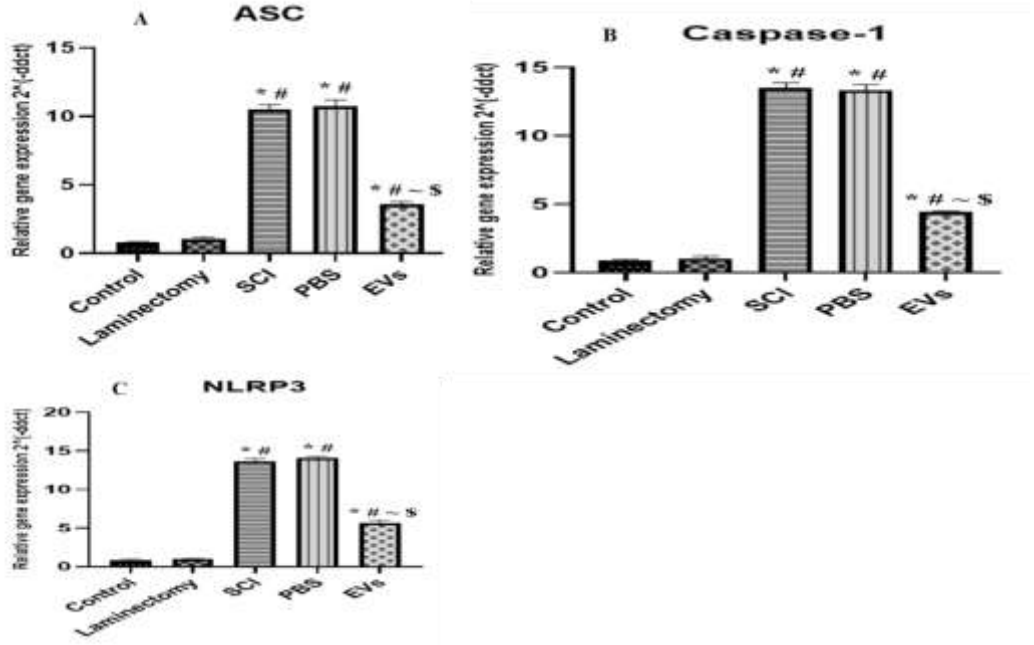

Figure 1: Effect of EVs on the inflammasomes

(A) mRNA levels of the ASC gene in control, laminectomy and SCI rats following injection of PBS and EVs intrathecally. * $P \leq 0.001$ VS. Control group, # $P \leq 0.001$ VS. Laminectomy group, ~ $P \leq 0.001$ VS. SCI group, $ $P \leq 0.001$ VS. PBS group. Data represent mean±SD. **(B)** mRNA levels of the Caspase-1 gene in control, laminectomy and SCI rats following injection of PBS and EVs intrathecally. * $P \leq 0.001$ VS. Control group, # $P \leq 0.001$ VS. Laminectomy group, ~ $P \leq 0.001$ VS. SCI group, $ $P \leq 0.001$ VS. PBS group. Data represent mean±SD. **(C)** mRNA levels of the NLRP3 gene in control, laminectomy and SCI rats following injection of PBS and EVs intrathecally. * $P \leq 0.001$ VS. Control group, # $P \leq 0.001$ VS. Laminectomy group, ~ $P \leq 0.001$ VS. SCI group, $ $P \leq 0.001$ VS. PBS group. Data represent mean±SD.

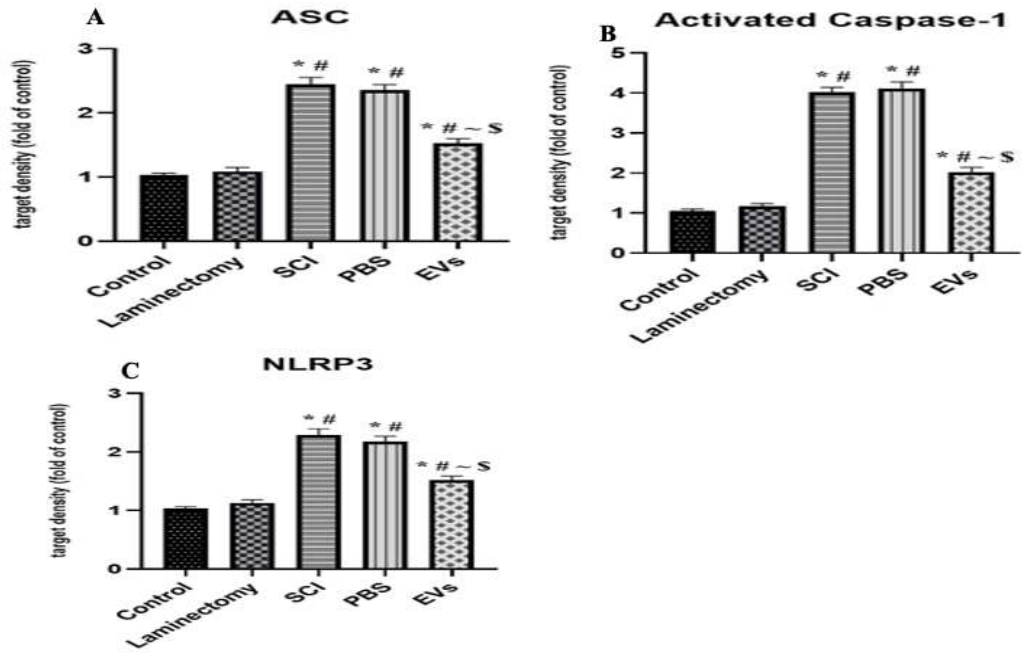

Figure 2: Effect of Extracellular vesicles on the inflammasomes protein level.
(A) Western blots of ASC proteins in the SCI rats injected with PBS and EVs. * P ≤ 0.001 VS. Control group, # P ≤ 0.001 VS. Laminectomy group, ~ P ≤ 0.001 VS. SCI group, $ P ≤ 0.001 VS. PBS group. Data represent mean±SD. **(B)** Western blots of Caspase -1 proteins in the the SCI rats injected with PBS and EVs. * P ≤ 0.001 VS. Control group, # P ≤ 0.001 VS. Laminectomy group, ~ P ≤ 0.001 VS. SCI group, $ P ≤ 0.001 VS. PBS group. Data represent mean±SD. **(C)** Western blots of NLRP3 proteins in the the SCI rats injected with PBS and EVs. * P ≤ 0.001 VS. Control group, # P ≤ 0.001 VS. Laminectomy group, ~ P ≤ 0.001 VS. SCI group, $ P ≤ 0.001 VS. PBS group. Data represent mean±SD.

DISCUSSIONS

SCI was induced on rats which up regulated the level of inflammasome complex NLRP3, ASC, and Caspase-1 in both gene expression and protein synthesis. Inflammasomes are protein complexes that known to form host immune responses to infections and most of cellular changes like SCI (Franchi et al., 2012). The mechanism of neuroinflammatory responses deals with the switching the pro-inflammatory interleukin 1 (IL1) group such as IL-1β and IL-18 that bring about apoptosis. IL-1β and IL-18 are both same, and are formed as inactive cytoplasmic precursor type (Schroder and Tschopp, 2010). Production as we as activation of pro- IL-1β and IL-18 require the activation of a multiprotein caspase-1-activating complex known as inflammasome (Schroder and Tschopp, 2010), which is the key factor of signaling platform that determines pathogenic stressors. Initiation of inflammasome complex in the immune cells of innate CNS is basically is one of the key important stages of inflammation of CNS sequentially activated by some disease stimuli as well as traumatic problems by pathogen-associated molecular pattern as well as damaged-associated molecular pattern molecules. Whenever NLRPs are activated it will result to the induction of ASC that has caspase activation and recruitment domains. Then ASC will associate with the CARD of pro-caspase-1. This mechanism will result to its transformation to active caspase-1 in which it will change pro types of IL-1β and IL-18 in to very functional types, these mechanisms will initiate an inflammatory response. In the current study SCI initiates formation and over expression of inflammasome complex ASC, NLRP3 and Caspase-1, in the current study the inhibition of inflammasome complex was targetted through injection of EVs in rats. Figure 1A-C shows gene expressions of the inflammasome complex ASC, NLRP3 and caspase-1of the groups of animals. Figure 2A-D shows over expression in protein synthesis of the inflammasome complex ASC, NLRP3 and Caspase-1. The results shows over expression of gene expression and protein synthesis of the inflammasome complex in SCI and PBS compared with control and laminectomy, this shows SCI can up regulate the inflammasome complex and initiate inflammation in the injured area which can lead to secondary injury and other complications in the body if care is not taken. This study is in line with different studies carried out that shows SCI can up regulate the production of ASC, NLRP3 and caspase-1, (Zendedel *et al*., 2015; Zendedel *et al.,* 2016; Nikmehr *et al.,* 2017; , Mohammadi *et al.,* 2018; Mohammadi *et al.,* 2019; Mosavi *et al.,* 2019; Mousavi *et al.,* 2019).

It was revealed that EVs involves in intercellular relationship and function in the modulation of stem cell continuity, tissue immunosurveillance as well as tissue repair (Benito-Martin *et al.,* 2015; Rani *et al.,* 2015). Extracellular vesicles has ability in transporting molecules as well as modulating cell functions in other cells (Logozzi *et al.,* 2019). The superiority as well as multi functions of EVs like its characteristic of tiny particle have bring about the possible chances for their application in diagnosis, therapeutic and screening purposes for various diseases and trauma. In this study EVs was used as therapeutic agent in SCI on rat model, at the end the results shows a great success in down regulation of inflammasomes complex there by stopping the secondary damages following SCI on rat model.

Result from a recent study indicated that miR-133b has a great impact in CNS apoptosis, neuronal outgrowth as well as differentiation in the CNS (Xia *et al.,* 2016). Another study shows that higher expression of miR-133b promotes motor function improvement following stroke in an experimental animals (Xin *et al.,* 2017). Exosomes, aa a great cell communicator, have been in application as cellular agents for systemic and local transporter of miRNAs in the therapy of

different condition which include Parkinsons's disease and stroke (Haney *et al.,* 2015). Extracellular vesicles are very tiny particles produced by cells that may likely promote cell-cell signaling by transferring bioactive lipids, proteins and RNA (Morel *et al.,* 2004, Mause and Weber, 2010). The genesis of this vesicle was identified based on the specific pattern of expression of surface antigens (Schorey and Bhatnagar, 2008; Mause and Weber, 2010). EVs released by neuronal cells show success in the therapy of neurodegenerative diseases, inflammatory as well as ischemic diseases (Zhang *et al.,* 2017; McCulloh *et al.,* 2018; Vogel *et al.,* 2018).

An evidence from the previous study shows that there is possibility an EVs to be a potential regenerative agent for therapy since it can evade the problems of straight stem cell transplantation such as tumorigenesis, low and slow survival and problems in differentiation (Baglio *et al.,* 2012). Application of precursor cell for treatment may likely function predominantly via paracrine processes involving EVs (Camussi *et al.,* 2013, Katsuda *et al.,* 2013). SVZ derived extracellular vesicles has many important characteristics possessed different lipid membrane content, prevent the inner components from digestion as well as degradation (Marote *et al.,* 2016; Yang *et al.,* 2017). However, EVs may also enter freely in to cerebrospinal fluid (CSF), which will help to reach the injury area of the spinal cord. All these characteristics are vital for the recovery of motor function following spinal cord injury (Liu *et al.,* 2019). In this research work, it was postulated that direct injection of EVs intrathecally can resolve the problems related with direct stem cell injection and suppress the formation of inflammasome as well promoted motor function recovery following spinal cord injury in rats, The current study shows that injection of EVs intrathecally to SCI animals down regulated gene expression and protein synthesis as shown in fig 1 and 2. This shows that injection of EVs in SCI patients can arrest the secondary damages. This is in conformity with several studies conducted that shows injection of EVs, neuronal cell, MSC or other agents can arrest the formation of inflammasome complex formation and leads to secondary damages. Research conducted by Rong *et al.,* 2019 revealed that NSC-sEVs can successfully decrease neuroinflammation and neuronal apoptosis, thereby enhancing functional recovery in SCI injured rats model (Rong *et al.,* 2019). Yanhui et al., shows that bone mesenchymal stem cell derived extracellular vesicle (BMSC-EV) can successfully hinder the migration of pericytes, and help to keep the blood spinal cord barrier intact after SCI. This also followed by axonal regeneration, reduction in the death of neuronal cell and motor function recovery; they also suggested that EVs might possibly be a good agent for the treatment of SCI (Lu *et al.,* 2019). Mohammadi *et al.,* 2019 shows that intrathecal injection of Wharton's jelly mesenchymal stem cells (WJ-MSCs) into an induced SCI rats, the results shows SCI elevated the mRNA gene and protein synthesis of inflammasome complex but after injection of WJ-MSCs intrathecally to experimental rats, the expression of mRNA as well as protein synthesis level of inflammasome complex decreased drastically (Mohamadi *et al.,* 2019). A study carried out by Zendedel *et al.,* 2015 revealed that SCI overexpressed the level on inflammasomes NLRP3, ASC, caspase-1, TNF-α, IL-1β and IL-18 in rat model. But the level significantly reduced as a result of transplanting of Stromal derived factor-1a (SDF-1a) intrathecally in injured rats and shows a good recovery of motor function in the experimental rats (Zendedel *et al.,* 2016).

Another research conducted by Sahar *et al.,* 2019 revealed that spinal cord injury can over express the inflammasomes, but after intrathecal transplantation SVZ-Derived extracellular vesicles, it down regulated the level of inflammasome complex and brought about neuronal recovery in SCI rats (Ijaz *et al.,* 2019), this is in conformity with the current study. Another study carried by Ibrahim et al., 2020 shows that SCI up regulated the level of inflammasome complex, but after intrathecal injection SVZ-Derived EVs it down regulated the level of inflammasome complex which inhibit the secondary damages and results to neuronal recovery in SCI rats(Mohammed *et al.,* 2020), results of this research is in agreement of current research.

CONCLUSIONS

Results of this study concluded that spinal cord injury can up regulate the level of inflammasome complex, also up regulation of inflammasome may lead to secondary damages and total neuronal damages. From the results of this study it was concluded that inflammasome complex should be the main target for inhibition of secondary injury and neuronal damages. It was also concluded that neuronal stem cell is the good and best therapeutic agent for SCI, intrathecal injection is best method of injection.

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