Therapeutic Effect of Stem Cells on Spinal Cord Injuries in Albino Rats

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ABSTRACT

BACKGROUND: Management of spinal cord injuries represents a major challenge. Spinal cord injury (SCI) is a debilitating event that can cause severe motor and sensory paralysis. Stem cell research has advanced from basic science to clinical practice, generated therapeutic tools, and gained attention as a core of modern medical research. SCI, which is not amenable to cure by conventional treatment, is one of the most active field s of stem cell research.

OBJECTIVES: The work aims to evaluate the effect of mesenchymal stem cells (MSCs) transplantation on an induced spinal cord injury in rat model and to detect the route by which mesenchymal stem cells (MSCs) transplantation produces cord recovery whether intralesional or intravenous.

MATERIAL AND METHODS: A prospective study on 60 adult female albino rats which was performed at Anatomy department, animal house department and clinical chemistry & stem cells research lab at biochemistry department at faculty of medicine, Zagazig University during two-year duration (from January 2015 to January 2017). The rats were randomly assigned and equally divided into three groups: **Group A**: (control group): the rats of this group received spinal cord injury only. **Group B**: (intravenous injected group): the rats of this group were injected with stem cells at the third day following SCI via intra-venous (IV) route. **Group C**: (intralesional injected group): the rats of this group were injected with stem cells at the third day following SCI via intra-lesional (IL) route. Behavioral improvement and the expression of neurotrophic factors of the transplanted groups were analyzed and compared with those of the control group.

RESULTS: At 6 weeks post-injury, the mean BBB motor scales in the control, IL and IV groups were 5.94 ± 0.28, 6.38 ± 0.50, and 9.63 ± 0.50, respectively. Regardless of the delivery route, the MSCs transplantation following spinal cord injuries presented better behavioral improvement. Moreover, the expressions of neuronal growth factor were significantly higher in the IL group and IV group than those in the control.

CONCLUSION: Transplantation of MSCs in the early stage of spinal cord injury gives a significant clinical improvement and higher expressions of neuronal growth factor.

KEY WORDS: spinal cord injury, mesenchymal stem cells

Introduction

Cell transplantation for the regeneration of an injured spinal cord would be one of the promising regenerative trials (**Sasaki et al., 2011**). Cumulative research has demonstrated its feasibility and various stem cells have been tried to protect against the secondary damage and to enhance the regeneration of a damaged spinal cord **(Wright et al., 2012)**. As one trial of this cell therapy, mesenchymal stem cells (MSCs) have been highlighted because they cannot only be easily harvested, expanded and transplanted, but they can also be directly harvested and transplanted, which obviates the ethical and immune rejection problems (**Nandoe et al., 2009**). MSCs are known to have a homing effect and to be neuro-

protective following SCI when they are injected in the early stage of SCI (**Lindvall & Kokaia, 2010**). The suggested neuroprotective effects of MSCs for SCI are that they act as an inductor of neurotrophic factor, a modulator of inflammation. Moreover, they are suggested to be able to replace damaged cells by trans-differentiation (**Vaquero & Zurita, 2013**).

Materials and Methods

Animal Model and group allocation:

This prospective study was carried on 60 adult female albino rats with body weight ranged from 300- 350gm. The rats were housed under standard conditions of light and dark cycles with free access to food and tap water under controlled temperature (28ºC- 30ºC). They were observed in this environment for seven days prior to surgery ensuring adequate adaptation. Animal housing conditions and all experimental procedures were in accordance with national institutes of health guidelines on animal care. Rats were randomly assigned and were equally divided into three groups as follows: Group A: (control group): the animals of this group received injury only. Group B: (intravenous injected group): the animals of this group were injected once at the third day following SCI via intra-venous (IV) injection. Group C: (intralesional injected group): the animals of this group were injected once at the third day following SCI via intra-lesional (IL) injection.

Induction of spinal cord injury:

For induction of spinal cord injury; Rats were anesthetized by intraperitoneal injection of 50 mg/kg pentobarbital with intramuscular injection of 0.8 mg/kg atropine sulfate. Hair was removed from the T8-T9 region of the vertebral column with an electric razor and skin was disinfected with povidone-iodine. Rats were placed prone on the surgical table and a midline skin incision was made over the T8-T9 spinous processes. The spinal cord was exposed by laminectomy at the T8-T9 level, with great care to a void iatrogenic damage. a moderate contusion model injury was then performed by clamping of the spinal cord with vascular clamp for 30 seconds. Following injury, muscle and skin were sutured w ith 3-0 Vicryl. Gentamycin (0.6cc/kg) was injected intramuscularly once a day for 1 week after surgery. Body temperature was maintained constant at 37 °C with a heating pad during surgery and the recovery period. Urine was removed manually by pressurizing the bladder for 7–14 days after the injury until the bladder reflex was recovered.

Preparation of allogenic mesenchymal stem cells

The femoral bone was used to obtain bone marrow. After anesthesia, the femoral bone was harvested and both ends of the femoral bone were cut. Bone marrow was aspirated with an 18-guage needle and then, diluted to 25 mL with Dulbecco' Eagles medium (DMEM) (Sigma, St. Louis, MO, USA) supplemented with 10% heat inactivated fetal bovine serum (FBS) (GibcoBRL, Grand island, NY, USA), 2 mM L-glutamate (Sigma), 100 U/mL penicillin and, 0.1 mg/mL streptomycin (Sigma). The bone marrow aspirates was plated and then incubated in a humidified atmosphere of 5% CO 2 at 37°C. For selecting the MSCs, the non-adherent cells were eliminated by replacing the medium 48 hr after cell seeding.

Behavioral assessment:

The functional recovery of rats was monitored once weekly for successive six weeks by two observers following injury by the use of **Basso, Beattie and Bresnahan (BBB)** motor scale.

Growth factor analysis:

Reverse transcription polumerase chain reaction (RT-PCR) test was used to assess the expression level of neurotrophic factors: brain-derived neurotrophic factor (BDNF) and neuronal growth factor (NGF) in the spinal cord tissue of the 3 study groups as 3 rats were sacrificed from each group at 1,3 and 6 weeks after injury.

Statistical analysis:

The data were managed through the use of statistical package of social sciences (SPSS) version 19 (SPSS Inc., Chicago, Illinois, USA). All the values in the figures and text are expressed as means± standard deviations (SDs). The results were analyzed by one-way ANOVA followed by a Bonferroni post-hoc test for multiple comparisons. A P value less than 0.05 was considered to be statistically significant.

Results:

Behavioral assessment:

All the injured rats manifested complete hindlimb paraplegia immediately after the operation. In all the groups, the rats gradually recovered varying degrees of motor function over the time of observation (**Fig. 1**). At 6 weeks post-injury, the mean BBB motor scales in the control, IL and IV groups were 5.94 ± 0.28, 6.38 ± 0.50, and 9.63 ± 0.50, respectively (**table 1**). Regardless of the delivery route, the MSCs transplantation following spinal cord injuries presented better behavioral improvement.

Growth factor analysis:

BDNF and NGF levels in the spinal cord tissue were measured in the 3 groups at 1,3 and 6 weeks post-injury as demonstrated in (**Figs. 2&3**) and which showed a statistically significant difference ($p< 0.05$) between the intra-lesional group and the control group at 1,3 and 6 weeks post injury for both BDNF and NGF levels. Also, a statistically significant difference ($p< 0.05$) was found between the intravenous group and the control group at 1and 3 weeks post injury for both BDNF and NGF levels. At 6 weeks post-injury, BDNF and NGF levels were higher in the intravenous than the control group but without a statistically significant difference ($p> 0.05$).

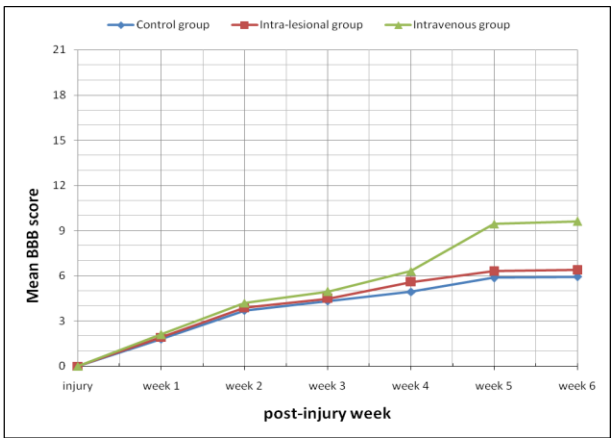

Fig. (1): locomotor assessment using Basso-Beattie-Bresnahan (BBB) scale tested at every week after spinal cord injury.

Table (1): BBB scores of rats in the 3 study groups over 6 weeks after injury.

Group / BBB score	Control group	Intralesional group	Intravenous group
At 1 week post-injury (n=20 rats in each group)			
Mean	1.80	1.93	2.11
Standard deviation	0.22	0.38	0.55
Median	1.87	1.88	2
Range	0.5 (1.5-2)	1 (1.5-2.5)	1.5 (1.5-3)
At 2 weeks post-injury (n= 17		15	15
Mean	3.81	3.82	4.2
Standard deviation	0.23	0.32	0.72
Median	3.75	3.75	3.75
Range	1 (3-4)	1.25 (3-4.25)	2 (3.75-5.75)
At 3 weeks post-injury (n=17		15	15
Mean	4.43	4.46	4.95
Standard deviation	0.43	0.68	0.64
Median	4	4.25	5
Range	1 (4 - 5)	1.75 (4- 5.75)	2 (4-6)
At 4 weeks post-injury (n= 14		10	10
Mean	4.94	5.59	6.31
Standard deviation	0.44	0.76	0.79
Median	5	6	6.5
Range	1 (4.5-5.5)	2 (4.5-6.5)	2 (5-7)
At 5 weeks post-injury (n= 14		10	10
Mean	5.87	6.30	9.44
Standard deviation	0.28	0.44	0.48
Median	5.87	6	9.25
Range	0.75 (5.5-6.25)	1 (6-7)	1.5 (9-10.50)
At 6 weeks post-injury (n= 14		10	10
Mean	5.94	6.38	9.63
Standard deviation	0.28	0.50	0.50
Median	5.88	6	10
Range	0.75 (5.5-6.25)	1 (6-7)	1 (9-10)

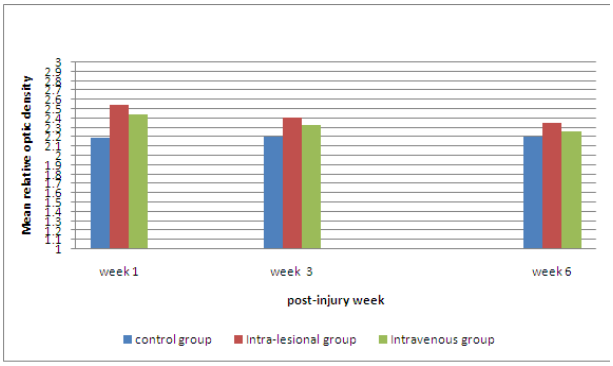

Fig. (2): NGF expression over time at 1, 3 and 6 weeks post-injury.

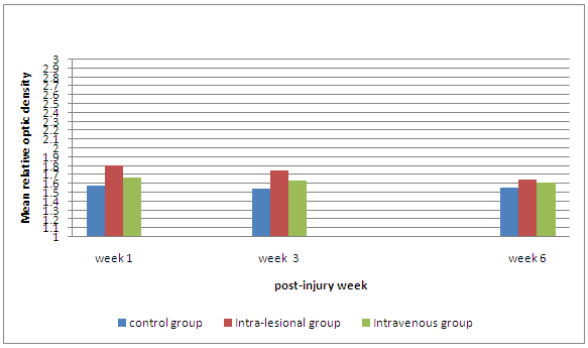

Fig. (3): NGF expression over time at 1, 3 and 6 weeks post-injury.

Discussion:

There is no doubt that cell based therapy, including stem cells, is an attractive and promising therapeutic strategy for many clinical conditions that currently lack efficacious treatment. However, there are many issues and concerns to be addressed before its clinical translation. As one of the efforts to address these topics, we tried different transplantation routes of stem cells for SCI. The efficacy and fate of the transplanted cells were observed according to different transplantation routes (**Takeuchi et al., 2012; Parr et al ., 2013 and Jung et al., 2016**)

Studies on applying MSCs application for various central nervous disorders have demonstrated that transplantation of MSCs alleviated further tissue damage and it yielded significant clinical improvement (**Yoo et al., 2008; Azizi et al., 2009; Nomura et al., 2011; Osaka et al., 2014 and Yoshihara et al., 2015**). These results were explained by the possibilities of a neuroprotective function and a tissue repair by the transplanted cells. However, these studies did not present the differences depending on the transplantation route.

A few studies that focused on comparing the efficacy following MSCs transplantation for SCI demonstrated more efficient engrafting of transplanted cells into lesion site when grafting by the intralesional or lumbar puncture routes (**Swanger et al., 2009; Paul et al., 2012 and de Haro et al., 2015**). These studies demonstrated this difference of efficacy only through examining the engrafting MSCs volume as counted by histological or radioisotope labeling examination. Clinical assessment or the differentiation of transplanted cells has not been addressed.

As suggested in many studies (**Nomura et al., 2011; Osaka et al., 2014 and Yoshihara et al., 2015)** intravenous delivery has inherent concerns of its efficacy. Although the IV route has the advantages of easy and safe delivery, trapping of the transplanted cells in the other organs and the high chance of exposure to an immune reaction limits its clinical utility. In the present study, as predicted and suggested by other previous studies, IV delivery showed more effective clinical improvement as compared to that of the control group and the IL group.

Therefore, IV delivery could be an effective delivery route for early MSCs transplantation following SCI. Homing of the MSCs to the disrupted blood-spinal cord barrier tissue and avoidance of additional injury that can be

caused by intralesional delivery could account for these results.

In agreement with numerous previous studies (**Song & Tuan, 2010; Kamada et al., 2011; Neuhuber et al., 2013 and Imitola et al., 2015**) our results showed that the degree of behavioral improvement was better in the IV group. This phenomenon suggests that the neuroprotective effects of early transplanted MSCs do not merely depend on the absolute number of the engrafted cells.

Although it is controversial, some studies have suggested the possibility of replacement of damaged tissue by the transplanted MSCs (**Krampera et al., 2010 and Ha & Kim, 2014**). However, the secretion of neurotrophic factors (BDNF, NGF), the modulation of inflammation and immune reactions and enhancement of axonal sprouting in the pathologic condition following SCI have been recently suggested as the primary effects of MSCs transplantation at the early stage, which is beyond their potential to differentiate to form glial and neural lineage cells (**Lee et al., 2014 and de Haro et al., 2015**).

In terms of the neurotrophic factors expression, the IL group showed higher BDNF and NGF expression compared to those in the control and IV groups. As suggested by the previous studies (**Jeong et al., 2010; Kamada et al., 2011; Neuhuber et al., 2013; Osaka et al., 2014 and Imitola et al., 2015)** ,this might be related to the absolute number of the engrafted MSCs. However, in this study, the clinical improvement was not correlated to the absolute number of the engrafted MSCs,and the expression of BDNF and NGF. These findings might be also related to the additional injury during the transplantation in the intralesional injections.

Conclusion:

In conclusion and according to our results, we suggest that early delivery of allogenic MSCs following SCI provided favorable behavioral improvement and higher expression of neuronal growth factors compared to the control group.

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التأثير العلاجى للخلايا الجذعية على اصابات الحبل الشوكى فى الفئران البيضاء

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المقدمة: تم التعرف على الخلايا الجذعية منذ أكثر من مائة و عشرون عاما على إنها من ضمن الخلايا المنتجة للدم السائدة فى نخاع العظام. هذه الخلايا تمد الجسم بخلايا الدم الحمراء و البيضاء الجديدة عند الإصابة بالأمراض الحادة و المزمنة و هذة الخلايا تعيش لأيام أو أسابيع قليلة و يجب أن يحل مكانها سلسلة من الخلايا الأسلاف فى نخاع العظم, وعلى الرغم من ذلك فهناك ايضا مجموعة من الخلايا الجذعية فى نخاع العظم التى توفر خلايا أخرى غير خلايا الدم و يطلق عليها الخلايا الجذعية المتوسطية, ولهذه الخلايا صفة جذابة و هى القدرة على التميز إلى عدد من أنواع الخلايا الناضجة شاملة الخلايا الليفية، الخلايا الليفيعضلية، الخلايا العظمية ،بعض الخلايا العصبية ، الخلايا الغضروفية ، الخلايا الدهنية،الخلايا العضلية والخلايا الطلائية، وأيضا لديها القدرة على التجدد التلقائي. وقد تم فيما بعد الحصول على هذة الخلايا الجذعية و زراعتها من الفئران والأرانب والبشرو جميعها لها نفس الخصائص البيولوجية المتشابهة.

الهدف من الدراسة:

- تهدف هذة الدراسة الى تقييم تأثير زرع الخلايا الجذعية المتوسطية على نموذج إصابة الحبل الشوكي المستحث فى الفئران البيضاءز

الطرق و ادوات البحث:

مكان البحث: معمل الخلايا الجذعية وقسم الكيمياء الحيوية وقسم التشريح وبيت الحيوان بكلية الطب- جامعة الزقازيق.

العينة و طريقة الاختبار: فى هذا البحث تم استخدام ستين من اناث الفئران البالغة يزن الواحد منها 300جرام تقريبا و تم تقسيمهم عشوائيا الي ثلاثة مجكوعات متساويه تحتوي كل مجوعة علي عشرين فأرا وكانت المجمزعات كالاتي: المجموعة (أ : المجموعة الضابطة):تم احداث اصابة بالحبل الشوكي ولم تتلقي علاجا بالخلايا الجذعية ، المجموعة (ب): وهذه المجموعة قد تلقت الخلايا الجذعية بواسطة الحقن المباشر في مكان الاصابة بالحبل الشوكي اما المجموعة (ج) فقد تلقت الخلايا الجذعية بواسسة الحقن في وريد الذيل. تم متابعة الأداء الوظيفي الحركي للفئران بالثلاثة مجموعات مرة اسبوعيا ولمدة ستة اسابيع مابعد الإصابة. كذلك تم ذبح عدد ثلاثة فئران من كل مجموعة بعد اسبوع وثلاثة اسابيع وستة اسابيع بعد الإصابة وذلك لقياس مستوي التعبير الجيني للعوامل المحفزه للانسجة العصبية في نسيج الحبل الشوكي لدراسة مدي تأثير الخلايا الجذعية علي مستوي تلك العوامل.

النتائج:

بعد جمع البيانات تم تحليلها بالكمبيوتر باستخدام برامج الاحصاء واستعمال الطرق الاحصائية المناسبة اللازمة وعرضها فى جداول واشكال بيانية تبين ان استخدام الخلايا الجذعية عبر الحقن المباشر في مكان الاصابة او عن طريق الوريد الذيلي كان مصحوبا بمعدل اعلي في مستوي التحسن الحركي للفئران خلال فترة مابعد الإصابة حيث وجدت فروق ذات دلالة احصائية مابين المجموعتين (ب) و (ج) بالمقارنة بالمجموعة الضابطة (أ) وكان معدل التحسن الحركي اكثر ارتفاعا مع المجموعة (ج). كذلك بينت الدراسة وجود فروق ذات دلالة احصائية بين مستوي التمثيل الجيني للعوامل المحفزة للنسيج العصبي في كلتي المجموعتين (ب) و (ج) بالمقارنة بالمجموعة الضابطة.

الخلاصة: وفقا لنتائج هذه الدراسه نجد ان استخدام الخلايا الجذعية مبكرا بعد اصابة الحبل الشوكي قد يساعد في استعادة بعض وظائف الحبل الشوكي مما يستلزم دراسات مستقبلية تشمل عينات اكبر عددا وفصائل متعددة للوصول لأفضل توقيت وطريقة لإستخدام الخلايا الجذعية في علاج اصابات الحبل الشوكي.