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Research Article

Musculoskeletal Trauma Treated With Bone Marrow Mesenchymal Stem Cells in Wistar Albino Rat

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Abstract: Stem cell research could lead to a new bone repair therapy in musculoskeletal trauma (MST). Regenerative medicine attempts to restore living tissue, which has been lost or damaged. To generate bone cells for regenerative medicine remains a significant challenge. Bone marrow mesenchymal stem cells (BMSCs) harvested from wistar albino rat was transplanted into traumatized site. It is highly interdisciplinary field, which involves isolation, characterization, differentiation and transplantation of bone marrow mesenchymal stem cells with CT and Histopathology observations. In this study 9 male and 9 female rat with age above one year and weight between 150 to 250 grams were required for the study. The animals were divided in to 3 groups (6 each.) group-A -MST Control, group-B -MST treated with Stem cells, group-C -MST treated with Stem cells and PLGA scaffold. CT was taken on 3rd, 10th & 30th day to confirm the progress of healing. When compared the control A with group C there is a statistical significance in callus formation in 3rd day($p<.01$), 10th day($p<.01$), and 30th day($p<.01$). At the end of 30th day the histopathology observation confirms with computer tomography observations. Autologous intravenous/bone marrow mesenchymal stem cell therapy favours' faster and complete healing in musculoskeletal injuries.
Keywords: Musculoskeletal trauma (MST), Bone marrow mesenchymal stem cells (BMSCs), Fluorescence activated cell sorting (FACS), Poly lactic co-glycolic acid (PLGA).

INTRODUCTION

Bone marrow mesenchymal stem cells (BMSCs) play a major role in tissue repair. Stem cell research could lead to a new bone therapy and replaces conventional methods. BMSCs have the capacity to differentiate into a variety of connective tissue cells: Bone, Cartilage, Tendon, Muscle, Adipose tissue and Nerve this differentiation potential makes BMSCs an excellent candidate for cell based tissue engineering. The proliferation and differentiation of BMSCs into skeletal cells can aid the tissue engineering process. This study aims at autologous transplantation of (BMSCs) bone marrow mesenchymal stem cell with polylactic-co-glycolic acid (PLGA) scaffold will help in effective and faster healing of tissues without immune reaction [1]. It is an advanced technique that scores over the other conventional methods of fracture treatment. By use of BMSCs, synthetic materials like metal rods, plate's composites, internal and external distracters can be avoided. Synthetic recombinant bone morphogenetic proteins (rh-BMP) are advanced, yet expensive. Autologous bone grafts in large defects because donor site morbidity, can be replaced by BMSC therapy. Regenerative medicine is an effective alternate treatment for musculoskeletal crush and gunshot injuries, which are very common in defence services [2]. BSMCs therapy can replace the existing conventional treatments because of immune previledge nature and minimal hospital stay. Since, healing is delayed in old age; it serves as an alternative method. The injured, matured MSK cells may play a critical role in the differentiation of transplanted mesenchymal cells to regenerate, the tissue of demand i.e. Bone and Muscle. Matured MSK cells in the traumatized area and extra cellular environment may play a role in determining transplanted BMSCs fate. Tissue injury induces the BMSCs differentiation and understanding the mechanism is essential [3].

MATERIALS AND METHODS

Randomly breed albino rat of both sexes (150-200gms) are maintained at Biomedical Research Unit and Laboratory Animal Centre (BRULAC) of Saveetha University were used for the study. Rats were kept in

polypropylene cages (3 per cage) with sterilized and dry paddy husk as bedding material. The animals were fed with commercial laboratory animal feed (TANUVAS-Chennai) and purified water *ad libitum*. The care and maintenance of the animals were as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA, India). This study has the approval of institutional animal ethical committee (SU/SMC/RD/10/2012; DT: 21ST Feb 2012).The animal was anaesthetised under xylozine 10mg/kg and ketamine 80mg/kg cocktail. The procedure is done under the laminar flow. Femur and tibia was removed and muscles connective tissues were cleaned .The cut bones were rinsed in the phosphate buffer solution. The cells were retrieved and passaged. From the first passage to second passage, cells take longer time to adapt in-vitro condition. Third to sixth passage, duration is shorter which indicates cells adaptation to new environment. The 6^{th} passage cells were analysed by flow cytometry for the expression of CD 44, CD90, CD29 positivity and CD45 negative expression. All these are specific markers for BMSCS. The 6^{th} passage cells were transplanted to muscular skeletal traumatised site.

Musculoskeletal truma treated with BMSCs

For musculo skeletal trauma (MST) study 9 male and 9 female rats with age above one year and weight between 150 to 250 grams were used. The animals were divided in to 3 groups, 6 each. All rats maintained in same environment and management condition. Group A – MST control, Group B –MST treated with Stem cells, Group C – MST treated with stem cells and PLGA scaffold. The animals were anaesthetized under xylozine 10 mgs /kg ketamine 80mgs/kg cocktail. Anaesthetized animal was placed in a sterile surgical table. The surgical area is right lower limb, was shaved with a sterile razer and disinfected with betadine solution. The right lower was exposed in the dorsal aspect, and then 2 to 3 inches longitudinal incision was made over the middle of the thigh. Later, a crush injury was created by dropping iron rod weight of 20 gms dropped from the height of 12.5mm. After the injury BMSCS were loaded in microliter syringe at a concentration of 1 x 10^5 cells /µl Injected in to the injured site with all aseptic precautions. In group A only saline injected, group B only stem cells and in group C stem cell with PLGA scaffold injected in to the site of injury. The surgical wound was dressed with betadine; immobilization of fracture was done by plaster of Paris externally. The limb movements were monitored on day to day basis. The rate of recovery was observed in all 3 groups by computerized tomography (CT) and Histopathology. CT was taken on 3^{rd}, 10th & 30th day to confirm the progress of healing. At the end of 30^{th} day the animals were sacrificed and bone and muscle were taken for histopathology observation.

RESULTS

Characterization of BMSCs by FACS analysis reveals stem cell positive for CD90, CD29, and CD44 and negative for CD45.

Table 1: FACS Analysis

Antibody	Status	P1 Expression	P2 Expression
CD 90	OK	NIL	99.9%
CD29	OK	NIL	44%
CD44	OK	NIL	100%
CD45	OK	90.6%	0.3%

The 6^{th} passage cells on flow cytometry exhibits Positive expression (P2) for CD90, CD29, CD44 and negativity expression (P1) for CD 45 labelled BMSC specific markers.

Table 2: CT results of musculoskeletal trauma treated with BMSCs

Sl. No.	Experiment	CT 3^{rd} day	CT 10^{th} day	CT 30^{th} day
1	MST control (Group – A)	MST confirmed	Negligible callus seen	Callus seen moderately in Axial, Coronal & 3D view
2	MST treated with stem cells (Group – B)	MST confirmed with mild callus around the fracture site	Callus seen moderately in Axial, Coronal & 3D view	Marked callus (firm in consistency, bony mass) and in some, bony union were also seen in Axial, Coronal& 3D view
3	MST treated with stem cells & scaffold (Group – C)	MST confirmed and multiple callus seen around the fracture site	Marked callus seen in the fracture site	Marked union of bone formation seen in all planes and Bone Remodelling were observed.

In Group C multiple callus formation seen on 3^{rd} day itself, around the fracture site and 30^{th} day of CT Marked bone union observed.

Table 3: Comparisons of group B with group A

			GROUP		Total
			Group B	Group A	
30th day	Mild	Count	0	1	1
		% within group	0%	16.7%	8.3%
	Moderate	Count	2	5	7
		% within group	33.3%	83.3%	58.3%
	Marked	Count	4	0	4
		% within group	66.7%	0%	33.3%
Total		Count	6	6	12
		% within group	100.0%	100.0%	100.0%

When compared the control A with group B there is a statistical significance in callus formation in 3rd day ($p<0.01$), 10th day ($p<0.01$), and 30th day ($p<0.05$)

Table 4: Comparisons of group C with group A

			GROUP		Total
			Group C	Group A	
30th day	Mild	Count	0	1	1
		% within group	0%	16.7%	8.3%
	Moderate	Count	1	5	6
		% within group	16.7%	83.3%	50.0%
	Marked	Count	4	0	5
		% within group	83.3%	0%	41.7%
Total		Count	6	6	12
		% within group	100.0%	100.0%	100.0%

When compared the control A with group C there is a statistical significance in callus formation in 3rd day ($p<0.01$), 10th day ($p<0.01$), and 30th day ($p<0.01$)

Table 5: Comparisons between group B and group C

			GROUP		Total
			Group B	Group C	
30th day	Moderate	Count	2	1	3
		% within group	33.3%	16.7%	25.0%
	Marked	Count	4	5	9
		% within group	66.7%	83.3%	75.0%
Total		Count	6	6	12
		% within group	100.0%	100.0%	100.0%

Statistical analyses between group B and Group C, there is no statistical significance in callus formation in 3rd, 10th and 30th day respectively. In 10th day Group C, marked callus was seen (66.7%) compared to Group B(33.3%).On 30th day in Group C, marked callus was seen ie.83.3% when, compared to group B (66.7%).

Table 6: Histological findings of musculoskeletal trauma in Wistar Albino rat

Sl. No.	Experiment	Bone	Muscle
1	MST control (Group – A)	Degeneration of cortical surface &Decreased Cellularity in Marrow cavity	Degeneration & Necrosis of muscle fibbers'
2	MST treated with stem cells (Group-B)	Trabeculae laided with Osteocyte&Osteoblast with Basophilic Cytoplasm	Muscle cell swollen With Central Nucleus and AmphiphilicCytoplasm.
3	MST treated with stem cells &Scaffold (Group-C)	Differentiated Trabecullae with Matured Osteocyte, Osteoblast &Basophilic Cytoplasm	Muscle cell size smaller with peripheral nuclei. Cytoplasm slowly changes from basophilic to Amphiphilic and to Eosinophilic.

New bone formation with trabeculae laided osteocyte and myocytes with Eosinophilic cytoplasm were seen in Group C.

DISCUSSION

BMSCs have the potential to differentiate into different lineages including bone, cartilage, tendon, muscle and neuron [4-6]. This differentiation potential makes BMSCs an excellent candidate for cell based tissue engineering [6].

6 weeks old rat used for isolation of BMSCs as suggested [7]. Saravana Kumar Sampath Kumar [7] showed that FACS analysis 99.9% of cells were positive for CD90, CD44 (100%) and 90.6% were negative for CD45.

Effect of BMSCs in the trauma has been studied by dividing the animals into 3 groups. MST treated with stem cell group B, is compared with the group C, stem cells treated with scaffold. Perumal Saraswathi *et al*. [8] in 2010 stated that at the end of 30^{th} day CT group C, callus is marked than group B. The union by callus and bone formation was marked in group C. Group B & Group C is compared with MST control group. In adults stem cells and progenitor cells act as a repair system for the body replenishing adult tissues. There was no significant difference between group B & group C in CT. But there is a marked difference between control and experimental group B & C in CT. The MST control group was used to study the recovery of the group B & C. The injured MSK cells would play a critical role in differentiation of transplanted BMSCs to regenerate the tissue of demand i.e. Bone, Muscle, Nerve & Blood vessels. In MST control group after 30 days only callus formation seen clearly. Whereas in group B, callus formation seen on 10^{th} day itself and group C, multiple callus seen on 3^{rd} day itself. Within the experimental group B & C there is no significant difference in the callus formation in the 3^{rd}, 10th and 30^{th} day as shown in statistical analysis. When compare the control with group B there is statistical significant in callus formation in 3^{rd} day ($p<0.01$), 10^{th} day ($p<0.01$), and 30^{th} day ($p<.05$), and with group C there is a statistical significance in callus formation in 3^{rd} day ($p<0.01$), 10^{th} day ($p<0.01$), and 30^{th} day ($p<0.01$)

Chai C and Leong KW reported that the biomaterials approach to expand and direct differentiation of stem cells [9]. Therefore the force generated intrinsically within the MSK cells in response to injury and extra cellular environment may play a role in determining the BMSCs fate [10]. There is no significant difference between groups B&C this could be probably due to the repair and replenishing property of BMSCs. The difference in callus formation between groups B & C is due to Bio-scaffold. In order to repair quickly and effectively, PLGA had been seeded with cells at the site of injury**.** In MST control group, microscopically in bone section, marrow filled with fatty tissue, muscle degeneration with hematoma observed CT findings were well correlated with the histopathology findings.

Satoru Morikawa *et al*. [11] stated that new bone formation with trabecullae laided osteosite in bone. No fibrosis seen in muscle was observed in group B & C. Microscopically much difference were not seen in group B&C, this may be due to restricted resolution of light microscope. There would be a difference intra cytoplasmically in electron microscope (EM) where the resolution is high.

CONCLUSION

Autologous intravenous/bone marrow mesenchymal stem cell therapy favours' faster and complete healing in musculoskeletal injuries, which is common during training and operations of Army, Navy and Air force staff. This is also beneficial to civilians in road accidents and old age osteoarthritis.

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