



Potential Healing Effect of Topical Stem Cell Transplantation and Methandrostenolone on Induced Cutaneous Wounds in Dogs

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Received: June 24, 2015 / **Accepted:** November 24, 2015

Abstract: This study was done to explore the influence of stem cells on cutaneous wounds in comparison with treatment with methandrostenolone drug. Cutaneous wound was induced on the back of each dog using disposable dermal biopsy punches. Twenty five dogs were divided into five groups: 1st group (T1) was control. 2nd group (T2) was induced without any treatment. 3rd group (T3) was induced wound and treated with phosphate buffer saline, 4th group (T4) was induced and treated with 5mg/kg/B.W. of Methandrostenolone, 5th group (T5) was induced and treated with stem cells 1×10^6 cell/250 μ L of phosphate buffer saline. After 7 and 15 days post treatment level of Hb and RBCs count in T2 and T3 group were decline as compared with control group. The WBCs, Alkaline phosphatase, acid phosphatase in T2 and T3 showed enhance as contrast with T1. After 7 days, Hb and RBCs in T4 and T5 showed important increase as compared with T2. Moreover the WBCs, alkaline phosphatase, acid phosphatase and total protein levels significantly elevated in the T4 and T5 as compared with T1. After 15 days of treatment the Hb, RBCs, levels of Alkaline phosphatase, acid phosphatase and total protein levels in T4 showed significant increase as compared with T1, The Hb, RBCs, levels of Alkaline phosphatase, acid phosphatase and total protein in T5 showed no significant changes as compared with control group (T1). The histopathological section in (T2) explained less collagen and more inflammatory cells, the section of wound tissue in (T4) appeared incomplete healing wound tissue and presence little inflammatory cells, the histopathological section of wound treated in (T5) showed return the wound to normal tissue. From this result it can be concluded that the MSCs have ability to access healing of the wound in comparison with methandrostenolone without any side effect.

Keywords: Stem cells transplantation, methandrostenolone, cutaneous wounds, dogs.

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Introduction

Healthy skin taking from the pond of adults stem cells such as of epidermal

coat stem cells and bone marrow stem cells, for its continuance (1). Consequent skin wound, intricate sequence of trials progress, concerning

homeostasis, inflammation, cellular propagation, angiogenesis and extracellular matrix construction. Cytokines, chemokines and growth factors, created as an inflammatory cells, skin potency cells, fibroblasts (2). The pathological circumstances, examples diabetes mellitus, chronic steroid treatment or immune inhibition, with next big wounds or flames, injury healing is late or not fulfilled (3). Stem cells transplantation in animal have confirmed the therapeutic effects of more fast injury therapeutic and enhanced skin renewal (4).

Methandrostenolone is one of the mainly trendy anabolic steroids and presents as enhance drugs (anabolic-androgenic-steroids) and synthetic testosterone analogs, this steroid is an extremely anabolic and androgenic drugs to supply small pro-gestational action. It has been profound that the effect on protein metabolism also connected with development of mood, self-esteem, appetite, and normalize effect on all functions of the body by enhancement calcium and potassium levels and contribution support in the discharge of insulin as well show of feeler alerts, it is also connected with glycogenolysis, nitrogen storage, protein production, muscle mass, muscle functions, and body force gains that are almost enduring, even-though methandrostenolone have suitable therapeutic uses, nontherapeutic abuse also occurs. Non therapeutic users often get anabolic-androgenic steroids information and harvest from a variety of doubtful sources and make questionability health decision (5).

The reason of this study was exploring the effects of intra-dermal stem cells

transplantation and methandrostenolone for injury healing and skin renewal in dogs.

Materials and Methods

Dogs Cutaneous Wound

The males local breed dogs aged one year were utilized for this trial. After the night fasting, the trial dogs were pre-anesthetic medicine consist of atropine sulfate (0.02 mg/kg of B.W.) subcutaneously. Under general anaesthesia using intramuscular xylazine HCl (1mg/kg of B.W.), followed by ketamine (5mg/kg of B.W.), thickness spherical injuries of 7 millimeter in distance were formed on the back of each dog use disposable dermal biopsy punches, the canine cutaneous wound model according to Kim, *et al.*(6).

Bone Marrow Aspiration

It is approved from the femoral bone of all animals in fifth group only, depending on Wellman and Radin (7), specific spine withdraw of bone marrow by sterile syringe contain heparin.

Harvesting of MSCs

Separation and sanitization of MSCs is approved depending on Dodson (8). Bone marrow specimens are centrifuge (8°C) going on eight-thousand rpm at five minutes, the lipid plus blood coats were useless, with cellular's pellet recharged at five ml of mesenculti® (Iscovics Modified Dulbecco's Medium (IMDM), Invitrogen-USA), in addition, ten percentages of fetal bovine serum (FBS). Marrow cells are laminate

culture container in twenty ml of IMDM having ten percentages of FBS and one percentages Penicillin / Streptomycin (100 U/mL). Additional cells were frozen in medium having ten percentages Dimethyl-sulfoxide (DMSO). Non-attach cells were detached following 72 hours of culture with alter of medium. Then, half of the media is changed two times a week; Following 2-3 weeks, cultured cells are dividing by 0.05% trypsin, and Ethylene diamine tetra acetic acid (EDTA), bathing via centrifugation, and extended to three flasks. After that it is reaching confluences (2-3 weeks), cells are isolating via 0.05% trypsin, EDTA and tested in favor of MSCs purpose in vitro. Frozen marrow cells are defrosted and bathing in IMDM, and care for the similar way as fresh marrow cells (8, 9).

Cytometry Investigation of Dog MSCs

MSCs canine in the three ways were immunologically tested for surface markers of MSCs. the MSCs were first detach as of the petri-dishes with trypsin plus EDTA after that centrifuged. Three hundred thousand cells are staining through every respective antibody. Rat monoclonal anti-canine CD90 (AbDseroitic, Kidlington, UK) with rabbit anti-rat FITC secondary antibody and monoclonal anti-canine CD44 conjugate with allophycocyanine (APC) (Minneapolis, USA) were used as MSCs positive markers. Fluorescently-labeled MSCs were finally washed once, fixed with 1% paraformaldehyde in PBS and stored in the dark at 4°C until analysis. In smallest amount twenty thousand of MSCs are utilized to test the incidence of every cell surface

marker, utilizing run of cytometry (Biosciences, Franklin Lakes, USA) (10).

Experimental Design

One day after wound creation, twenty five males local breed dogs were divided into five groups and the period of treatment was 15 days.

-First group (T1) negative control group (without induction wound and any treatment).

-Second group (T2) induced wound without any treatment.

-Third group (T3) induced wound and treated with phosphate buffer saline (PBS) weekly.

-Fourth group (T4) induced wound and injected 5mg/kg of Methandrostenolone drug subcutaneously giving once every three days, the injection was made ventral to the incision.

-Fifth group (T5) induced wound and treated with stem cells 1×10^6 MSCs/250 μ L of PBS administrated weekly.

-Third and fifth groups were injected intradermal into injures (100 μ L for direct injection into the injury bed and 200 μ L for injections spaced in a radial pattern around the injury edging).

Hematologic Parameters

Blood samples were collection from cephalous vein in EDTA tubes for the evaluation of diverse hematological factors on specific days of post-healing for RBCs counts, WBCs count, and Hb according to Coles (11).

Biochemical Test

The blood specimens were collected from all experimental the animals at 7

and 15 days. For estimation of different biochemical factors which includes serum total protein levels, alkaline phosphatase levels, acid phosphatase levels by using commercial kits (12,13,14).

Histological Study

Biopsy sample was taking the injury site from each animal by harvesting the skin part into 10% formalin and processes procedure by routine regularly in histokinette. Masson's trichrome stain mark was used for the expression of collagen fiber in segments (15,16).

Statistical Analysis

Analysis of variances (ANOVA) one way and least significant differences (LSD) ($p < 0.05$) has been used to contrast the data of different groups throughout the experiment (17).

Results and Discussion

The utilizes of mesenchymal culture medium mesencult[®] with fetal bovine serum provided best time (seven days) to get great amount of cells, also some cells were floating and further cells begin to attach in container wall with time. The MSCs propagated of the culture media in the lasting of week, they appearance cluster of attach cells, and extended, attached with spare, also, they appeared fibroblast and spindle, look separated of dog bone marrow. The attachments of MSCs begin to reproduce in three days after subculturing in mesenicult[®]. The cluster of spindles looks the cells were as encircled by the same cells, which offered form of more thickly cellular

masses. These cells attached to the culture dish via twenty-four hours of culture. Next to way three, assumed MSCs were cultured and examined for expression of MSCs surface markers and inhabitants doubling time. These MSCs were spoken mesenchymal stem cells markers (CD 44 and CD 90) was positive marker in favor of it. Gross result was evidenced at specific days after therapeutic. Group T4 proved injury liquid on 7th day while the injury liquid was clear decrease in T5 which well adhered to the wound surface. Complete access healing was observed within 15 days in T5 group whereas T4group took slowly and incomplete healing in15 days. Canine mesenchymal stem cells was separated from dog's bone marrow, these specific cells were illustrated as multi-powerful stem cells because they have the ability of discrimination into mesodermal line, the results was agreement with (18,19). The separation, recognition and distillation of dog MSCs newly happen to an vital subject for the medical utilize of MSCs (19).The specific of canine surface antigen expressed mesenchymal stem cells markers (CD 44 and CD 90). This fact is single of the hallmarks touching the utilization of MSCs for medical utilize and given that MSCs are placed in the bone marrow, the majority researchers have separated MSC utilizing their ability to attach artificial culture dish, the MSCs can trans discrimination *invivo* to broad multi-powerful with discrimination into a number of cells lines, including osteocytic, chondrocytic, and keratinocyte lineage (19, 20). Complete access healing was observed within 15 days in T5 group while T4 group took slowly and incomplete healing in15

days this may be attributed to the MSCs enhance the fibroblast proliferation which can be obtained as a creation of fresh blood vessels which is essential to continue granulation tissue (21,22), in addition to MSCs promote re-epithelialization, and quicker epithelialization, follow bone marrow derivative stem cell transplantation for cutaneous wound therapeutic, have been confirmed in previous researches in other species when they mention the functions of MSCs can comprise help of keratinocyte passage with propagation (23,24, 25).

The results after 7 and 15 days of treatment of Hb and RBCs in T2 and T3 groups explained significant decline ($P<0.05$) as compared with T1, in otherwise the WBCs, Alkaline phosphatase, Acid phosphatase in T2 and T3 groups showed significant raise ($P<0.05$) as contrast with T1 (table 1A,B).

Decrease of Hb and RBCs level in T2 and T3 groups may be regarded to the induced wound lead to loss of blood in otherwise the enhance of WBCs, alkaline phosphatase, acid phosphatase and total protein in T2 and T3 groups may be revealed to damage tissues lead to increase fibroplastic activity and cellular infiltration activity of collagen (26,27). In addition to reduced phagocytosis, the alkaline phosphatase played an essential responsibility in forming alkaline surroundings at injury place which is necessary for fibroplastic action toward produce collagen while acid phosphatase was caused acidic surroundings to organize over produce of collagen with initiated the development remodel of collagen (28).

After 7 days Hb and RBCs in T4 and T5 groups showed important increase ($P<0.05$) as compared with T2 while the WBCs, Alkaline phosphatase, Acid phosphatase and total protein significantly elevated ($P<0.05$) in the T4 and T5 groups as compared with T1 groups. (Table 1A). After 15 days of therapeutic the Hb, RBCs, levels of Alkaline phosphatase, Acid phosphatase and total protein in T4 group showed significant raise ($P<0.05$) as compared with T1 group. In otherwise the Hb, RBCs, levels of Alkaline phosphatase, Acid phosphatase and total protein in T5 groups return to normal levels as compared with T1 group (table 1B).

The results of methandrostolone (androgenic anabolic steroid) can be attributed to increase the concentration of erythropoietin hormone which stimulate bone marrow to increase production of RBCs (29, 30), in addition, it is well known to induce inflammatory responses, which lead to increases in WBCs count (31). The increase in total protein levels may be attributed to methandrostolone are raising of male hormone which leads to stimulate muscle cells to maintain a high concentration of nitrogen which would create the cell to retain a larger amount of protein that works to build muscle mass and large this steroid hormone is one of the derivatives industry of testosterone this results agree with the study conducted by Moussa and Bashandy (32). While the T5 group revealed no significant difference as compared with T1 group after 15 days of treatment this result can be attributed to the MSCs have the ability of transdifferentiation *in vivo* to extensive multipotency with

differentiation into a number of cell lineages, including osteocytic, chondrocytic, and keratinocyte lineage (19, 20). Therefore the MSCs treated injuries preserve favorable for progressing the quality of the cured injures; as a result, MSCs appeared to influence the degree of wound grown,

in addition to prevent of extreme injury reduction or hypertrophic scar formation is vital for maintain regular action of the skin, also the capability of MSCs to normalize collagen construction lead to heal large skin wounds (28).

Table (1): Effect of stem cells, methandrostenolone, and PBS on the hematological and biochemical parameters levels in all groups:

A-After 7 Days from Therapeutic

Parameters Groups	RBCs count $\times 10^6/\mu\text{l}$	Hb g/dl	WBCs count $\times 10^3/\mu\text{l}$	Alkaline phosphatase (I.U)	Acid phosphatase (I.U)	Total protein (gm/dl)
T1	6.54 \pm 0.30a	13.2 \pm 0.20a	14 \pm 0.23a	20.20 \pm 0.11 a	5.32 \pm 0.12a	6.7 \pm 0.22a
T2	4.2 \pm 0.11b	10.0 \pm 0.25b	17 \pm 0.26b	33.15 \pm 0.20b	7.20 \pm 0.40 b	4.22 \pm 0.26b
T3	4.4 \pm 0.31b	11.2 \pm 0.24b	16 \pm 0.36b	34.20 \pm 0.26b	7.32 \pm 0.54b	5.51 \pm 0.22b
T4	8.2 \pm 0.38c	15.57 \pm 0.14c	19.8 \pm 0.12c	30.20 \pm 0.45c	8.30 \pm 0.25c	9.20 \pm 0.30c
T5	8.8 \pm 0.44c	15.8 \pm 0.10c	20.4 \pm 0.12c	31.12 \pm 0.23c	8.10 \pm 0.11c	8.30 \pm 0.11c

B-After 15 Days of Therapeutic

Parameters Groups	RBCs count $\times 10^6/\mu\text{l}$	Hbg/dl	WBCs count $\times 10^3/\mu\text{l}$	Alkaline phosphatase (I.U)	Acid phosphatase (I.U)	Total protein (gm/dl)
T1	6.56 \pm 0.37a	13.5 \pm 0.27a	14 \pm 0.21a	20.24 \pm 0.12a	5.36 \pm 0.13a	6.6 \pm 0.24a
T2	4.6 \pm 0.40 b	11.3 \pm 0.20 b	18.4 \pm 0.15 b	39.15 \pm 0.23b	9.11 \pm 0.40b	4.02 \pm 0.26b
T3	4.8 \pm 0.43 b	11.6 \pm 0.13 b	17.5 \pm 0.22 b	38.10 \pm 0.26b	8.40 \pm 0.54b	5.22 \pm 0.22b
T4	8.0 \pm 0.50 c	15.41 \pm 0.10 c	16.0 \pm 0.14 b	28.20 \pm 0.45c	6.72 \pm 0.25c	10.11 \pm 0.30c
T5	6.6 \pm 0.31a	13.15 \pm 0.15 a	13.8 \pm 0.12 a	20.15 \pm 0.23a	5.25 \pm 0.11a	6.80 \pm 0.11a

*mean \pm SE, No. of animals =5/ group *Dissimilar small letters mean significant diverse (p <0.05) between column numbers

The results of histopathological alters after 15 days of treatment the histopathological section of induced wound without any treatment explained less collagen and more inflammatory cells and odema as in figure (1), this result can be attributed to the injury therapeutic is a compound and active procedure of return cellular construction and tissue coats in injured tissue this results agreed with results supported by Gallicchio, *et al.*, (29), while the section of wound tissue after treatment with Methandrostenolone subcutaneously appeared incomplete healing wound tissue, presence little inflammatory cells, fibroblast and high collagen as in figure (2), the side effect of

methandrostenolone in delayed healing of wound, this result can be regarded to the last stage of injury therapeutic, the injury suffers reduction resultant in a lesser quantity of obvious scar tissue this results agreed with results recorded by Midwood *et al.*(33) in otherwise the histopathological section of wound treated with MSCs showed return the wound to normal tissue and disappear inflammatory cell as in figure (3). The MSCs may be returned to that MSC act as transdifferentiation essentially to mesodermal lineages, lead to increase keratinocyte activity and faster epithelialization, following bone marrow derivatived stem cell transplant in favor of cutaneous injury therapeutic,

have be established in previous researches in other species, so that the functions of MSCs may including

support of keratinocyte migration and production (23,24, 25).

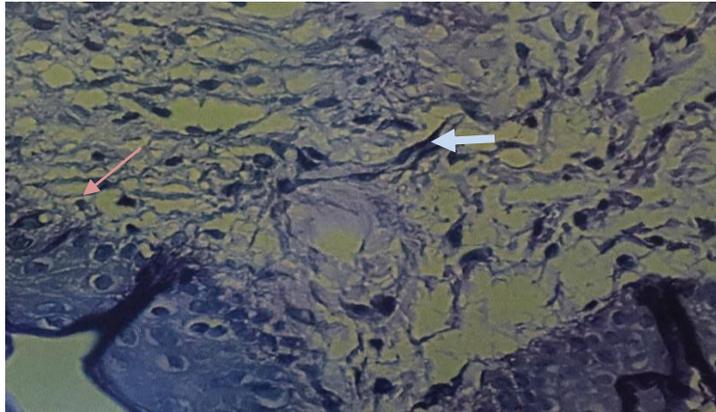


Figure (1): Histopathological section of induced wound without any treatment showed less collagen ← and more inflammatory cell → and odema after 15 days of treatment (Masson's trichrome stain X200).

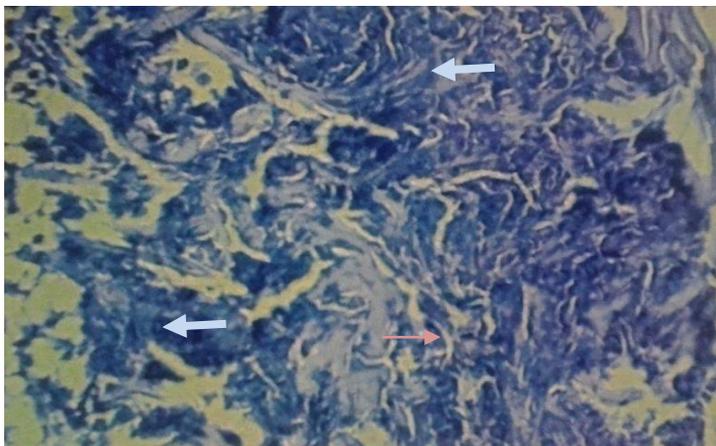


Figure (2): Histopathological section of treated wound with Methandrostenolone showed incomplete healing wound tissue, presence little inflammatory cells → and fibroblast with high collagen ← after 15 days of treatment (Masson's trichrome stain X400).

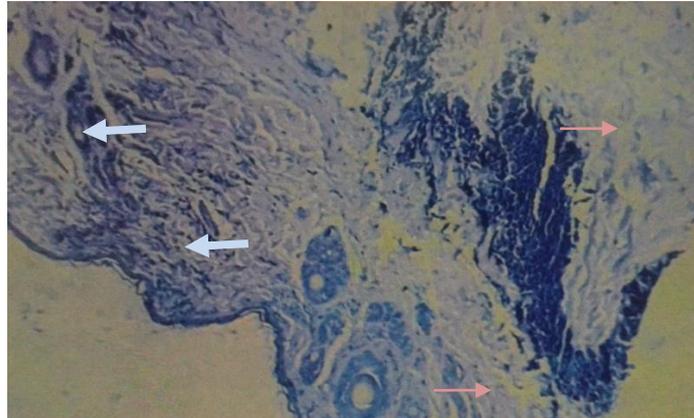


Figure (3): Histopathological section of wound treated with MSCs showed return the wound to normal tissue ← and disappear inflammatory cell → after 15 days of treatment (Masson's trichrome stain X200).

Conclusion

It can be concluded that the MSCs have ability to access healing of the wound in comparison with Methandrostenolone without any side effect.

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