JJBS

Jordan Journal of Biological Sciences

Volume 3, Number 4, December 2010
ISSN 1995-6673
Pages 185 - 192

In vitro Study of Adult Bone Marrow Mesenchymal Stem Cells Derived from Albino Rats and their Cardiomyogenic Differentiation

Intissar N. Waheed [a], Intidhar M. Mnati [b] and Abdul Hussain H.Kadhim [b]

[a] *College of Pharmacy, University of Duhok;* [b]*College of Education (Ibn Al-Haitham), University of Baghdad, Iraq*

Abstract

Mesenchymal stem cells (MSCs) are pluripotent adult stem cells residing within the bone marrow (BM) capable of self renewal, production of large number of differentiated progeny and regeneration of tissues. This study aimed at isolating the adult rats BM-MSCs and stimulating their *in vitro* differentiation into cardiomocytes. The BM, isolated from young male white rats *(Rattus norvegicus albinos)* and maintained in culture at 37C˚ and 5%CO_2. The culture system which permits to direct differentiation of the MSCs into cardiomyocyte-like characteristics was treated with rat embryonic heart muscle extract (HME), and differentiated cells were detected with immunocytochemistry examination using anti - myosin and anti - cardiotin antibodies as markers. The MSCs had fibroblast like morphology before HME treatment, but their morphology began to change after treatment. Treated MSCs increased in size and had formed a ball and a stick like morphology and were connecting with adjoining cells after one week and began to form myotube like structures at the end of second week. After three weeks of treatment, the differentiated cells could be distinguished by the presence of a number of branches and began to interface with each other to form cardiac like cells. The differentiated cells were positive for myosin and cardiotin. The morphological changes were not seen in control cells. We can conclude that HME may contain certain growth factors that stimulate the differentiation of BM-MSCs to the muscular pathway.

الملخص

تتواجد الخلايا الجذعية اللحمية ضمن البيئة الدقيقة لنقي العظم وهي خلايا جذعيه بالغة متعددة القوى، لها القدرة على التكاثر والتجدد وتكوين أعداد كبيرة من الخلايا المتمايزة إضافة إلى قابليتها على تجديد الأنسجة التالفة. تهدف هذه الدراسة إلى عزل وإنماء الخلايا الجذعية اللحمية البالغة لنقي عظم الجرذان ومن ثم تحفيز نموها وتمايزها خارج الجسم الحي إلى الخلايا العضلية القلبية. عزلت الخلايا الجذعية اللحمية لنقي العظم من ذكور الجرذان البيضاء اليافعة *(Rattus norvegicus albinos)* وتم إدامتها في الوسط ألزرعي وبدرجة حرارة 37C˚ و 5% غاز ثاني اوكسيد الكربون.ولتوجيه تمايز هذه الخلايا إلى خلايا شبيهة بالخلايا العضلية القلبية تم معاملتها بمستخلص عضلات قلوب أجنة الجرذان. وبعدها تم الكشف على هذه الخلايا المتمايزة باستعمال الفحص الكيميائي الخلوي المناعي وذلك باستعمال معلمات (واسمات) متخصصة لذلك مثل anti-myosin and anti-cardiotin. تبدو الخلايا الجذعية اللحمية قبل المعاملة بمستخلص عضلات القلب بشكل شبيه بالأرومة الليفية ولكن يبدأ شكلها بالتغاير بعد المعاملة ، حيث تبدأ الخلايا بالزيادة في حجمها وتكون شكل شبيه بالكرة ومن ثم يتغير شكلها ويصبح شبيه بالقضيب وبعد أسبوع واحد من المعاملة تبدأ هذه الخلايا بالارتباط بالخلايا المجاورة لها ، وفي نهاية الأسبوع الثاني تبدأ بتكوين تراكيب شبيهة بالأنبوب العضلي، ولكن بعد ثلاثة أسابيع يصبح بالإمكان تمييز هذه الخلايا المتمايزة وذلك بوجود عدد من التفرعات المتصلة مكونة خلايا شبيهة بالخلايا القلبية. ولكن هذه التغيرات لم تلاحظ في مجموعة السيطرة. أظهرت الخلايا المتمايزة استجابة موجبة لكلا النوعين من الواسمات. من خلال نتائج هذه الدراسة ممكن أن نستنتج بان مستخلص عضلات القلب قد يحتوي على عوامل نمو معينة والتي بدورها تقوم بتحفيز تمايز الخلايا الجذعية اللحمية لنقي العظم إلى الاتجاه العضلي.

Keywords: Bone marrow, Cardiomyogenic differentiation, Mesenchymal stem cells, Cell culture..

1. Introduction

Bone marrow stem cells (BMSCs) have myogenic potential and are therefore promising candidates for multiple cell-based therapies for myocardial diseases (Orlic *et al*., 2001). Recent attention has focused on BM as a source of stem cells which can be collected from adults and used for transplantation without posing ethical questions or creating problems of tissue matching and rejection (Hassink *et al*., 2003).

Mesenchymal stem cells (MSCs) are pluripotent adult stem cells residing within the BM microenvironment (Makino *et al*., 1999). Mesenchymal stem cells have an adherent nature and are expandable in culture and can differentiate into osteoblasts, chondrocytes, neurons, skeletal muscle cells (Prockop, 1997) and cardiomyocytes (Wang *et al*., 2000). Cardiomyogenic differentiation of stem cells has been vastly reported (Tomita *et al*., 1999; Kehat *et al*., 2001). The cells undergoing cardiomyogenic

* Corresponding author. intissar1960@yahoo.com.

differentiation achieve the cardiomyocyte phenotype through the expression of specific genes encoding various transcriptional factors and structural and regulatory proteins (Peng *et al*., 2002).

The potential of MSCs to differentiate into myogenic cells was first reported by (Wakitani *et al*., 1995) and then by a number of other investigators (Tomita *et al*., 1999; 2002; Xu *et al*., 2004). Early *in vitro* studies of the primary culture of rat MSCs treated with 10µmol/L 5-azacytidinem (a DNA demethylating chemical compound used to induce cardiomyogenic differentiation), showed that after 2 weeks of treatment, murine BM-MSCs formed myotube like structure and expressed myocardial specific proteins, such as cardiac troponin I and cardiac myosin heavy chain (MHC). These data suggest that stromal stem cells including MSCs differentiate into cardiomyocytes under appropriate culture conditions (Tomita *et al*., 1999). The same results were also demonstrated by Makino *et al*., 1999; Fukuda, 2001 and Bittira *et al*., 2002. At a concentration of 3 µmol/L for 1 week, 5-azacytidine induced BMCs into cardiomyogenic cells. These cells stained positive for myosin, actin and desmin and showed spontaneous beating at 3 weeks after treatment. Electron microscopy revealed a cardiomyocyte like structure including typical sarcomeres, a centrally positioned nucleus and a trial granule.

An interesting study from Xu *et al*., 2004 showed that the MSCs of human BM when treated with 10µmol/L 5-azacytidine appeared spindle shaped with irregular processes and the myogenic cells differentiated from MSCs were positive for beta–MHC, desmin and alpha cardiac actin.

After-wards, various strategies have been adopted for directed differentiation of BM-MSCs into cardiomyocytes by culturing BM-MSCs in *vitro* using culture media supplemented with retinoic acid (RA), dimethyl sulphoxide (DMSO) and 5-azacytidine (Heng *et al*., 2004; Antonitsis *et al*.,2007). The study of AL-Jumely, (2006) showed that mice BM-Hematopoetic stem cells (HSCs) when treated with heart muscle extract (HME) of newborn mice differentiated into muscular pathway *in vitro*, and these cells are stained positive for myosin.

The current study aimed at isolating and cultivating the BM-MSCs from young rats and stimulating their growth and differentiation *in vitro* into cardiomyocytes using HME.

2. Materials and Methods

Young male white rats (50-55 day old) *(Rattus norvegicus albinos)* weighing 180-200gm were used for the isolation of MSCs from the BM. These animals were obtained from the animal breeding house of the Medical Research Unit - College of Medicine - Al-Nahrain University - Baghdad - Iraq.

2.1. Isolation of bone marrow derived mesenchymal cells

Bone marrow derived mesenchymal stem cells cultures were prepared according to the protocol of Wakitani *et al*., 1995. Briefly, under sterile conditions, the femur and tibiae of the rats were excised, with special attention given to remove all connective tissue attached to bones. Bone marrow was extruded from these bones by flushing the BM cavity using a syringe with 20-gauge needle filled with culture medium (Minimum Essential Medium (MEM) supplemented with 10% fetal calf serum (FCS)). The harvested BMCs were gently pipetted to break up cell clumps in order to obtain cell suspension. After a homogenous cell suspension was achieved, the cells were centrifuged at 2000 rpm for 10 minutes and the cell pellet was resuspended in 3ml of culture medium.

The cell suspension was loaded carefully onto 5ml of 60% percoll in sterile conical tube, and then centrifuged for 20-25 minutes at 2000 rpm at 8Cº. The mononuclear cells (MNCs) were retrieved from the buffy coat layer by sterile Pasteur pipette and placed in 5ml sterile conical tube. The cells were washed two to three times with PBS to remove the percoll and centrifuged at 2000 rpm for 10 minutes at 8 Cº.

2.2. Culturing and expansion of MSCs

The cell suspension was seeded in 50cm plastic tissue culture flasks with 5 ml culture medium and maintained at 37C˚ in a humidified atmosphere with 5% CO_2 for two weeks. Cultures of MSCs were inspected and refeed every three days and passaged when the MSCs have reached approximately 80% confluence (Javazon *et al*., 2001). The mesenchymal population was isolated on the basis of its ability to adhere to the culture plate.

2.3. Cardiomyocytes differentiation of BM-MSCs in vitro:

To stimulate the differentiation of MSCs *in vitro* to the cardiomyocytes progeny, the second passage of rat BM-MSCs were resuspended after trypsin treatment and seeded into 4-well culture plates at a density of $1X10^6$ cell \ well. The second day after seeding, the tissue culture plates were divided into two groups as follows:

- Control group: which were treated with MEM +10% FCS only without HME.
- Treated group: which were treated with MEM +10% FCS and 0.1µl HME per 1ml medium. The cells of this group were cultured for three different periods: 1week, 2weeks and 3weeks.

The HME were prepared from the hearts of rat embryos of (18-19 days old) following the general principle of embryo extract preparation as described by New (1966) and modified by (Hammash and Waheed, 2004; and AL-Jumely, 2006).

The medium was changed twice a week until the experiment was terminated. After that, the changes in morphology of treated cultures were reported and compared with the untreated (control) cultures. The cells in both groups were fixed with 4% phosphate buffered formalin for 10 minutes. The cells were detected using immunocytochemistry examination which was performed with primary monoclonal antibodies against anti-cardiotin and anti-myosin (Pochampally *et al*., 2004; Xu *et al*., 2004).

3. Results

3.1. Effect of HME treatment on MSCs

The results of the current study showed that the MSCs before HME treatment appeared elongated and flattened with a fibroblast like morphology (spindle like uni-polar or bipolar shape) (Fig. 1A). But after HME treatment, the

cells began to proliferate and differentiate during the first week (Fig. 1B). Approximately, 50% of all adherent cells had enlarged or increased in size and had formed a ball like appearance (Fig. 1C) or lengthened in one direction and formed a stick like morphology, these cells began to connect with adjoining cells in one week (Fig. 1D).

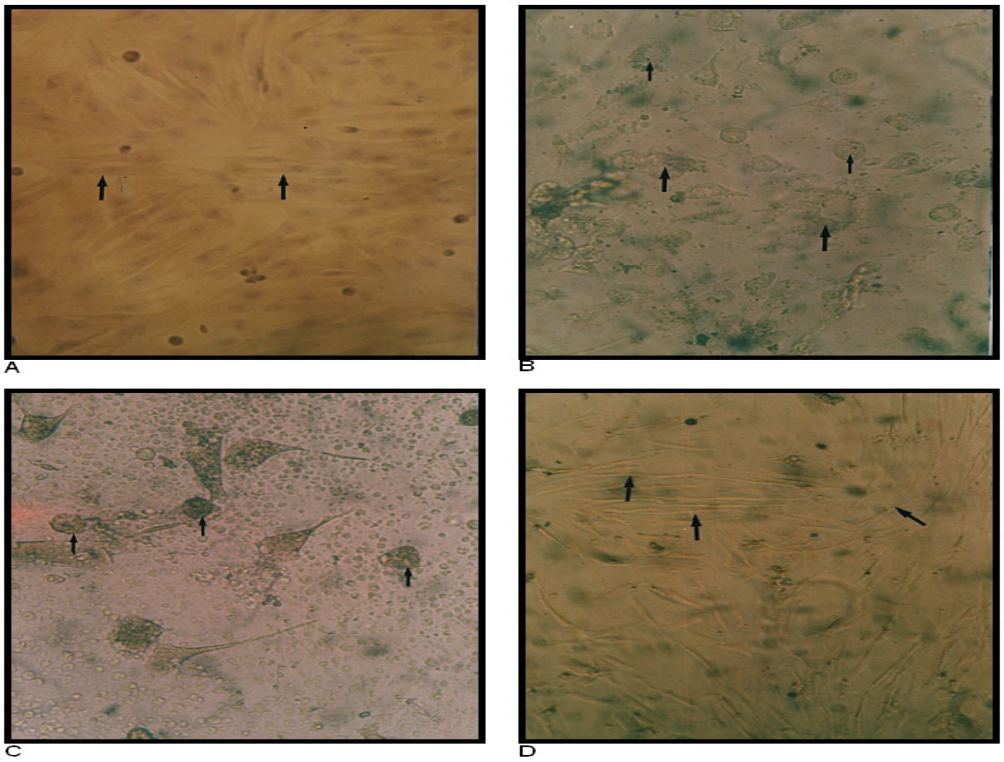

Figure1: The morphology of differentiated MSCs *in vitro* in the first week of the second passage (before and after treatment with HME). (A): MSCs had fibroblast-like morphology (arrows) before HME treatment (X100.8). (B): the cells treated with HME began to proliferate (arrows) (X160). (C): some of adherent cells treated with HME enlarged and formed a ball-like appearance (arrows) (X160). (D): most of adherent cells lengthened in one direction and had formed stick-like morphology (arrows) and began to connect with adjoining cells (X100.8).

At the end of second week, the cells began to connect to each others and then formed myotube-like structure (Fig. 2A). After three weeks, we noticed that most of the cells are mononuclear and some of them are binuclear but a few are extremely multinucleated (Fig. 2 B, C). The differentiated cells can be distinguished by the presence of a number of branches (Fig. 3 A, B), and these cells began to interface with each other to form cardiac-like cells (Fig. 3 C). These morphologies changes of BM-MSCs during exposure to HME in treated groups during different exposed periods were not seen in control groups.

3.2. Immunocytochemical Examination for Differentiation of Mesenchymal Stem Cells in vitro

To determine if HME treatment can induce MSCs to express cardiac muscle specific or related proteins, an immunocytochemistry examination was performed with monoclonal antibodies against myosin and cardiotin (Fig. 4, Table 1).

The immunostaining of the differentiated MSCs with anti-myosin and anti-cardiotin at two and three weeks after HME treatment showed that about 80% of the resulting differentiated cells expressed these proteins which was found in the longitudinal sarcoplasmic reticulum of mature cardiomyocytes (Fig. 4 A, B, C).

In contrast, the control groups expressed negative responses for these general (myosin) and specialized (cardiotin) (Fig 4 D).

4. Discussion

4.1. Effects of HME on MSC differentiation

Mesenchymal stem cells which have been isolated from BM can be expanded and induced either *in vitro* or *in vivo* to terminally differentiate into osteoblasts, chondrocytes, neural cells, myotubes and hematopoietic-supporting stroma (Dennis and Charbord, 2002). The BM-MSCs can also be differentiated into skeletal and cardiac muscles using appropriate environmental conditions plus several growth factors (Tomita *et al*., 2002; Xu *et al*., 2004; Yoon *et al*., 2008). To direct the differentiation of MSCs into specialized population, the growth conditions of MSCs need to be changed in specific ways, such as adding growth factors to the culture medium or changing the chemical composition of the surface on which MSCs grow (Odorico *et al*., 2001). Consequently, we substituted these growth factors using rat embryonic HME as a crude source of stimulating factors for directing the differentiation of MSCs *in vitro*.

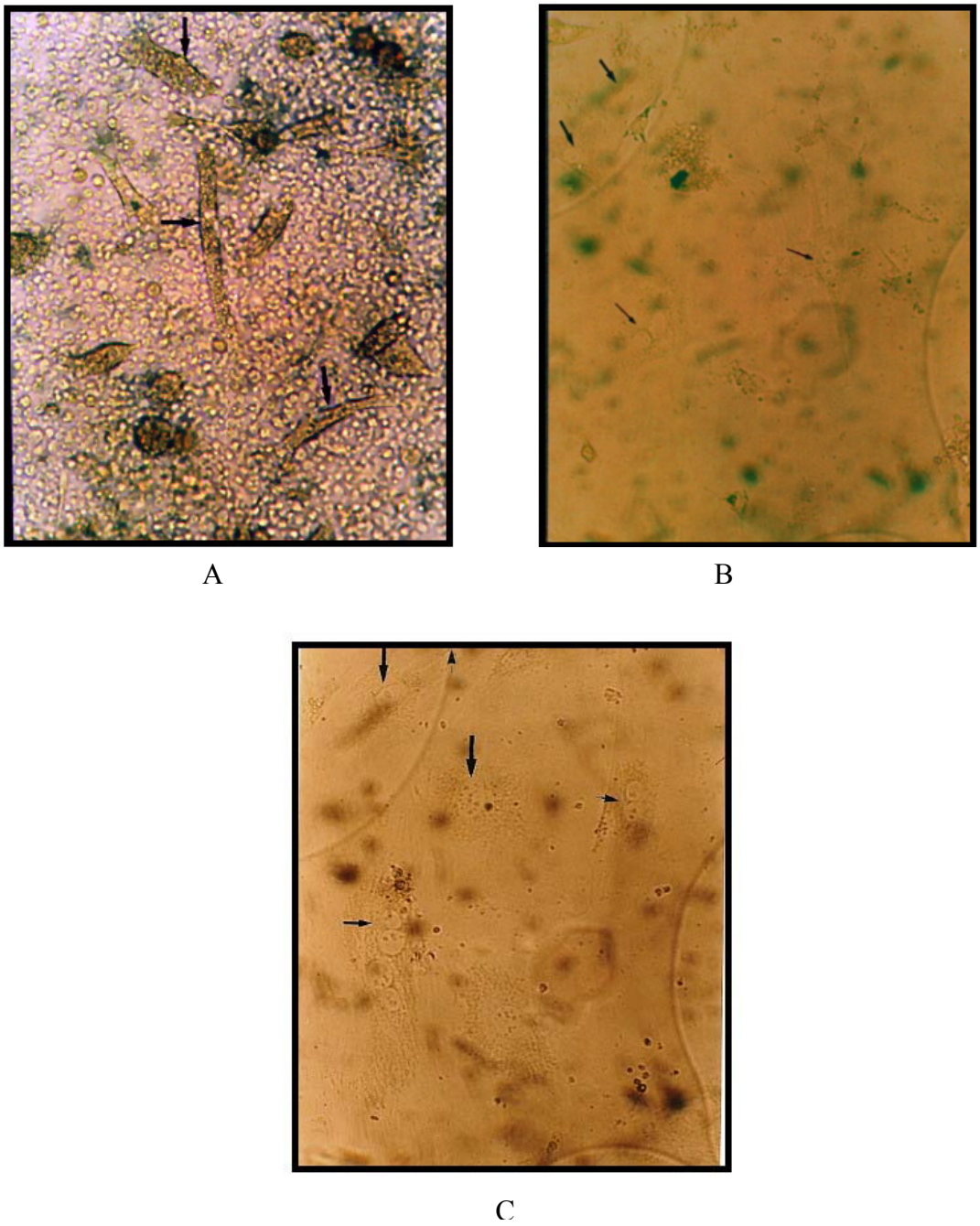

Figure 2: The morphology of differentiated MSCs *in vitro* at second passage after treatment with HME. (A): at the end of second week of culturing, the MSC cells connected with adjoining cells and began to form myotube-like structure (arrows) (X160). (B): at the third week of culturing, most of the cells are mononuclear (thick arrows) and some are binuclear (head arrow) but few are multinuclear (thin arrow) (X100.8). (C): the cells at third week, some of cells are mononuclear (thick arrows) and the other are binuclear (thin arrows) (X100.8).

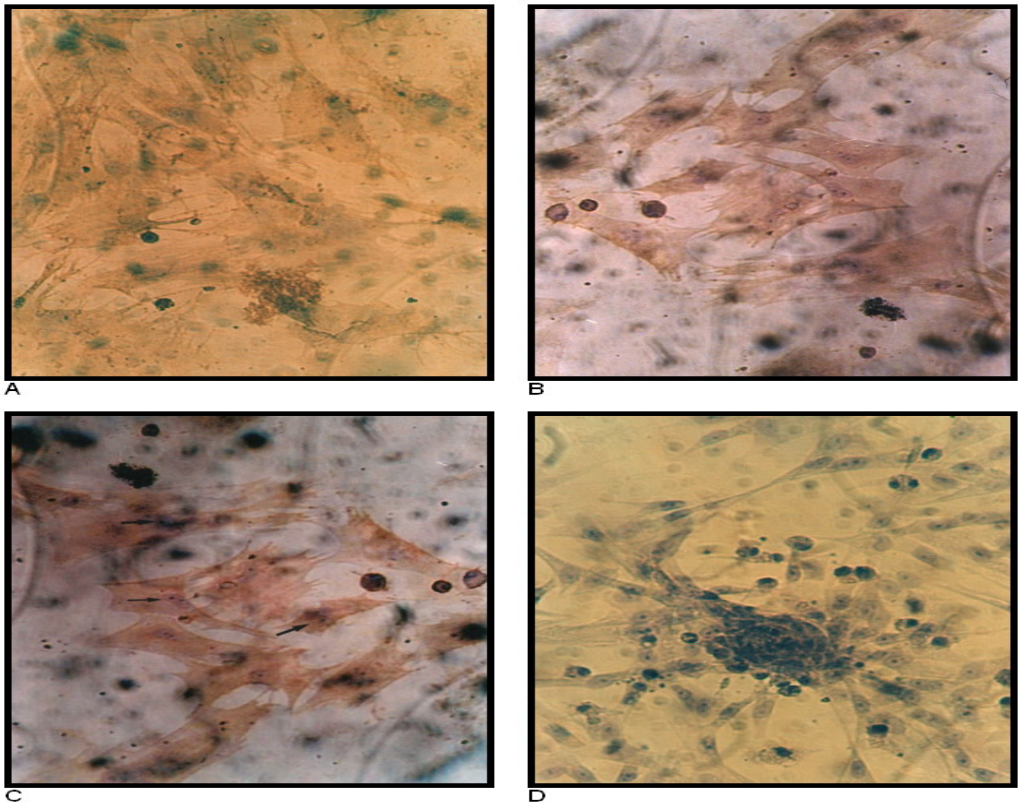

Figure4: Immunocytochemical analysis for differentiation of MSCs in vitro in treated groups with HME at second passage. (A): The most of differentiated cells were positive for anti-myosin marker (brown color) (X100.8). (B): the most of differentiated cells were positive for anti-cardiotin marker (X100.8). (C): the differentiated cells at three weeks after HME treatment were positive for anti-cardiotin marker and some of these cells appeared binuclear (arrows) (X160). D: the MSCs in control groups were negative for anti-cardiotin marker (X100.8).

Table1: Markers commonly used in the present study and characterized differentiated cell types.

Markers	Dilution	Cellular distribution	Properties
Anti-myosin	1:10	Myoblasts, Myocytes	A component of structural and contractile protein found in myocyte
Anti-cardiotin	1:50	Neonatal and mature cardiomyocyte	A component of structural and contractile protein found in cardiomyocyte

The direction of differentiation of MSCs has been performed for the first time after treatment with HME at a concentration of 0.1µl/1mL media, which is considered as the optimal concentration for differentiation into myogenic cells with cardiomyocyte-like characteristics for different exposure periods. From the results of the present study, we observed the differentiation of BM-MSCs into cardiac-like cells in treated groups compared with control groups. This is confirmed with immunocytochemical analysis and the results of the present study are consistent with many of prior reports (Wakitani *et al*., 1995; Makino *et al*., 1999) that suggested that using 5-azacytidine induced BM-MSCs to differentiate into myogenic cells. These adherent and differentiated cells increased in size and formed a ball like appearance or formed a stick like morphology, and then formed myotube like structure which is a feature that was not present or detected in the control groups. The effects of HME treatment in stimulation and differentiation of MSCs in culture was similar to the effect of several materials such as Amphotericin-B (Wakitani *et al*., 1995) or the drug 5-azacytidine (Makino *et al*., 1999).

The precise mechanism of how to induce BM-MCs to differentiate into muscle cells is unknown. In studies on myogenic differentiation of the mouse embryonic cell line with 5-azacytidine, Konieczy *et al*., (1984) proposed that these cells contain a myogenic determination locus in a methylated state with a transcriptionally inactive phase, which becomes demethylated and transcriptionally active with 5-azacytidine causing the cells to differentiate into myogenic cells .

The use of HME to stimulate the differentiation of MSCs took three weeks of treatment which is longer than the period which caused the differentiation of MSc using the 5-azacytidine; the latter was for 24 h only (Makino *et al*., 1999; Xu *et al*., 2004).

The role of these embryonic extract and as mentioned by Leor *et al*., (1996) is that most embryonic tissues are regarded as an important source of extracting factors that stimulate the growth and differentiation of stem cells into

special direction. The newly differentiated cells can then be used for therapeutic angiogenesis.

The critical role of extract also was observed by Waheed (Un-published data) when the induction of muscle differentiation from embryonic stem cells (ESCs) was carried in media containing New Born Bovine Serum (NBBS) and embryonic muscle extract. Additionally, the study made by (AL-Jumely, 2006) demonstrated that the differentiation of HSCs into muscle like cells *in vitro* could be done treating the colony of HSCs with HME of new born mice.

4.2. Immunocytochemical Examination for Differentiation of Mesenchymal Stem Cells in vitro

The results of the immunocytochemistry examination showed that the differentiated cells were positive when detected by anti-myosin antibody. This result is similar to different studies such as (Makino *et al*., 1999; AL-Jumely, 2006) who suggested that the expression may be associated with activation of the myosin gene.

Myosin is known to be an early marker of myogenic differentiation and myosin filaments are very important structures of muscle tissues that play an important role in contraction (Grigoriadis *et al.,* 1988; Yablonka-Reuent, 2005). The immunostaining analysis using anti-cardiotin marker demonstrated that most of the differentiated cells expressed this protein. These findings are similar to that described by Pochampally *et al*., (2004).

Cardiotin is a high molecular weight protein complex (300KDa) located in the longitudinal sarcoplasmic reticulum (SR) of cardiac muscle. The cardiotin structure consists of subunits of 60KDa and 100KDa. During cardiac contractile dysfunction, a decreased in SR activity is detected (Schaart *et al*., 1993).

According to the results of (Yoon *et al*., 2002) study, it was concluded that the cells from BM-MSCs co-cultured with rat neonatal cardiomyocytes expressed cardiac troponin I and other cardiac-specific proteins. When the rat cardiomyocytes were removed from the culture medium, the differentiation did not occur. These results confirm the important results obtained from the current study. We observed that MSCs when exposed to the culture medium without adding HME, the cells did not differentiate and were negative for anti-cardiotin marker.

References

Al-Jumely BA. 2006. Long-term culture of adult bone marrow stem cells in albino mice. (MSc thesis). Baghdad (Iraq): College of Science, Baghdad University. 112p. (In Arabic)

Antonitsis P, Ioannidou-Papagiannaki E, Kaidoglou A and Papakonstantinou Ch. 2007. *In vitro* cardiomyogenic differentiation of adult human bone marrow mesenchymal stem cells. The role of 5-azacytidine. Interact CardioVasc Thorac Surg. **6**:593-597.

Bittira B Kuang JQ Al-Khaldi A Shum-Tim D and Chiu RCJ. 2002. *In vitro* pre-programming of marrow stromal cells for myocardial regeneration. Ann. Thorac. Surg. **74**: 1154-1160.

Dennis JE and Charbord P. 2002. Origin and differentiation of human and murine stroma. Stem Cells **20**: 205-214.

Fukuda K. 2001. Development of regenerative cardiomyocytes from mesenchymal stem cells for cardiovascular tissue engeneering. Artif. Org. **25**: 187-193.

Grigoriads AE, Heersch JNM and Aubin JE. 1988. Differentiation of muscle, fat, cartilage and bone from progenitor cells present in a bone- derived clonal cell population: effect of dexamethasone. J. Cell Biol. **106**: 2139-2151.

Hammash MH and Waheed IN. 2004. Embryonic stem cells differentiation into neural like cells *in vitro* and *in vivo* after intracerebral transplantation. Iraqi JMS. **3**:100-108.

Hassink RJ, de la Riviere AB, Mummery CL and Doevendans PA. 2003. Transplantation of cells for cardiac repair. J. Am. Coll. Cardiol. **41**: 711-717.

Heng BC, Haider KH, Sim EK, Cao T and Ng SC. 2004. Strategies for directing the differentiation of stem cells into the cardiomyogenic lineage *in vitro*. Cardiovasc. Res. **62**: 34-42.

Javazon EH Colter DC Schwarz EJ and Prockop DJ. 2001. Rat marrow stromal cells are more sensitive to plating density and expand more rapidly from single – cell derived colonies than human marrow stromal cells. Stem cells. **19**:219-225.

Kehat I, Kenyagin-Karsenti D, Snir M, Segev H, Amit M, Gepstein A, Livne E, Binah O, Itskovitz-Eldor J and Gepstein L. 2001. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. J. Clin. Invest. **108**: 407-414.

Konieczy SF Emerson CP and Riley GP. 1984. 5-azacytidine induction of stable mesodermal stem cell lineages from 10T1/2 cells: evidence for regulatory genes controlling determination. Cell **38**: 791-800.

Leor J Patterson M Quinones MJ Kedes LH and Kloner RA. 1996. Transplantation of fetal myocardial tissue into the infarcted myocardium of rat. A potential method for repair of infarcted myocardium?. Cir. **94**: II332-II336.

Makino S Fukuda K Miyoshi S Konishi F Kodama H Pan J Sano M Takahashi T Hori S Abe H Hata JI Umezawa A and Ogawa S. 1999. Cardiomyocytes can be generated from marrow stromal cells *in vitro*. J. Clin. Invest. **103**: 697-705.

New DAT. 1966: **The Culture of Vertebrate Embryos**. Logos press, Academic press, Great Britain and London.

Odorico JS Kaufman DS and Thompson JA. 2001. Multilineage differentiation from human embryonic stem cell lines. Stem Cells. **19**: 193-204.

Orlic D Kajstura J Chimenti S Limana F, Jakoniuk I Quaini F Nadal-Ginard B Bodine DM Leri A and Anversa P. 2001. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc. Natl. Acad. Sci. USA. **98**:10344-10349.

Peng CF, Wei Y, Levsky JM, Mc Donald TV, Childs G and Kitsis RN. 2002. Microarray analysis of global changes in gene expression during cardiac myocyte differentiation. Physiol. Genomics **9**: 145-155.

Pochampally RR Neville BT Schwarz EJ Li MM and Prockop DJ. 2004. Rat adult stem cells (marrow stromal cells) engraft and differentiate in chick embryos without evidence of cell fusion. PNAS. **101**: 9282-9285.

Prockop DJ. 1997. Marrow stromal cells as stem cells for non hematopoietic tissues. Sci. **276**: 71-74.

Schaart G Vander Ven PF and Ramaekers FC. 1993. Characterization of cardiotin, a structural component in the myocard. Eur. J. Cell. Biol. **62**: 34-48.

Tomita S Li RK Weisel RD Mickle DAG. and Jia ZQ. 1999.Autologous transplantation of bone marrow cells improves damaged heart function. Circ. **100:** II247-256.

Tomita S, Mickle DAG, Weisel RD, Jia ZQ, Tumiati LC, Allidina Y, Liu P and Li RK. 2002. Improved heart function with myogenesis and angiogenesis after autologous porcine bone marrow stromal cell transplantation. J. Thorac. Cardiovasc. Surg. **123**:1132- 1140.

Waheed IN. Formation of muscle-like cell during differentiation of embryonic stem cell *in vitro* by treatment with muscle extract (un published data).

Wakitani S Saito T and Caplan AI. 1995. Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. Muscle Nerve **18**: 1417-1426.

Wang JS Shum-Tim D Galipeau J Chedrawy E, Eliopoulos N and Chiu RCJ. 2000. Marrow stromal cells for cellular cardiomyomyoplasty: feasibility and potential clinical advantages. J. Thorac. Cardiovasc. Surg. **120**: 999-1006.

Xu W Zhang X Qian Zhu W Sun X Hu J Zhou H and Chen Y. 2004. Mesenchymal stem cells from adult human bone marrow differentiate into a cardiomyocyte phenotype *in vitro*. Exp. Biol. Med. **229**: 623-631.

Yablonka-Reuent Z. 2005. Development and postnatal regulation of adult myoblasts. Wiley Inter Science **30**: 366-380.

Yoon YA Murayama T and Tkebuchawa T. 2002. Clonally expanded bone marrow derived stem cells differentiate into multiple lineages *in vitro* and can attenuate myocardial dysfunction post myocardial infarction. Circ. **106** : II-S1.

Yoon J Choi Seung-Cheo Park Chi-Yeon Choi Ji-Hyun Kim Yang-In Shim Wan-Joo and Lim Do-Sun. 2008. Bone Marrow-derived side population cells are capable of functional cardiomyogenic differentiation. Mol. Cells **25**: 216-223.