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BONE BIOLOGY

The influence of parathyroid hormone 1-34 on the osteogenic characteristics of adipose- and bone-marrow-derived mesenchymal stem cells from juvenile and ovarectomized rats

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Objectives

Mesenchymal stem cells (MSCs) are of growing interest in terms of bone regeneration. Most preclinical trials utilize bone-marrow-derived mesenchymal stem cells (bMSCs), although this is not without isolation and expansion difficulties. The aim of this study was: to compare the characteristics of bMSCs and adipose-derived mesenchymal stem cells (AdMSCs) from juvenile, adult, and ovarectomized (OVX) rats; and to assess the effect of human parathyroid hormone (hPTH) 1-34 on their osteogenic potential and migration to stromal cell-derived factor-1 (SDF-1).

Methods

Cells were isolated from the adipose and bone marrow of juvenile, adult, and previously OVX Wistar rats, and were characterized with flow cytometry, proliferation assays, osteogenic and adipogenic differentiation, and migration to SDF-1. Experiments were repeated with and without intermittent hPTH 1-34.

Results

Juvenile and adult MSCs demonstrated significantly increased osteogenic and adipogenic differentiation and superior migration towards SDF-1 compared with OVX groups; this was the case for AdMSCs and bMSCs equally. Parathyroid hormone (PTH) increased parameters of osteogenic differentiation and migration to SDF-1. This was significant for all cell types, although it had the most significant effect on cells derived from OVX animals. bMSCs from all groups showed increased mineralization and migration to SDF-1 compared with AdMSCs.

Conclusion

Juvenile MSCs showed significantly greater migration to SDF-1 and significantly greater osteogenic and adipogenic differentiation compared with cells from osteopenic rats; this was true for bMSCs and AdMSCs. The addition of PTH increased these characteristics, with the most significant effect on cells derived from OVX animals, further illustrating possible clinical application of both PTH and MSCs in bone regenerative therapies.

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Keywords: Parathyroid hormone, Mesenchymal stem cell, Bone marrow, Adipose

Article focus

- Bone-marrow- (bMSC) and adipose-derived mesenchymal stem cell (AdMSC) osteogenic capacity.
- Ovarectomized (OVX)-derived and juvenile-derived mesenchymal stem cell characteristics.
- The role of parathyroid hormone (PTH) on osteogenic capacity.

Key messages

- bMSCs have a greater osteogenic capacity than AdMSCs.
- Juvenile cells have a greater osteogenic and adipogenic differentiative capacity than cells derived from OVX cells.
- PTH increases the osteogenic capacity of both AdMSCs and bMSCs.

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Strengths and limitations

- A comparison of osteogenic characteristics from two viable cell sources.
- Investigation of the underlying genomic changes would be beneficial.

Introduction

Multiple influences alter the osteogenic capabilities of undifferentiated mesenchymal stem cells (MSCs); studies have compared the functional differences between cells from adolescent and aged animals, as well as the role of cell source and osteoporosis on activity.[1-4]

Postmenopausal oestrogen deficiency leads to an uncoupling of the bone remodelling cycle, where upregulated osteoclast activity is matched only with aberrant osteoblast activity, resulting in net resorption.[5,6] This is characterized by a reduction in bone mass and altered trabecular microarchitecture, hence fragility.[7]

The majority of bone regeneration studies have used MSCs derived from bone marrow (bMSCs). This is not without problems, including morbidity associated with obtaining cells from iliac crest puncture, low cell yield, and reduced potency following extensive passage.[8] Zuk et al[9] initially described the use of cells obtained from subcutaneous adipose liposuction aspirate as a source rich in MSCs (AdMSCs). Moreover, unlike periosteal cells or cells obtained from myogenic sources, adipose tissue is readily available, harvesting carries very limited morbidity, and cell yield is much greater than that found from other sources.[10,11] Reports on the osteogenic capacity of AdMSCs compared with bMSCs are contradictory; bMSC characteristics are thought to be affected by age, unlike AdMSCs, where cells are thought to retain all characteristics regardless of the age of the source. Cell yield is also a fundamental difference: bMSCs yield 6×10^6 nucleated cells per millimetre of aspirate, with a maximum of 0.01% being MSCs, while 2×10^6 cells can be isolated from 1 gm adipose tissue, 10% of which are stem like.[12,13]

Studies, including those conducted on postmenopausal women, demonstrate a profound anabolic effect of parathyroid hormone 1-34 (PTH).[14,15] Moreover, *in vitro* data have shown PTH to mediate MSC fate, increasing not only the number of MSCs, but also their preferential osteogenic differentiation over adipogenesis.[16] Interestingly, these findings have predominantly been reported in bMSCs, with very little data on the effect of PTH on AdMSCs.

In addition to anabolic effects, PTH has also been shown to effect cell mobilization. The stromal cell-derived factor-1 (SDF-1)/C-X-C chemokine receptor type 4 (CXCR4) axis has been found to be an important regulator of stem cell migration. SDF-1, also known as C-X-C motif chemokine 12 (CXC1L2), is produced by a multitude of tissue types including fracture endosteum and in its active form is bound to the CXCR4 receptor found on MSCs. Granero-Moltó et al[17] demonstrated dynamic stem cell migration to the fracture site in a stabilized tibial osteotomy model being CXCR4-dependent. The clinical significance of the SDF-1/CXCR4 axis has further been alluded to, whereby the overexpression of CXCR4 on MSCs led to increases in bone density,[18] with increased SDF-1 expression following PTH treatment *in vitro*.[19]

As such, although comparisons have been made between the osteogenic potential of different stem cell sources, a very limited body of work compares these differences across juvenile, adult, and ovarectomized (OVX) animals, nor does this work elucidate their capacity to migrate to SDF-1. We completed this study with the purpose of comparing varying sources of MSCs in the presence of PTH, hypothesizing that MSCs isolated from the adipose tissue of OVX rats will have lower osteogenic capacity, proliferation, and migration than cells isolated from the bone marrow of juvenile counterparts, and that coculture with intermittent PTH will upregulate these characteristics.

Materials and Methods

Female Wistar rats were used throughout this study. Animals were classed as 'juvenile' at two to four weeks or 'adult' at six to nine months. One group of animals ('OVX') were supplied immediately following bilateral ovarectomy. These animals were housed for 16 weeks in pairs and osteopenia was confirmed by assessing their femora, lumbar third and fourth vertebrae, and humeri mineral density with peripheral quantitative CT (pQCT) compared with age-matched non-OVX controls. A reduction of 22% in bone mineral density was confirmed; as such, our model was one of osteopenia rather than osteoporosis. Additionally, OVX animals were aged between ten and 13 months at the time of use experimentally.

Bone marrow cell isolation. Following gaseous euthanasia, all animals were processed within 60 minutes to maintain cell viability. Within a laminar flow hood, dissected femora were washed twice with phosphate buffered saline (PBS; ThermoFisher, Hemel Hempstead, United Kingdom) to remove remaining external debris. Ends were transected at the diaphyseal-metaphyseal junction leaving a diaphyseal portion that was flushed three times with 5 ml of Dulbecco's Modified Eagle Medium (DMEM, ThermoFisher) high glucose, with the aspirate collected and cultured in DMEM, 20% foetal calf serum, and 1% penicillin-streptomycin ('standard media').

Adipose-derived mesenchymal stem cell isolation. AdMSCs were isolated from the abdominal subcutaneous fat, avoiding perinephric and visceral fat. Under aseptic conditions, adipose samples were washed three times with PBS following the removal of other soft tissues and weighed. Subsequently, specimens were minced with sterile scissors; 8 ml of warmed 0.1% collagenase-type II (Sigma-Aldrich, Gillingham, United Kingdom) was then

added to the adipose tissue and agitated in a 37°C water bath for 60 minutes. Samples were centrifuged and the supernatant was aspirated and discarded. The pellet was resuspended in 5 ml of fresh standard media, and the suspension was cultured and used for experimental procedures at passages 3 to 4.[20]

All cell work was repeated in triplicate, from the bone marrow and adipose tissue of three animals from each group (OVX, juvenile, and adult).

Passaged cells were seeded at a density of 4500/cm^3 in 48-well plates (Corning, Ewloe, United Kingdom) and cultured in standard media for 24 hours, following which media were discarded and the cells were washed with PBS to remove nonadherent cells. Culture in standard media continued until 80% confluence, after which media was supplemented with 1×10^{-7} M water-soluble dexamethasone (Sigma-Aldrich), 1×10^{-4} M ascorbic acid (Sigma-Aldrich), and 1×10^{-2} M beta-glycerol phosphate (Sigma-Aldrich), herein referred to as 'osteogenic media'.

Alizarin red staining. At days 7, 14, and 21, mineralization was assessed by staining calcium deposits with alizarin red. Cells were fixed in formalin and stained with 100 µl of alizarin red solution. The plates were covered with foil and incubated at room temperature for 15 minutes. The stain was then aspirated and the cells were washed multiple times with PBS until the solution ran clear to remove nonspecific staining. Cells were then left in 100 µl of PBS and images were taken.

To quantify staining, 10% cetylpyridinium chloride (CPC) was added to 10 mM of sodium phosphate to obtain a working solution of pH 7. Following imaging, PBS was discarded from wells and 200 µl of the CPC solution was added to each well for 15 minutes, agitated at room temperature, and covered in foil. Absorbance was then read on a plate reader at 570 nm (Tecan Infinite Pro; Tecan Trading, Männedorf, Switzerland). A standard curve was made by serial dilution of alizarin red working solution in CPC and read again at 570 nm. Data were normalized for cell numbers, which were quantified using DNA Hoechst.

Alkaline phosphatase activity. Alkaline phosphatase (ALP) data were collected at days 3, 7, 14, and 21. In total, 50 µl of each sample (repeated in duplicate) was combined in a 96-well Nunc microplate reader with 50 µl of p-Nitrophenol phosphate (Sigma-Aldrich) and agitated at 37°C in the dark for 30 minutes. Plates were then read at a fluorescence of 405 nm and measurements expressed in U/l.

For adipogenic characterization, cells were passaged and seeded as described for osteogenic differentiation. At 80% confluence, media was removed and cells were cultured with standard media supplemented with 0.5 mM isobultyl-1-methylxanthine, 10 ug/ml insulin, and 0.1 mM dexamethasone (all Sigma-Aldrich), herein termed 'adipogenic media'.

Oil Red O staining. At days 7, 14, and 21, lipid production was assessed by staining droplets with Oil Red O. For fixed cells, 100 ul of 60% isopropanol was added to each well and incubated for 15 minutes at room temperature. Cells were then washed twice with PBS and incubated in Oil Red O working solution for 15 minutes at room temperature. After staining, cells were washed carefully with 60% isopropanol and analyzed. To quantify staining, 200 ul of 100% isopropanol was added to each well and the plate was agitated at room temperature for 15 minutes. The supernatant was then collected and absorbance was read on a plate reader at wavelength 510 nm.

A standard curve was made by serial dilution of Oil Red O working solution in isopropanol and read again at 510 nm.

Cell morphology. Passage 2 and 3 cells from the three groups for both sources were cultured in standard media and their morphology was assessed by measuring their aspect ratio, whereby the ratio of the length of a cell to its width was calculated using ImageJ software (National Institutes of Health, Bethesda, Maryland).

Characterization of stem cells – flow cytometry. A total of 100 000 cells from the bone marrow and adipose of juvenile, adult control, and OVX rats were analyzed for their cluster differentiation (CD) markers: CD29, 90, 45, 106, 146, and 34. Cells were labelled with antimouse/rat CD29-fluorescein (ThermoFisher Scientific, San Diego, California), 17 antimouse/rat CD90-allophycocyanin (APC; ThermoFisher Scientific), antirat CD45-APC (ThermoFisher Scientific), and CD34-phycoerythrin (PE; Abcam, Cambridge, United Kingdom). The CD expression was compared with the isotype control. Cells were fixed in 4% formalin for 15 minutes at room temperature, washed with 0.5% bovine serum albumin (BSA), and stained with the conjugated primary antibody for one hour at room temperature in the dark. After one hour, the cells were washed with 0.5% BSA and analyzed on flow cytometer (CytoFLEX; Beckman Coulter, Brea, California).

Human parathyroid hormone 1-34 (Teriparatide; Bachem, Saint Helens, United Kingdom) was dissolved in 4 mM hydrochloric acid solution containing 0.1% BSA to a stock concentration of 5 mM, which was subsequently stored at -20°C. Intermittent regimens involved the culture of cells in the PTH containing media for six hours in every 48-hour cycle at 50 nmol based on a preliminary study comparing dosing regimens and concentrations.[20] We repeated the osteogenic differentiation of all groups derived from both adipose and bone marrow, with the addition of PTH, again assessing with ALP, alizarin red, and osteocalcin immunocytochemistry.

The effect of PTH 1-34 on cell proliferation. A total of 10 000 cells derived from the adipose and bone marrow of juvenile, adult, and OVX rats (n = 3 for each group) were incubated in DMEM, 20% foetal calf serum, and 1% penicillin streptomycin with intermittent PTH. At three, seven, ten, and 14 days, 10% Alamar Blue assay (AbD Serotec, Kidlington, United Kingdom) was added to the culture media for four hours; the resultant media was

Table I. Mean cluster differentiation (CD) marker expression (SD 1)

CD marker	Mean expression, % (SD)	
	Adipose	**Bone marrow**
Juvenile		
CD29	*95.8* (1.6)	*95.1* (2.1)
CD90	*91.6* (0.7)	*96.6* (3.2)
CD106	*86.0* (2.4)	*88.0* (2.9)
CD146	*90.2* (0.6)	*91.0* (8.1)
CD34	*2.7* (1.9)	*2.6* (7.6)
CD45	*9.1* (0.6)	*10.9* (8.2)
Adult		
CD29	*90.9* (1.6)	*91.1* (9.4)
CD90	*94.7* (4.9)	*97.0* (1.1)
CD106	*85.0* (1.6)	*85.0* (6.0)
CD146	*89.6* (1.2)	*89.0* (7.2)
CD34	*3.6* (0.5)	*3.8* (3.1)
CD45	*12.9* (0.7)	*9.0* (1.1)
Ovarectomized		
CD29	*97.2* (0.6)	*99.1* (0.1)
CD90	*91.9* (1.4)	*94.4* (4.2)
CD106	*87.1* (0.6)	*87.0* (7.8)
CD146	*89.7* (1.9)	*90.0* (1.2)
CD34	*1.7* (0.8)	*1.7* (0.4)
CD45	*9.7* (1.2)	*11.1* (6)

read at an excitation of 560 nm and an emission of 590 nm using a Tecan plate reader. The mean absorbance was determined from triplicate samples. The absorbance was then normalized to DNA assays and a comparison was made between groups as per our previous studies.[21]

The effect of PTH 1-34 on cell migration to SDF-1. At passage 3, cells were again seeded at a concentration of 4500 cells/cm^2 in 48-well plates and cultured with either osteogenic media or osteogenic media supplemented intermittently with 50 nM of PTH 1-34 for 21 days. A total of 10000 cells from each group were loaded in plain DMEM without supplements in the upper compartments of a 5 mm pore size Boyden chambers (Corning). The lower compartment of the chambers was filled with 100 ng/ml SDF-1 (Peprotech, London, United Kingdom) in standard media, and incubated at 37°C, 5% CO_2.

After 16 hours, the cells that migrated to the opposite side of the membrane were fixed with 10% formalin and stained with Toluidine blue.

Following fixation, the chambers were rested in 200 ul of the toluidine solution for three minutes. Subsequently, wells were washed three times with distilled water, after which wells were analyzed under the microscope, where cells that had migrated were counted by selecting six random fields at ×20 magnification and calculating the mean percentage number of cells. For the control, both the top and bottom of the chamber were filled with standard media with no SDF-1 and the cells were loaded in the upper chamber as described.

Statistical analysis. Values are expressed as the mean and standard deviation. Data were found to be nonparametric following Shapiro–Wilkinson testing, and as such Mann–Whitney U tests were used for analysis in GraphPad software (GraphPad Software, Inc., San Diego, California).

Results

Expression of CD markers. There was no significant difference in CD marker expression by cells obtained from any group, regardless of tissue source. Mean CD marker expressions (SD 1) are outlined in Table I.

Cell morphology. Both AdMSCs and bMSCs from juvenile rats demonstrated a tight spindle-like morphology, with no significant difference in mean aspect ratios (bMSC 18.66, AdMSC 19.1). The mean ratios in adult cells were significantly smaller (bMSC 4.99, AdMSC 5.31), although again there was no difference between different tissue sources. Mesenchymal stem cells from OVX rats had the smallest aspect ratio compared with the other cell types (bMSC 2.25, AdMSC 1.80).

Proliferation. Although plotted growth curves all showed time-dependent growth up to day 14, no significant effect on cell metabolic activity or on proliferation when normalized against DNA was seen secondary between groups. This was despite tissue or age/ovarectomy status of the source.

Osteogenic differentiation. Mineralization increased in all groups over the 21-day experimental period. At day 7, juvenile bMSCs produced significantly more calcium phosphate than OVX cells ($p = 0.038$; this trend continued over the 21-day period. There was no difference between calcium phosphate deposition from juvenile- and adult-derived bMSCs at any timepoint; this was also the case for AdMSCs. Juvenile AdMSCs at all timepoints had significantly greater mineralization than OVX cells ($p = 0.042$. When comparing tissue source, bMSCs deposited significantly more calcium phosphate then AdMSCs; this difference was most profound for OVX cells (Fig. 1).

On the addition of PTH, cells showed a significant increase in alizarin red staining compared with untreated groups at all timepoints for bMSCs. This effect was noted to be most profound on OVX cells that showed a nearly two-fold increase on calcium phosphate deposition compared with untreated cells at day 21 ($p = 0.044$) (Figs 2a and 2b); this effect was also seen in OVX AdMSCs.

bMSCs demonstrated the most significant reaction to PTH compared with AdMSCs by day 21 ($p = 0.044$).

No difference between ALP expression from AdMSCs or bMSCs was seen, but as with calcium phosphate deposition, juvenile and adult cells expressed significantly more ALP than OVX cells at day 14, when production peaked for cells from both tissue sources ($p = 0.033$). Similarly, PTH led to increased ALP production for all cell types compared with untreated cells ($p = 0.041$); this affected AdMSCs and bMSCs to the same degree, with no difference in the magnitude of the effect independent of age or ovarectomy.

Adipogenic. At days 14 and 21, adipocytic differentiation was significantly greater in MSCs isolated from juvenile animals compared with adult control and OVX groups for both tissue sources. MSCs from juvenile rats accumulated significantly greater amounts of lipid from day

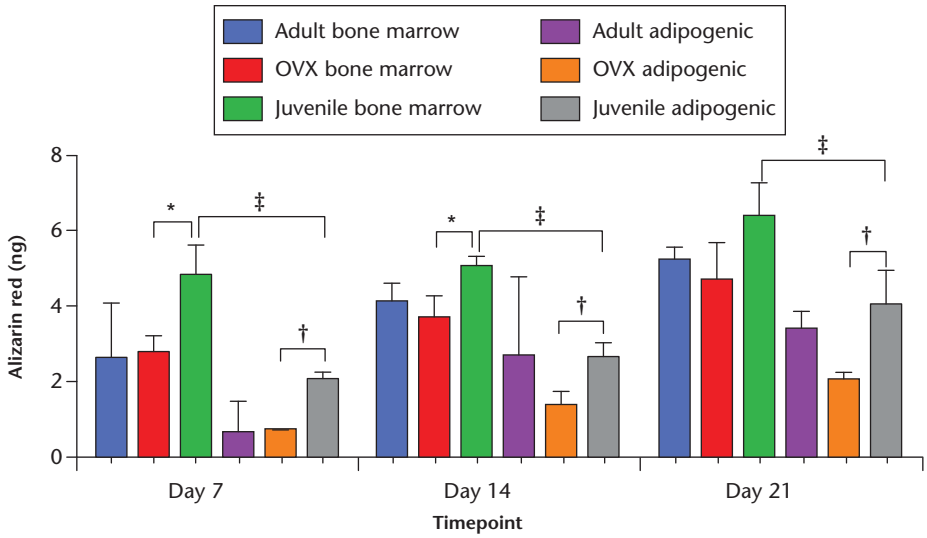

Fig. 1

Graph of alizarin red staining of adipose- and bone-marrow-derived cells. From both sources, juvenile groups demonstrated greater alizarin red staining than ovarectomized (OVX) groups (*p < 0.04; †p < 0.05). Bone-marrow-derived juvenile cells showed greater alizarin staining than adipose-derived cells at all time-points (‡p < 0.03).

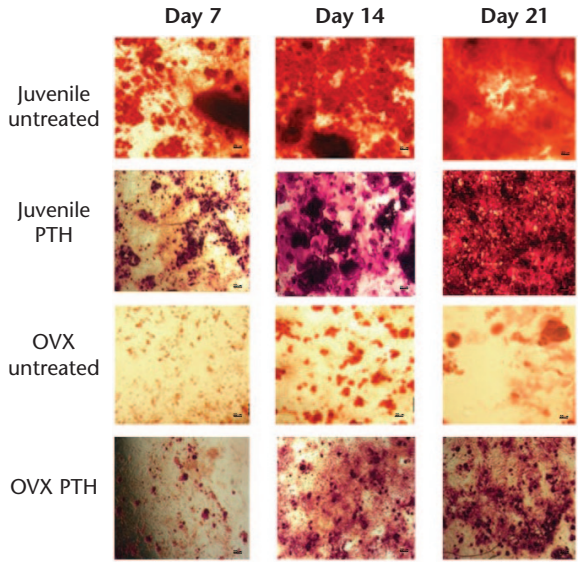

Fig. 2a

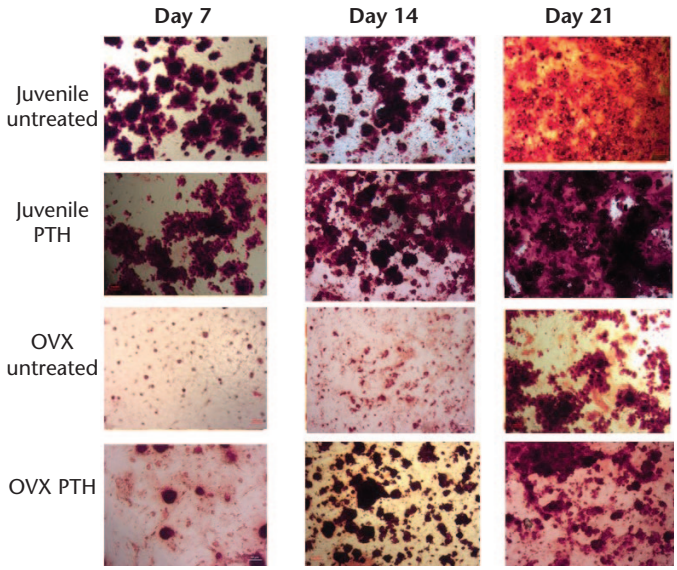

Fig. 2b

a) Images of calcium phosphate deposition stained with alizarin red from adipose-derived cells. b) Images of calcium phosphate deposition stained with alizarin red from bone-marrow-derived cells. PTH, parathyroid hormone; OVX, ovarectomized.

7 compared with the other two groups of cells. The rate of lipid accumulated from day 7 onwards was greater in cells isolated from juvenile rats. Cells isolated from adipose tissue and bone marrow, regardless of donor age or whether they were derived from OVX rats, continued to show increased lipid formation over the 21-day period, although juvenile cells were always more productive than those in the adult and OVX groups.

When comparing bMSCs with AdMSCs, there was no difference in adipogenic differentiation, but the reduction in microdroplet formation was more significant between adipose juvenile and OVX cells compared with bMSCs ($p = 0.037$).

Cell migration to SDF-1. In bMSCs, the migration of juvenile cells to SDF-1 was significantly greater than for OVX- or adult-derived cells ($p = 0.046$) and was nearly twice as high as the migration of OVX cells ($p = 0.047$). In AdMSCs, the migration of MSCs from young rats was significantly less than with bMSCs, although the pattern of these cells migrating more than cells from OVX animals was continued ($p = 0.032$) (Figs 3 and 4). On the addition of PTH, all cell types demonstrated increased migration

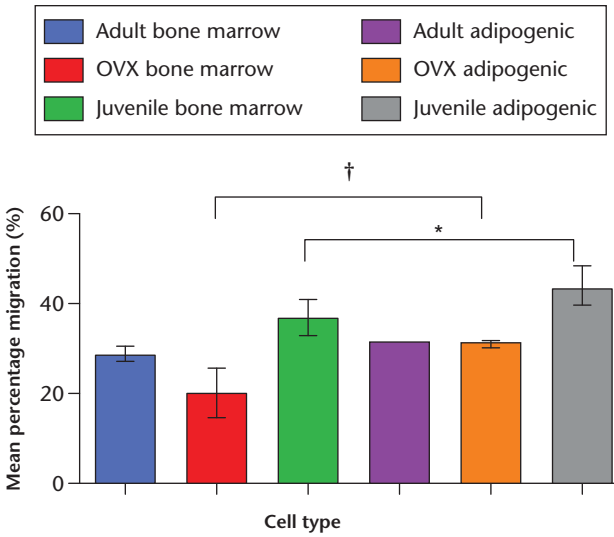

Fig. 3

Migration of bone-marrow-derived cells to stromal cell-derived factor-1 (SDF-1). On the addition of parathyroid hormone (PTH), juvenile cells (*p < 0.05) and ovarectomized (OVX) cells (†p < 0.04) showed enhanced migration. iPTH, intermittent parathyroid hormone.

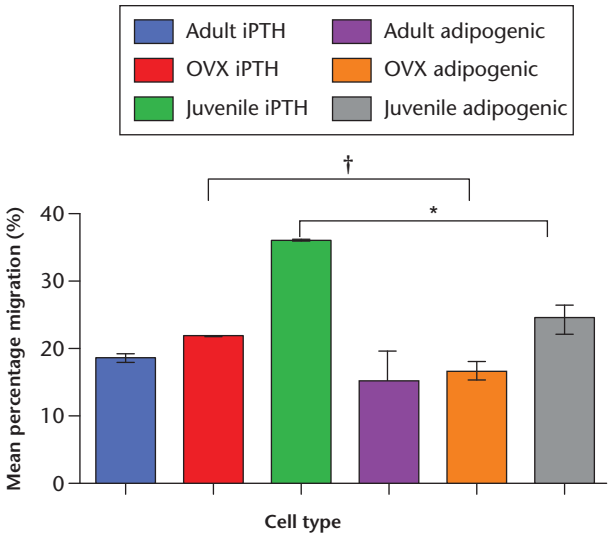

Fig. 4

Migration of adipose-derived cells to stromal cell-derived factor-1 (SDF-1). On the addition of parathyroid hormone (PTH), juvenile cells (*p < 0.04) and ovarectomized (OVX) cells (†p < 0.05) showed enhanced migration. iPTH, intermittent parathyroid hormone.

compared with their untreated counterparts and this treatment affected AdMSCs and bMSCs equally.

Discussion

This study examines the capacity of AdMSCs and bMSCs to differentiate into adipocytes and osteoblasts, as well as the effects of PTH 1-34 dosing regimens on cellular osteogenic characteristics and migratory capacity. We found that AdMSCs demonstrated poorer calcium phosphate deposition, osteocalcin expression, and migration along the CXCR4/SDF-1 axis compared with bMSCs, and that these differences were more profound for cells derived from OVX animals than for their juvenile counterparts. We also found differences between cells isolated from juvenile and OVX animals for each cell source, with comparative reductions in osteogenic and migrative characteristics in OVX-derived cells, although these cells showed similar proliferative capacity and CD marker expression to juvenile counterparts.

We found no difference in CD marker expression from the cells independent of source, age, or OVX status of animal. Other studies have demonstrated a higher expression of CD34 from AdMSCs at early culture;[11] this CD marker is important for cell-to-cell adhesion, as well as cell extracellular matrix deposition. Similarly, several reports suggest higher CD106 and CD146 from bMSCs. Our findings were contrary to this, whereby all samples were negative for CD34, and there was equal expression of CD106 and CD146. This may be explained by the use of the cells at passage 3, particularly as previous work has demonstrated a reduction in CD34 expression at later passage.[11] The lack of difference between OVX- and juvenile-derived cells and CD markers is in keeping with other works,[21] although the heterogeneity in study methodologies means only a limited inference can be made between the expression of CD markers and the *in vivo*/*in vitro* activity of cells. Similarly, we found no difference morphologically between AdMSCs or bMSCs; both demonstrated the same spindle-like phenotype from juvenile populations, and both moved morphologically to a more flattened phenotype from aged and OVX animals. However, again the value of morphology is limited in isolation.

Asumda and Chase[22] demonstrated reduced osteogenic and adipogenic differentiation ability of bMSCs from senile and juvenile rats. However, Singh et al[23] found no observable difference in osteogenic and adipogenic differentiation between cells from these groups. Similarly, in a rabbit study, Beane et al[24] showed no difference in ALP expression or alizarin red staining between bMSCs from juvenile and senile rabbits, but they found that age affected the adipogenic differentiation of the same cells and also led to reduced adipogenic differentiation in AdMSCs. The reason for the disparate findings between the studies is likely to be multifactorial, and may be a result of varying culture practices and cell isolation techniques. Moreover, we use explanted cells, which even at passage 3 are a heterogeneous cell group; although they do conform to standard definitions of being 'stem-like', one wonders if pure or clonal-cultured MSCs may demonstrate different characteristics. In addition, although differences were seldom seen between adult and juvenile cells, due to the time taken for osteopenia to develop, the OVX animals we used were older

than all other groups. As such, differences may be secondary to their 'senile' nature rather than solely ovarectomy.

A fundamental advantage of using AdMSCs in bone regeneration, rather than bMSCs, is based on the evidence that the proliferative and osteogenic capacity of AdMSCs is not affected by age. In the present study, there was no difference in proliferation between any MSCs independent of age, OVX, or source. This is in contrast with other studies assessing bMSCs, whereby MSCs from older rats have significantly lower proliferation compared with MSCs from young rats.[10,25-27] Again, Beane et al[24] looked at MSCs from young and old rabbits derived from the bone marrow, muscle, and fat, where the cells from bone marrow demonstrated a reduction in proliferation with age, whereas cells from the other two sources did not. Georgen et al[28] found that MSCs from OVX rats had a lower proliferation rate than their control counterparts and concluded that the low proliferation rate would correlate with reduced self-renewal capacity, which might cause a gradual depletion of MSC sources in the bone marrow of OVX animals. When comparing the effects of intermittent PTH on the osteogenic capacity of AdMSCs and bMSCs, we found significant differences. Although PTH affected all OVX cells, this change was most significant with cells derived from bone marrow, where mineralization at day 7 for OVX and young bone marrow cells had increased 2.1- and 1.9-fold compared with comparative cells from adipose tissue, which had increased 1.6- and 1.5-fold, respectively.

The stimulation, proliferation, and differentiation of bone-marrow-derived osteoprogenitor cells by PTH 1-34 has been well documented in the literature.[29] The anabolic window is based on findings of intermittent dosing regimens. Contrary to the larger body of literature, we did not find an increase in proliferation of cells from any group after PTH dosing, although our exposure cycle was greater than the documented 30 to 60 minutes that has been reported in works as selectively upregulating of the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathways.[30]

We demonstrated that MSCs from OVX rats, whether derived from bone marrow or adipose, have a lower *in vitro* migration compared with MSCs from juvenile and adult rats. Overall, AdMSCs had poorer migrative capacity than those from bone marrow.[31,32] SDF-1 is a chemokine receptor for CXCR4 and the SDF-1/CXCR4 biological axis plays an important role in the migration of stem cells and the wound repair of tissues and organs. The impaired migration capacity of MSCs from rats four months after ovariectomy may be due to their low expression of CXCR4. This could explain the impaired bone formation in osteoporotic patients, as these cells have a reduced capacity to migrate to the site of bone loss. SDF-1 is produced in the periosteum of injured bone and encourages endochondral bone repair by recruiting MSCs to the site of injury. Therefore, mobilization of osteoblastic progenitors to the bone surface is an important step in osteoblast maturation and formation of mineralized tissue. Very little work has explored the effect of PTH on AdMSCs and their migration; we found that these cells also reacted to PTH in a similar way to bMSCs; the percentage increase in migration was greatest in OVX cells compared with untreated cells.

Interestingly, *in vivo* studies have yielded mixed results on the efficacy of AdMSCs in bone formation. Niemeyer et al[33] and Hayashi et al[34] showed significantly poorer fracture healing in sheep and rat defects, respectively, compared with the osteogenesis and full bone bridging achieved with implanted bMSCs. Indeed, implanted undifferentiated AdMSCs tended to differentiate into cells with an adipose-like morphology and thus hindered healing. This is converse to Kang et al[35] and Stockmann et al[36] who, in porcine and canine models, respectively, found no difference in bone formation with bMSCs and AdMSCs. Yet, heterogeneity of subcutaneous *versus* intra-abdominal fat, cell number, cell culture techniques, and fracture model makes comparisons between studies difficult, and thus renders evaluation of the true potential of AdMSCs *in vivo* difficult.

We found that cell migration and osteogenic differentiation is reduced when derived from osteopenic animals, and that bMSCs have greater calcium phosphate deposition than AdMSCs. Yukata et al[37] reported the reduced efficacy of PTH on periosteal stem cells in an aged osteopenic mouse compared with a juvenile model; conversely, our findings demonstrated increased sensitivity of OVX cells to PTH. This may have implications for clinical applications. If allogenic cells from younger patients are incompatible for use in the aged osteoporotic population, then can the addition of PTH improve the ability of OVX-derived cells to migrate and differentiate, thus rendering them effective for bone regeneration? Moreover, although we demonstrated bMSCs to have superior osteogenic capabilities compared with AdMSCs, in the presence of PTH 1-34, AdMSCs also showed improved mineralization capacities and migration to SDF-1. As such, this study also highlights the potential utility of AdMSCs when the morbidity associated with bone marrow aspiration is too high.

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Ethical review statement
- The animal aspects of the study were completed in accordance with the Home Office Animals Scientific Procedures Act 1986.