Osteogenic differentiation of mesenchymal stem cells from osteopenic rats subjected to physical activity with and without nitric oxide synthase inhibition

N.M. Ocarino a, J.N. Boeloni a, A.M. Goes b, J.F. Silva a, U. Marubayashi c, R. Serakides a,∗

a Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinatrias, Universidade Federal de Minas Gerais, Avenida Presidente Antônio Carlos, 6627-CEP, 30161-970, Belo Horizonte, Minas Gerais, Brazil
b Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
c Departamento de Biofísica e Fisiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais Belo Horizonte, Minas Gerais, Brazil

A R T I C L E   I N F O

Article history:
Received 7 April 2008
Revised 7 August 2008
Available online 3 September 2008

Keywords:
Mesenchymal stem cell
Osteogenic differentiation
L-NAME
Nitric oxide

A B S T R A C T

Physical activity has potent and complex effects on bones. We hypothesized that physical activity has a positive effect upon osteopenic rat bones because it stimulates osteogenic differentiation of bone marrow mesenchymal stem cells (MSCs). We also postulated that local nitric oxide concentrations mediate the effects of physical activity on bones. The objective of this study was to investigate the osteogenic differentiation in vitro of MSCs from osteopenic female rats subjected to physical activity with and without nitric oxide synthase inhibition. We used MSCs from the femurs of Wistar female rats divided into six groups: Group 1, sham-operated (control); Group 2, sedentary osteopenic; Group 3, active osteopenic; Group 4, sham-operated with L-NAME; Group 5, sedentary osteopenic with L-NAME; and Group 6, active osteopenic with L-NAME. The cells were cultured at 37 °C and 5% CO2. Cells were phenotypically characterized with anti-CD45, anti-CD90, anti-CD73, and anti-CD54 using a FACScan cytometer. MSCs were cultured in osteogenic medium for 7, 14 and 21 days. Alkaline phosphatase activity, the capacity of dimethylthiazol conversion in formazan crystals, collagen synthesis and the number of mineralized nodules were analyzed. The means of all of the variables were compared using the SNK test. MSCs did not express CD45 in 96.94% of the cells, but there was expression of CD73, CD54 and CD90 in 93.99%, 95.10% and 86.77% of the cells, respectively. MSCs from osteopenic rats showed less osteogenic differentiation. Surprisingly, physical activity increased the osteogenic differentiation of MSCs in osteopenic rats. Inhibition of nitric oxide synthase in vivo had a negative effect upon the osteogenic potential of MSCs from normal rats and from osteopenic rats subjected to physical activity. Our results suggest that nitric oxide stimulates MSCs osteogenic differentiation and that nitric oxide mediates the beneficial effects of physical activity upon MSCs osteogenic differentiation.

© 2008 Published by Elsevier Inc.

As life expectancy has increased, the incidence of osteoporosis has risen in both women and men worldwide [1,2]. Despite much research into this area, the etiopathogenesis of osteoporosis has not been totally elucidated. The effects of sex steroid deficiencies [3] and physical inactivity in the genesis of post-menopausal osteoporosis are well acknowledged [4–6]. Physical activity has potent and complex effects on bones. The effects of physical activity may be directly mediated via mechanical forces or indirectly mediated by hormones, growth factors, cytokines and nitric oxide [3,7–12]. It has previously been shown that daily physical activity on an automatic treadmill promotes an increase in the bone mass of the whole skeleton of osteopenic rats by morphologically and functionally altering bone cells [13]. Exercise has been shown to increase the number of connections between osteoblasts–osteocytes and osteocytes–osteocytes and to increase the number of osteoblasts and their activity. Osteoblasts are cells that originate from osteogenic differentiation of bone marrow MSCs [14]. It has also been shown that, if cultures of MSCs are vibrated while they grow, there is more osteogenic differentiation [15]. However, the effects of physical activity in vivo upon MSC osteogenic differentiation in vitro are not known.

We hypothesized that physical activity has positive effects on osteopenic rat bones since it stimulates the osteogenic differentiation of bone marrow MSCs. The factors that stimulate MSC differentiation into osteoprogenitor cells in vitro have been previously studied [16]. In addition, it has been demonstrated that physical activity stimulates the local and systemic production of nitric oxide and that the autocrine action of nitric oxide stimulates bone matrix synthesis [12,17]. Nitric oxide (NO) is also important for osteoblastic differentiation of MSCs in vitro [18]. Our second hypothesis was that local nitric oxide is a mediator of the effects of physical activity on MSCs and that it affects their differentiation into...
osteoprogenitor cells. Therefore, our aim was to study the osteogenic differentiation of MSCs in vitro from osteopenic female rats subjected to physical activity in the presence and absence of nitric oxide synthase (NOS) inhibition.

Materials and methods

Experimental design

Forty Wistar female rats were used in this study. Two-month-old animals were randomly divided into two groups, one ovariectomized and the other sham-operated (normal). Rats were submitted to bilateral ovariectomy as a standard procedure to induce osteopenia. To confirm induction of osteopenia, 3 months after the ovariectomy, five control rats and five ovariectomized rats were sacrificed. Osteopenia was confirmed by morphometric analysis of the bone mass. The ovariectomized rats showed a significant reduction in their trabecular bone volume in the whole skeleton when compared to the control group (data not shown).

Three months after ovariectomy, the rats were submitted to different treatments for the subsequent 3 months. The rats were divided into six experimental groups: Group 1, sham-operated (control) (n = 5); Group 2, sedentary osteopenic (n = 5); Group 3, active osteopenic (n = 5); Group 4, sham-operated with L-NAME (n = 5); Group 5, sedentary osteopenic with L-NAME (n = 5); and Group 6, active osteopenic with L-NAME (n = 5).

Animals from the same experimental group were housed, with five rats per cage, in a 12 h-light/dark cycle. They were fed with commercial rat chow containing 22% crude protein, 1.4% calcium and 0.6% phosphorus. Food and water were provided ad libitum to all animals. The rats were treated with the NOS inhibitor L-NAME by oralgastric probe (10 mg/animal/day) 40 min before being subjected to physical activity. The dose of L-NAME used was similar to those that have previously been found to influence skeletal metabolism in rats [19,20].

The active osteopenic groups performed controlled physical activity on a motor-driven treadmill at 15 m/min speed and 0° inclination once a day, 5 days a week for 3 months. The exercise lasted 15 min/day in the first week. From the 2nd week to the completion of this study, animals exercised for 30 min/day. This activity was considered to be of a moderate degree [21]. Electric shocks or other means of artificial stimulation were not used at any stage. Six months after surgery, the experimental groups were killed using an overdose of anesthetic (Tionembrutal 2.5%). The sedentary rats performed no daily physical activity on the motor-driven automatic treadmill beyond normal movement in their home cages.

Cell harvesting and culture

Rat bone marrow stromal cells were isolated from the femurs of animals from all experimental groups. The femurs were dissected from the attached muscle and connective tissue under aseptic conditions, and the epiphyses were removed. Bone marrow was flushed out with Dulbecco’s modified Eagle’s medium (DMEM) (Gibco, Grand Island, NY). The released cells were suspended in DMEM supplemented with 10% fetal bovine serum (Gibco) plus antibiotics (60 µg/L gentamicin, 25 µg/L B anfotericin, 100 µg/mL penicillin, and 100 µg/mL streptomycin) and collected in a 75 cm² culture flask containing 10 mL culture medium. The cells were grown at 37 °C and 5% CO₂ for 3 days. The non-adherent cell population was removed, and the adherent layer was washed once with fresh media. The culture medium was changed twice a week.

FACS analysis

Cells from the fourth passage were harvested with trypsin/EDTA and centrifuged at 1400 rpm for 10 min, and then resuspended at 1 x 10⁶ cells/well in phosphate-buffered saline (PBS). Cell aliquots were incubated with individual primary or control antibodies for 30 min at 4 °C. The samples were analyzed using a FACScan cytometer (Becton Dickinson), and the data were analyzed using CELLQUEST software (Becton Dickinson). The following primary antibodies were used, anti-CD45 (clone 69 mouse), anti-CD90 (clone Ox-7 mouse), anti-CD73 (clone 5 F/B9 mouse) and anti-CD54 (clone 1A29 mouse) (BD Biosciences, San Jose, CA, USA).

Osteogenic differentiation

After the fourth passage, the adherent cells were harvested by treatment with trypsin/EDTA. They were then counted and replaced in 6- and 24-well culture plates (1 x 10⁵ cells/well). The cells were cultured in osteogenic medium, which consisted of DMEM supplemented with 10% fetal bovine serum (Gibco), 60 µg/L gentamicin, 25 µg/L B anfotericin, 100 U/mL penicillin, 100 µg/mL streptomycin, 10 mM β-glycerophosphate, 50 µg/mL ascorbic acid (Merck, Germany), and 10 nM dexamethasone (Sigma). The cells were grown at 37 °C and 5% CO₂ for either 7, 14 or 21 days.

Viability assay

Briefly, the cells were incubated with MTT for 2 h at 37 °C. After incubation with MTT, they were treated for 12 h with a solubilization solution (SDS in 10% HCl), and the absorbance at 595 nm of the solubilized MTT formazan product was measured using a microtitrater plate reader. The mean absorbance and standard deviation were determined in triplicate for each experimental group.

Alkaline phosphatase activity

Briefly, the cells were incubated with BCIP/NBT (5-bromo,4-chloro,3-indolylphosphate-nitroblue tetrazolium) solution (Gibco) for 2 h at 37 °C. After incubation, the cells were treated for 12 h with solubilization solution (SDS in 10% HCl). The absorbance at 595 nm was measured using a microtiter plate reader. The mean absorbance and standard deviation were determined in triplicate for each experimental group.

Collagen production assay

The cultures were washed in PBS and fixed in Bouin for 2 h at 37 °C. After incubation, they were washed with deionized water. The cells were stained with sircol red for 30 min at room temperature. The cell culture plates were then washed with a 0.01 N HCl solution, air dried, and stained with sircol red and then examined by light microscopy. They were treated with 0.5 M NaOH solution, and the absorbance at 540 nm was measured using a microtiter plate reader. The mean absorbance and standard deviation were determined in triplicate for each experimental group.

Mineralization assay

The cultures were washed in PBS, fixed in 70% ethanol and rinsed with deionized water. After addition of 5% silver nitrate solution, the wells were exposed to light for 2 h. Two plates were rinsed with deionized water, and the residual silver nitrate was neutralized by 5% sodium thiosulfate. The number of nodules...
was quantified in 50 fields using a 10× objective. The mean and standard deviation were then determined in triplicate for each group.

**Statistical analysis**

Delineation was entirely at random with a 6 × 3 factorial (six groups × three periods). The data were submitted to analysis of variance (ANOVA), and means were compared using the Student Newman Keuls Test (SNK) (Instat, version 3.00, 32 Win 95/NT; GraphPad Software San Diego, CA, USA). Differences were considered significant if \( p < 0.05 \).

**Results**

Phenotypic characterization of the bone marrow MSCs indicated that there was no CD45 expression in 96.94% of the cells. There was, however, expression of CD73, CD54, and CD90 in 93.99%, 95.10%, and 86.77% of the cells, respectively (Fig. 1).

The extent of osteogenic differentiation was confirmed by measuring the alkaline phosphatase activity, collagen synthesis and production of mineralized nodules.

The sedentary osteopenic group showed a significant reduction of the spectrophotometric absorption of formazan at the 7 day time-point in culture, but the spectrophotometric absorption of formazan in this group increased progressively up until 21 days in culture to equal the levels in the control group. At the 14 and 21 day timepoints, all of the groups treated with L-NAME showed a reduction of the spectrophotometric absorption of formazan (Table 1).

![Fig. 1. Flow cytometric evaluation of CD45, CD90, CD73, and CD54 (ICAM-1) frequencies in mesenchymal stem cells (MSCs) of rats. The fluorescence scale is on the x axis and is considerably positive when the cell peak is above 101. The empty black peak refers to unmarked stem cells (negative control). The full red peak refers to stem cells marked with antibodies: CD45, CD90, CD73 and CD54 (ICAM-1). For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.](image)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Days of culture (differentiation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 d</td>
</tr>
<tr>
<td>Sham-operated (control)</td>
<td>0.7161 ± 0.01Aa</td>
</tr>
<tr>
<td>Sedentary osteopenic</td>
<td>0.4000 ± 0.11Cb</td>
</tr>
<tr>
<td>Active osteopenic</td>
<td>0.7737 ± 0.01A</td>
</tr>
<tr>
<td>Sham-operated with L-NAME</td>
<td>0.5550 ± 0.05BCb</td>
</tr>
<tr>
<td>Sedentary osteopenic with L-NAME</td>
<td>0.6590 ± 0.13AbA</td>
</tr>
<tr>
<td>Active osteopenic with L-NAME</td>
<td>0.5761 ± 0.02Bab</td>
</tr>
</tbody>
</table>

Different capital letters in the same column and different small letters in the same line indicate a significant difference (\( p < 0.05 \)).

Interestingly, at 7 days, the osteopenic group subjected to physical activity showed a significant increase in alkaline phosphatase activity when compared to the normal and sedentary osteopenic groups. At the 14 and 21 day timepoints, however, there was no difference between the groups. The groups that had been subjected to NOS inhibition showed a significant reduction in MSC alkaline phosphatase activity at 7, 14 and 21 days (Table 2).

The groups that had not been treated with L-NAME, i.e., the sham-operated (control), sedentary osteopenic and active osteopenic groups, showed a significant progressive increase in collagen
synthesis up to the 21 day timepoint. At the 7 and 14 day timepoints, collagen synthesis in these groups was significantly lower than the groups that had been subjected to NOS inhibition, but after 21 days of culture, there was no significant difference in collagen synthesis between the groups that had been treated with L-NAME and those that had not (Table 3).

The cells from the control group had increased production of mineralized nodules during all the studied time periods as compared to cells from the experimental groups. Ovariectomy led to a significant reduction in the number of mineralized nodules. Surprisingly, physical activity significantly increased the capacity of the cultured MSCs from osteopenic rats to produce mineralized nodules (Table 4). In vivo NOS inhibition had negative effects upon

Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Days of culture (differentiation)</th>
<th>7 d</th>
<th>14 d</th>
<th>21 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated (control)</td>
<td>0.3355 ± 0.01Ba</td>
<td>0.4625 ± 0.04Aa</td>
<td>0.4970 ± 0.03Aa</td>
<td></td>
</tr>
<tr>
<td>Sedentary osteopenic</td>
<td>0.2978 ± 0.23Ba</td>
<td>0.3891 ± 0.07Aa</td>
<td>0.4728 ± 0.05Aa</td>
<td></td>
</tr>
<tr>
<td>Active osteopenic</td>
<td>0.6539 ± 0.16Aa</td>
<td>0.3071 ± 0.05Aa</td>
<td>0.4817 ± 0.06Ab</td>
<td></td>
</tr>
<tr>
<td>Sham-operated with L-NAME</td>
<td>0.1331 ± 0.03Cc</td>
<td>0.1100 ± 0.01Ca</td>
<td>0.2326 ± 0.03Bb</td>
<td></td>
</tr>
<tr>
<td>Sedentary osteopenic with L-NAME</td>
<td>0.1020 ± 0.02Ca</td>
<td>0.2300 ± 0.03Bc</td>
<td>0.2210 ± 0.02Ba</td>
<td></td>
</tr>
<tr>
<td>Active osteopenic with L-NAME</td>
<td>0.1065 ± 0.02Ca</td>
<td>0.1400 ± 0.03Ca</td>
<td>0.1850 ± 0.003Ba</td>
<td></td>
</tr>
</tbody>
</table>

Different capital letters in the same column and different small letters in the same line indicate a significant difference (p < 0.05).

Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Days of culture (differentiation)</th>
<th>7 d</th>
<th>14 d</th>
<th>21 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated (control)</td>
<td>0.1555 ± 0.005Cb</td>
<td>0.1765 ± 0.008Bb</td>
<td>0.2243 ± 0.001Aa</td>
<td></td>
</tr>
<tr>
<td>Sedentary osteopenic</td>
<td>0.1863 ± 0.013Cb</td>
<td>0.1913 ± 0.009Bb</td>
<td>0.2286 ± 0.013Aa</td>
<td></td>
</tr>
<tr>
<td>Active osteopenic</td>
<td>0.1647 ± 0.007Cc</td>
<td>0.1918 ± 0.007Bb</td>
<td>0.2305 ± 0.006Aa</td>
<td></td>
</tr>
<tr>
<td>Sham-operated with L-NAME</td>
<td>0.2425 ± 0.001Aa</td>
<td>0.2500 ± 0.001Aa</td>
<td>0.2326 ± 0.03Ab</td>
<td></td>
</tr>
<tr>
<td>Sedentary osteopenic with L-NAME</td>
<td>0.2173 ± 0.002Ba</td>
<td>0.2300 ± 0.04Aa</td>
<td>0.2210 ± 0.02Ba</td>
<td></td>
</tr>
<tr>
<td>Active osteopenic with L-NAME</td>
<td>0.2080 ± 0.004Bb</td>
<td>0.2400 ± 0.03Aa</td>
<td>0.1850 ± 0.003Ac</td>
<td></td>
</tr>
</tbody>
</table>

Different capital letters in the same column and different small letters in the same line indicate a significant difference (p < 0.05).

Table 4

<table>
<thead>
<tr>
<th>Variable</th>
<th>Days of culture (differentiation)</th>
<th>7 d</th>
<th>14 d</th>
<th>21 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated (control)</td>
<td>11.170 ± 1.22Aa</td>
<td>12.57 ± 0.9Aa</td>
<td>9.07 ± 1.0Ab</td>
<td></td>
</tr>
<tr>
<td>Sedentary osteopenic</td>
<td>0.510 ± 0.1Ca</td>
<td>1.70 ± 0.1Da</td>
<td>0.02 ± 0.03Dc</td>
<td></td>
</tr>
<tr>
<td>Active osteopenic</td>
<td>8.850 ± 0.9Ba</td>
<td>8.27 ± 2.7Ba</td>
<td>6.47 ± 0.3Bb</td>
<td></td>
</tr>
<tr>
<td>Sham-operated with L-NAME</td>
<td>0.000 ± 0.00Cbc</td>
<td>2.47 ± 0.06Da</td>
<td>3.80 ± 0.2Ca</td>
<td></td>
</tr>
<tr>
<td>Sedentary osteopenic with L-NAME</td>
<td>0.570 ± 0.03Cc</td>
<td>4.37 ± 0.5Cb</td>
<td>5.97 ± 0.7Ba</td>
<td></td>
</tr>
<tr>
<td>Active osteopenic with L-NAME</td>
<td>0.040 ± 0.02Ca</td>
<td>0.65 ± 0.04Da</td>
<td>1.17 ± 0.5Da</td>
<td></td>
</tr>
</tbody>
</table>

Different capital letters in the same column and different small letters in the same line indicate a significant difference (p < 0.05).

the in vitro production of mineralized nodules except in the sedentary osteopenic group. Treatment with L-NAME significantly reduced MSCs’ production of mineralized nodules in normal rats. Notably, L-NAME impeded the beneficial effect of physical activity upon mineralized nodule production in the active osteopenic rats. In the sedentary osteopenic group treated with L-NAME, however, the number of mineralized nodules was increased as compared to the untreated sedentary osteopenic group (Table 4).

Discussion

Bone marrow contains MSCs, hematopoietic cells [22] and fibroblasts [23]. Amongst other molecules, hematopoietic cells express CD45 [24,25], which can also be expressed in fibroblasts [26,27]. CD73 can be expressed in fibroblasts [21] and in mesenchymal stem cells [23]. CD90 [22] and CD54, however, which were expressed in more than 90% in the cells of this study, are not expressed in fibroblasts or hematopoietic cells [26].

The dimethylthiazolyl (MTT) assay is a sensitive and quantitative colorimetric method which measures cell viability and the status of cell activation. It is based on the ability of succinate dehydrogenase enzymes present in the mitochondria of viable cells to convert MTT into formazan crystals [27,28]. Since the MTT assay is dependent on mitochondrial metabolism [27,28], there may be at least two explanations for the observed interference of L-NAME treatment with the MTT assay: (1) the MSCs of animals treated with L-NAME have decreased viability when they are cultivated in osteogenic differentiation medium, or (2) the MSCs of animals treated with L-NAME have decreased mitochondrial enzymatic activity.

Interestingly, the estrogen deficiency caused by ovariectomy promoted a significant decrease in the extent of MSC osteogenic differentiation. Several in vivo studies have demonstrated that estrogen deficiency is a major pathogenic factor in bone loss associated with menopause [29]. If there is a deficiency in the production of sex steroids, there is a decrease in the expression of collagen I, non-collagen proteins and alkaline phosphatase in osteoblasts, which reduces synthesis and mineralization of the matrix [30]. In addition, researchers have demonstrated the existence of the estrogen receptor in osteoprogenitor cells from bone marrow [31]. When MSC cultures were grown in osteogenic differentiation medium supplemented with different concentrations of estrogen (E2), there was an increase in the levels of osteocalcin expression and calcium deposition [32]. Other studies have demonstrated that MSC primary cultures isolated from ovariectomized mice displayed a significant increase in protein expression, alkaline phosphatase activity and expression of collagen I after in vitro treatment with E2 [33].

We have demonstrated that NOS inhibition reduces the osteogenic differentiation of MSCs from normal rats, indicating that nitric oxide is an important factor in the induction of mesenchymal stem cell differentiation into osteoprogenitor cells. There is evidence in the literature to suggest that NO plays an important role in bone metabolism [17]. In vitro studies have indicated that NO has a biphasic effect on osteoblast and osteoclast activity: small amounts of NO may act as an autocrine stimulator of osteoblast activity and expression of collagen I after in vivo treatment with E2 [33].

The number of mineralized nodules was larger in the sedentary osteopenic group treated with L-NAME as compared to the sedentary osteopenic group. The increase in osteogenic capacity of MSCs found in the sedentary osteopenic rats treated with L-NAME agrees with the data obtained by morphometric analysis of the bone mass in vivo (data not shown). These observations pave the way for further studies to understand the underlying causes of the differences
between normal and osteopenic rats when nitric oxide synthase is inhibited.

Several studies have shown that the anabolic effect of estrogen on bone seems to be mediated by NO [37] once that the protective effect of estrogen on ovariectomy-induced bone loss in rats is inhibited by the NOS inhibitor L-NAME [38]. NO is also involved in mediating estrogen-induced stimulation of bone formation and NO production plays an important role in the regulation of osteoblastic function as assessed in vivo and in vitro [19]. In fact, estrogens stimulate the synthesis and release of NO through the stimulation of endothelial NOS gene expression [39].

Surprisingly, physical activity increased the osteogenic potential of MSCs from rats that had a deficiency in sex steroid production. Alkaline phosphatase activity is an early marker of osteogenic differentiation [40]; therefore, the effects of physical activity are evident even at early stages of osteogenic differentiation. Although there was no difference in collagen synthesis between the active and sedentary groups, the number of mineralized nodules was significantly increased in the active osteopenic group as compared to the sedentary osteopenic group.

The effect of physical inactivity in the genesis of post-menopausal osteoporosis is well known [4–6], but the effects of physical activity upon the osteogenic differentiation of MSCs in vivo are unknown. Until now, it has only been shown that MSC cultures submitted to vibrations exhibit a greater degree of osteogenic differentiation than those not vibrated [15]. It has previously been demonstrated that daily physical activity on an automatic treadmill promotes an increase in the bone mass of the whole skeleton of osteopenic rats [13]. The effects of exercise may be directly due to mechanical force, or they may be indirectly mediated by hormones, growth factors and cytokines [3,7–11]. The effects of these hormones explain the effects of physical activity on the morphology, number and activity of bone cells in the bones of osteopenic rats subjected to physical activity [13]. Our results show, however, that physical activity also has positive effects on the osteogenic differentiation of MSCs from osteopenic rats.

Increasing the osteogenic capacity of MSCs from osteopenic rats may be one of the mechanisms involved in the improvement of bone mass of female rats with a sex steroid deficiency subjected to daily physical activity.

It has previously been demonstrated that physical activity stimulates the local and systemic NO production and that the autocrine action of NO stimulates synthesis of bone matrix [12,17]. Studies have shown that the NO production by bone cells in response to fluid shear stress is rapid and rate-dependent. These results might provide a basis for a dynamic stimulation of adaptive bone formation for predicting bone loss under environments of unloading as in prolonged bed rest or microgravity [12]. The NO release has been determined as parameters of bone cell responsiveness, since signaling molecules have been shown to be essential for the anabolic response of bone to mechanical loading [20]. In addition, researchers have shown that NO synthesis can be induced in osteoblasts and osteocytes by mechanical strain and shear stress [41–43]. [13] showed that daily physical activity on an automatic treadmill promotes an increase in the bone mass of the whole skeleton of osteopenic rats due to osteoblastic hyperplasia. Our initial hypothesis was that local NO levels mediate the beneficial effects of physical activity and increase the extent of MSC differentiation into osteoprogenitor cells. Our experimental data support this initial hypothesis because the increase in MSC osteogenic potential from the osteopenic rats subjected to physical activity was not observed in active osteopenic rats that were treated with a NOS inhibitor.

Our results suggest that the NO effects in bone metabolism are also due to increased MSC differentiation into osteoprogenitor cells and that NO is a mediator of the beneficial effects of physical activity upon bone mass in osteopenic rats.

We conclude that NOS inhibition reduces the osteogenic potential of bone marrow MSCs from normal rats. Daily physical activity on an automatic treadmill increases the osteogenic potential of bone marrow MSCs from osteopenic rats. NOS Inhibition in vivo had a negative effect upon the osteogenic potential of MSCs from normal and osteopenic rats subjected to physical activity.

Acknowledgments

This work was supported by grants from the Fundação de Amparo à Pesquisa de Minas Gerais (Fapemig), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Edital Capes Pró-equipamentos Nº01/2007).

References


