

· 基础研究 ·

甲状旁腺素联合骨髓间充质干细胞对骨质疏松大鼠股骨颈骨密度相关指标的影响

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【摘要】 目的 探索骨髓间充质干细胞(BMSCs)联合甲状旁腺激素(PTH)与二者单独使用对骨质疏松性大鼠骨密度相关指标的影响。**方法** 将50只SD雌性大鼠分为A、B、C、D、E组,每组10只,B、C、D、E组切除双侧卵巢,A组大鼠仅切除卵巢周围同等大小的脂肪组织。术后12周各组抽取3只大鼠,检测大鼠的雌激素水平及骨密度相关指标骨小梁数量(Tb.N)、骨小梁厚度(Tb.Th)、骨小梁分离度(Tb.Sp)和骨体积分数(BV/TV)。脱颈法处死SD健康雄性大鼠取骨髓进行BMSCs细胞培养,流式细胞技术单标法检测BMSCs是否培养成功。术后12周,各组剩余7只大鼠。其中A和B组不做后期干预处理;C组按60 μg/kg剂量背部皮下注射Rat PTH 1-34,隔日1次,连续12周;D组显露双侧股骨颈,注入浓度为 5×10^8 /ml的BMSCs;E组在D组的基础上在皮下注射PTH,注射浓度、频率与C组一致。所有大鼠均在干预12周后处死,Micro-CT测量5组大鼠股骨颈Tb.N、Tb.Th、Tb.Sp和BV/TV,观察成骨情况。使用SPSS 20.0软件进行统计分析。组间比较采用单因素方差分析,有统计学意义再进行LSD两两比较。**结果** 术后12周,与A组比较,B、C、D及E组大鼠雌激素水平、Tb.N、Tb.Th及BV/TV显著下降,Tb.Sp明显升高,差异有统计学意义($P<0.05$),但B、C、D及E组各组间上述指标差异不明显($P>0.05$),可证明B、C、D及E组大鼠为骨质疏松模型。流式细胞技术单标法显示,P3代BMSCs表达CD29(99.86%)和CD90(99.73%),几乎不表达CD34(6.48%)和CD45(0.94%),证明BMSCs培养成功。干预12周后,Micro-CT数据显示,单独使用PTH或BMSCs及联合使用均可改善骨微观结构,但均未达到A组水平。与B组比较,C、D、E组的Tb.N、Tb.Th及BV/TV显著增加,Tb.Sp显著下降,差异有统计学意义($P<0.05$);与C或D组比较,E组Tb.N、Tb.Th及BV/TV显著增加,Tb.Sp显著下降,差异有统计学意义($P<0.05$)。**结论** BMSCs联合PTH治疗局部骨质疏松的效果优于单独使用治疗的效果。

【关键词】 骨髓间充质干细胞;甲状旁腺激素;骨质疏松

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Effect of parathyroid hormone combined with BMSCs transplantation on femur bone density in osteoporotic rats

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【Abstract】 Objective To explore the effects of bone marrow mesenchymal stem cells (BMSCs) transplantation or parathyroid hormone (PTH) alone or combined together on related indices of bone density in osteoporotic rats. **Methods** Fifty female SD rats were divided into groups A, B, C, D and E, 10 animals in each group. Bilateral ovaries were removed in the rats from groups B, C, D and E, while for those from group A, adipose tissue around the ovaries was only removed. In 12 weeks after operation, 3 rats were selected from each group, and then the estrogen level and bone density related indices such as trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp) and bone volume to tissue volume (BV/TV) were measured. SD healthy male rats were sacrificed to isolate and culture BMSCs, and the obtained cells were further identified with single labeling flow cytometry. At 12 weeks after operation, except those from groups A and B getting no intervention, subcutaneous injection of 60 μg/kg rat PTH 1-34 on the back (once every other day, for 12 consecutive weeks) was given to group C and the injection of 5×10^8 /ml BMSCs on the bilateral femoral neck were given to the group D, and group E were given the same intervention as group C on the basis of group D. The rest 7 rats in five groups were sacrificed in 12 weeks after intervention. Tb.N, Tb.Th, Tb.Sp and BV/TV of femoral neck were measured

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by micro-CT scanning. SPSS statistics 20.0 was used for statistical analysis. One-way ANOVA was employed for comparison among groups, and LSD test was applied for those with statistical significance. **Results** Compared with group A, the estrogen level, Tb. N, Tb. Th and BV/TV in groups B, C, D and E were decreased significantly, and Tb. Sp was increased significantly ($P < 0.05$), but there was no significant differences among groups B, C, D and E ($P > 0.05$), and the rats in group B, C, D and E were identified as osteoporotic rats. Single-labeling flow cytometry showed that CD29 (99.86%) and CD90 (99.73%) were expressed in the third passaged (P3) BMSCs, while CD34 (6.48%) and CD45 (0.94%) were rarely expressed, which proved that BMSCs were successfully cultured. After 12 weeks of intervention, micro-CT scanning showed that PTH or BMSCs alone, or combination improved bone micro-structure, but they did not reach the level of group A. Compared with group B, Tb. N, Tb. Th and BV/TV in groups C, D and E were increased significantly, while Tb. Sp was decreased significantly ($P < 0.05$). Compared with groups C or D, Tb. N, Tb. Th and BV/TV in group E were increased significantly and Tb. Sp was decreased significantly ($P < 0.05$). **Conclusion** The effect of BMSCs combined with PTH in the treatment of local osteoporosis is better than that of single use.

【Key words】 bone marrow mesenchymal stem cells; parathyroid hormone; osteoporosis

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骨质疏松症是因骨量丢失、骨强度降低及骨微观结构改变从而增加了脆性骨折风险的一种疾病,它给医学带来了挑战并对社会经济造成了威胁。骨质疏松患者的终生骨折风险高达40%,而发生骨折最常见的部位为脊柱、髋部及手腕,其他部位也可发生,如肱骨、肋骨等^[1]。骨质疏松症的药物治疗主要分为两类,一类为促骨形成类药物,如甲状旁腺素(parathyroid hormone, PTH),代表药物特立帕肽;另一类为抗骨吸收药物,如双磷酸盐类^[2]。从细胞水平、动物实验及患者临床的应用方面,单纯应用PTH治疗骨质疏松的有效性已被证实^[3-5],但PTH联合用药存在局限性,如不推荐与抗骨吸收类药物联用^[6],是否能寻找另一种非药物途径来联合PTH进行骨质疏松治疗是一个亟待解决的临床问题。骨髓间充质干细胞(bone marrow mesenchymal stem cells, BMSCs)广泛存在于人体骨髓及松质骨中,可在体内进行自我复制,并成为骨组织修复以及相关疾病的治疗选择^[7]。BMSCs具有多向分化能力,目前在体外的提纯与扩增技术已成熟。基于上述背景,本研究在体外构建组织工程扩增BMSCs,再将其注入骨质疏松大鼠模型的股骨颈内,随后单独或联合使用PTH,通过Micro-CT测量反映骨密度的相关指标变化,初步研究BMSCs联合PTH对骨质疏松性大鼠的局部成骨效果。

1 材料与方法

1.1 试剂与设备

大鼠雌激素ELISA试剂盒(美旋生物科研试剂上海有限公司);水合氯醛(天津盛鑫源化学试剂有限公司);胎牛血清(ScienCell生命科技有限公司);DMEM/F12 1:1培养基、无菌PBS溶液、青霉素-

链霉素双抗溶液、含EDTA的0.25%胰蛋白酶(武汉博士德生物工程有限公司);成骨细胞诱导液(Cyagen Biosciences,美国);Rat PTH 1-34(默克生命科学上海有限公司);全波长酶标仪Eppendorf(中国生命科技有限公司);Micro-CT(SCANCO Medical AG Inc,瑞士)。

1.2 实验动物及骨质疏松模型构建

SD IGS 雌性大鼠50只,2月龄,SPF级,体质量(210 ± 20)g,饲养于山西医科大学实验中心动物房,正常摄食、进水。将50只雌性大鼠按随机数字表法分为A、B、C、D、E 5组,每组10只。浓度8%水合氯醛腹腔麻醉,B、C、D、E组经大鼠腰背部切口进入腹腔切除双侧卵巢,A组仅切除卵巢周围同等大小的脂肪组织。手术后12周,分别在每组按抽样检验法抽取3只大鼠,心脏穿刺取血液2 ml,1000转/min离心10 min,收集血清加入大鼠雌激素试剂盒,450 nm酶标仪测定各孔吸光度值,分别计算各组大鼠雌激素平均浓度。随后将每组抽取的3只大鼠处死,采用Micro-CT检测股骨颈的骨密度相关指标,即骨小梁数量(trabecular number, Tb. N)、骨小梁厚度(trabecular thickness, Tb. Th)、骨小梁分离度(trabecular separation, Tb. Sp)和骨体积分数(bone volume to tissue volume, BV/TV)。

1.3 BMSCs培养与鉴定

健康SD IGS 雄性大鼠5只,4周龄,SPF级,平均体质量80 g,相关指标符合实验动物标准。脱颈法处死,碘伏全身浸泡消毒5 min,超净工作台上分离双侧股骨,剪掉两端骨骺,用含10% FBS的DMEM/F12 1:1完全培养基冲出骨髓至15 ml离心管中,反复吹打,1000转/min离心3 min,弃上清,加入8 ml完全培养基吹打混匀后接种在4个

25 cm² 规格的培养瓶中, 4 ml/瓶, 置 37℃、5%CO₂ 浓度的培养箱中培养。48 h 后半量更换培养基, 以后每 3 d 全量更换新鲜培养基。待细胞铺满培养瓶底至细胞融合成单层、密度为 80%~90% 时, 用 0.25% 胰酶消化, 按 1:2 的比例进行传代培养。待传至 P3 代时, 选择生长状态良好的细胞至离心管, 0.25% 胰酶消化, 4℃ 离心, 1000 转/min, 离心 3 min, 用 PBS(含 1% 胎牛血清) 充分洗涤细胞 3 次, 重悬细胞, 各管依次加入单克隆抗体 CD34、CD45、CD29、CD90, 流式细胞仪进行检测分析。

1.4 术后干预

术后 12 周, 每组剩余 7 只大鼠。A、B 组不做干预处理, 其中 B 组为骨质疏松空白对照组; C 组按 60 μg/kg 剂量背部皮下注射 Rat PTH 1-34, 隔日 1 次^[8], 连续注射 12 周(PTH 干预组); D 组显露双侧股骨颈, 采用旋转进针法注入 0.5 ml 浓度为 5×10⁸/ml 的 BMSCs(BMSCs 干预组); E 组在 D 组基础上背部皮下注射 Rat PTH 1-34, 注射浓度、频率与 C 组一致(PTH 联合 BMSCs 干预组)。

1.5 干预后大鼠股骨颈骨密度相关指标变化

各组剩余大鼠均在干预 12 周后处死。大鼠经解剖、分离双侧股骨, 小心剔除周围肌肉后经 Micro-CT 检测 Tb. N、Tb. Th、Tb. Sp 及 BV/TV, 并行三维图像重建。

1.6 统计学处理

采用 SPSS 20.0 软件进行统计分析。计量资料以均数±标准差($\bar{x}\pm s$)表示, 组间比较采用单因素方差分析, 有统计学意义再进行 LSD 两两比较。 $P<0.05$ 为差异有统计学意义。

2 结果

2.1 骨质疏松大鼠模型鉴定

术后 12 周, A 组大鼠雌激素浓度为 (496.2±14.7) ng/L, B、C、D 及 E 组大鼠雌激素浓度分别为

(361.7±17.3)、(359.3±15.3)、(362.8±13.9) 和 (360.9±16.5) ng/L, 与 A 组比较, B、C、D 及 E 组雌激素水平均显著下降, 差异有统计学意义($P<0.05$), 但 B、C、D 及 E 组间差异不明显($P>0.05$)。Micro-CT 检测 5 组股骨颈骨密度相关性指标发现, A 组 Tb. N、Tb. Th、BV/TV 均明显高于其余 4 组, Tb. Sp 明显低于其余 4 组, 差异均有统计学意义($P<0.05$), 但 B、C、D、E 组各组间骨密度相关性指标结果差异无统计学意义($P>0.05$; 表 1)。由此可以证明 B、C、D、E 组骨质疏松大鼠模型建立成功^[9]。

2.2 BMSCs 形态与鉴定

BMSCs 接种 24 h 后观察可见部分细胞贴壁, 48 h 后光镜下可见培养瓶底部有少量梭形细胞, 高倍镜下核仁清晰可见。3 d 后再次换液可见密集的细胞团, 呈漩涡状(图 1)。传代后细胞贴壁较原代快, 形态均匀, 生长迅速。流式细胞技术单标法显示, P3 代细胞表达 CD29(99.86%)、CD90(99.73%), 几乎不表达 CD34(6.48%)、CD45(0.94%)(图 2), 表明本研究成功获取并培养了 BMSCs。

2.3 干预 12 周后骨密度相关指标变化

Micro-CT 数据显示, 单独使用 PTH、注入 BMSCs 及联合使用二者均可改善骨微观结构, 但均未达到与 A 组相似的正常水平。与 B 组比较, C、D、E 组的 Tb. N、Tb. Th 及 BV/TV 显著增加, Tb. Sp 显著下降, 差异有统计学意义($P<0.05$); 与 C 或 D 组比较, E 组 Tb. N、Tb. Th 及 BV/TV 亦显著增加, Tb. Sp 显著下降, 差异有统计学意义($P<0.05$; 表 2)。各组 Micro-CT 骨形态学三维重建见图 3。

3 讨论

随着“老年世纪”的到来, 如何有效治疗骨质疏松症已成为当前临床的热点问题, 而成功构建骨质疏松的动物模型是实验进行的关键一步。切除卵巢后的大鼠模型与绝经后女性在骨质疏松生理变化过

表 1 术后 12 周各组大鼠股骨颈 Micro-CT 检测数据

Table 1 Micro-CT examination data of femoral neck of each group at 12 weeks after surgery ($n=3, \bar{x}\pm s$)

Item	Group A	Group B	Group C	Group D	Group E
Tb. N(1/mm)	1.761±0.031	0.943±0.036 *	0.952±0.029 *	0.937±0.044 *	0.983±0.027 *
Tb. Th(mm)	0.165±0.020	0.090±0.017 *	0.089±0.013 *	0.093±0.016 *	0.088±0.019 *
Tb. Sp(mm)	0.985±0.035	2.971±0.043 *	2.945±0.040 *	2.897±0.053 *	2.953±0.037 *
BV/TV(%)	0.199±0.023	0.122±0.017 *	0.131±0.019 *	0.119±0.016 *	0.120±0.014 *

A: sham operation group; B: osteoporosis group; C: PTH intervention group; D: BMSCs intervention group; E: PTH-BMSCs intervention group. Tb. N: trabecular number; Tb. Th: trabecular thickness; Tb. Sp: trabecular separation; BV/TV: bone volume to tissue volume. Compared with group A, * $P<0.05$.

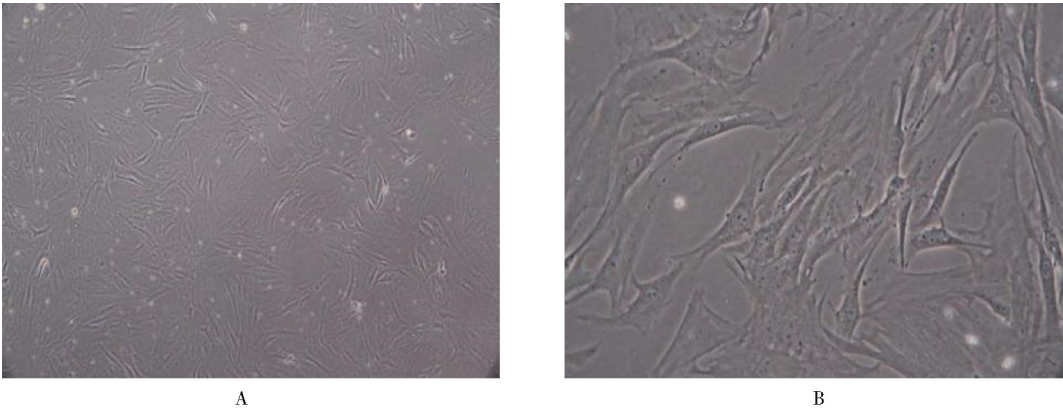


图 1 原代培养 BMSCs 的光学显微镜观察

Figure 1 Primary BMSCs under optical microscope

BMSCs: bone marrow mesenchymal stem cells. A; magnification $\times 100$; B; magnification $\times 400$.

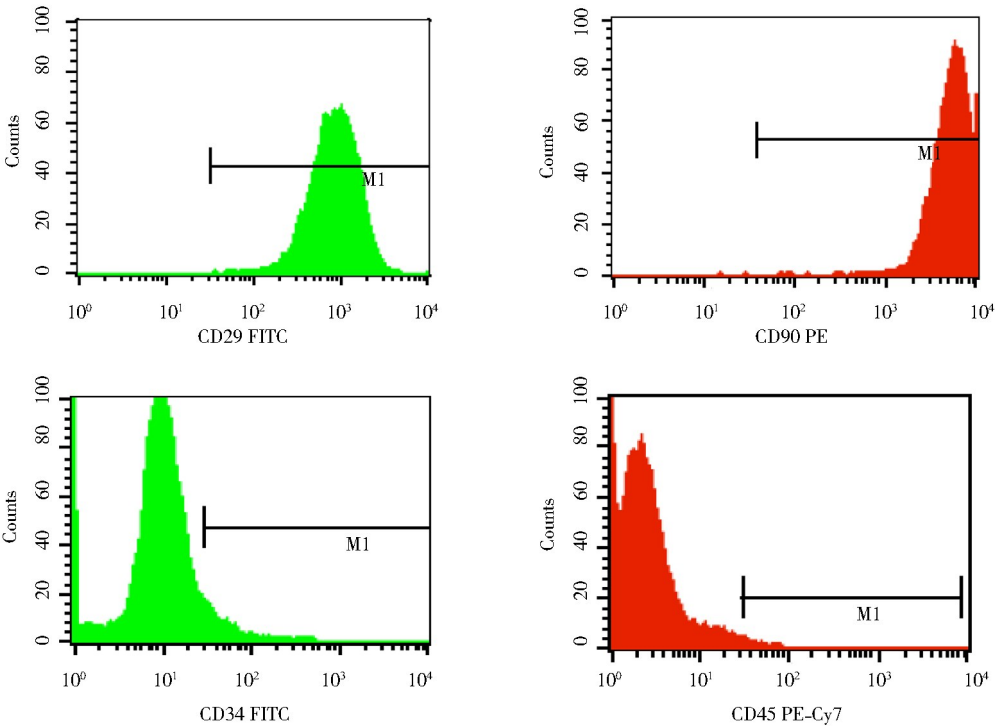


图 2 大鼠 BMSCs 流式细胞技术鉴定

Figure 2 Flow cytometry identification of BMSCs

BMSCs: bone marrow mesenchymal stem cells; FITC: fluorescein isothiocyanate; PE: P-phycoerythrin; PE-Cy7: P-phycoerythrin-Cyanine 7; M1: antibody-binding cells.

表 2 干预 12 周后各组大鼠股骨颈 Micro-CT 检测数据

Table 2 Micro-CT examination data of femoral neck in each group after intervention for 12 weeks ($n=7$, $\bar{x}\pm s$)

Item	Group A	Group B	Group C	Group D	Group E
Tb. N (1/mm)	1.671 $\pm$ 0.033	0.931 $\pm$ 0.049 [*]	1.240 $\pm$ 0.027 ^{*#}	1.114 $\pm$ 0.048 ^{*#}	1.341 $\pm$ 0.065 ^{*#$\Delta$$\blacktriangle$}
Tb. Th (mm)	0.162 $\pm$ 0.022	0.084 $\pm$ 0.017 [*]	0.122 $\pm$ 0.009 ^{*#}	0.100 $\pm$ 0.011 ^{*#}	0.134 $\pm$ 0.010 ^{*#$\Delta$$\blacktriangle$}
Tb. Sp (mm)	0.957 $\pm$ 0.038	2.987 $\pm$ 0.052 [*]	1.946 $\pm$ 0.050 ^{*#}	2.335 $\pm$ 0.083 ^{*#}	1.604 $\pm$ 0.014 ^{*#$\Delta$$\blacktriangle$}
BV/TV (%)	0.198 $\pm$ 0.040	0.118 $\pm$ 0.026 [*]	0.158 $\pm$ 0.029 ^{*#}	0.140 $\pm$ 0.017 ^{*#}	0.171 $\pm$ 0.011 ^{*#$\Delta$$\blacktriangle$}

A; sham operation group; B; osteoporosis group; C; PTH intervention group; D; BMSCs intervention group; E; PTH-BMSCs intervention group. Compared with group A, ^{*} $P<0.05$; compared with group B, [#] $P<0.05$; compared with group C, ^{Δ} $P<0.05$; compared with group D, ^{$\blacktriangle$} $P<0.05$.

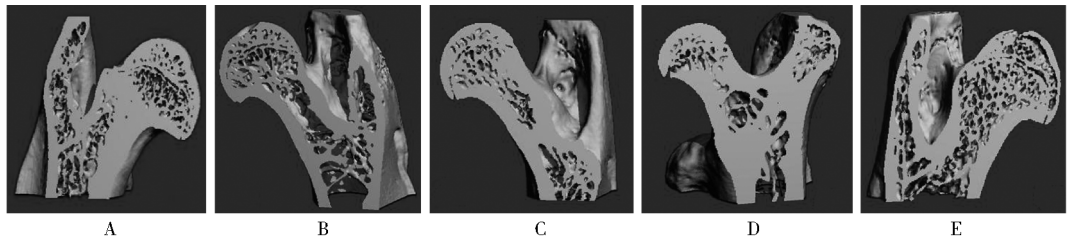


图3 各组大鼠股骨颈三维重建图像

Figure 3 Three-dimensional reconstruction of the femoral neck of each group

A: sham operation group; B: osteoporosis group; C: PTH intervention group; D: BMSCs intervention group; E: PTH-BMSCs intervention group.

程上相似,已成为建立骨质疏松的经典模型之一^[10]。

在正常的人体骨髓中,主要有两类细胞,即起源于造血干细胞的造血细胞和起源于 BMSCs 的间充质细胞^[11]。BMSCs 具有自我更新的多向分化潜能,在实验室中不同的诱导条件下,BMSCs 可分化为成骨细胞、成脂细胞、成软骨细胞等,其数量与活性在骨组织重建及维持骨量、增强骨密度等方面起着至关重要的作用^[12]。有研究表明^[13],老年人群中 BMSCs 的增殖与分化能力均有不同程度下降,导致骨形成速度减慢,其直接后果是骨量的丢失和骨微结构的破坏,这与骨质疏松的病理观察相符。经典胚胎学研究和体外成体器官研究表明,微环境在干细胞的自我更新和分化中起着决定性作用^[14]。本实验结果也表明,与骨质疏松空白对照组比较,BMSCs 干预组及 BMSCs 联合 PTH 干预组中 Tb. N、Tb. Th 及 BV/TV 显著增加,Tb. Sp 显著下降,差异有统计学意义($P<0.05$),这与文献报道一致^[15]。但如何使注入的 BMSCs 更多地 toward 成骨方面增殖、分化将是今后在研究详细机制方面的热点与难点。

目前,一种人工合成的 PTH 已被美国食品药品监督管理局批准,其促骨形成药物已在临床上使用多年。PTH 对骨代谢有双重调节效应,即小剂量间断性应用 PTH 能促进骨髓干细胞向成骨细胞的增殖、分化^[16],而大剂量连续使用则会上调核子- κ B 受体活化因子配体的表达,降低骨保护素的表达,促进破骨细胞的生成并增强其活性,导致骨吸收。本研究选择去势大鼠来模拟临床上的绝经后骨质疏松妇女,随后施加小剂量间断性 PTH(药物治疗)与局部注射 BMSCs(细胞治疗)干预,通过 Micro-CT 骨形态参数对比 PTH 与 BMSCs 单独或联合使用对骨质疏松的改善作用。实验结果显示,单独使用 PTH 或 BMSCs 均可以提高 Tb. N、Tb. Th 及 BV/TV,降低 Tb. Sp 等相关骨性指标,且二者联合使用在抑制骨质疏松大鼠骨量丢失、改善骨微观结构等方面总体效果更好,但其具体机制目前尚不清楚。查阅文

献^[17,18],我们推测大体机制如下。体外培养的 BMSCs 经局部注射后,在骨髓微环境及基质中各种因子的作用下进行自我扩增并向成骨方向分化,这一过程使得注射部位骨量及骨骼强度增加。而 PTH 则是一种能促进 BMSCs 向成骨方向分化的重要激素,可通过与成骨细胞表面受体结合,激活 cAMP/ Ca^{2+} /蛋白激酶 C/磷酸酶 C 等信号通路,增强 I 型胶原等成骨相关基因的表达,刺激成骨细胞的活性,从而促进骨再生,提高骨转换指标,增加新骨的形成,迅速增加骨量,进而提高骨密度,降低发生骨折的风险。

综上,PTH 联合 BMSCs 对骨质疏松大鼠模型的干预效果比单独使用二者效果显著。本研究创新点在于这种联合治疗的方法弥补了临床上对骨质疏松患者无合适联合药物治疗的局限,并已显示出传统治疗方法不可比拟的优势,随着研究的不断深入,相信该联合治疗方法将会为临床上越来越多的骨质疏松患者提供治疗选择。但也存在不足之处,如股骨颈注入 BMSCs 时无特定专用器械致细胞损失,缺乏相应的植入细胞标记及细胞植入后的活体示踪等,需在今后的研究中继续探讨改进,并尝试在椎体、胫骨等多部位注射进行验证。

【参考文献】

- [1] Gutowski JL. Compression fracture in postpartum osteoporosis[J]. J Bone Metab, 2014, 21(2): 161. DOI: 10.11005/jbm.2014.21.2.161.
- [2] 徐杰, 邹勇. 抑郁症的中西医研究进展[J]. 中国中医药远程教育, 2017, 15(16): 156-163. DOI: 10.3969/j.issn.1672-2779.2017.16.066.
- Xu Jie, Zou Y. Research progress of traditional Chinese medicine and western medicine on depression[J]. Chin Med Mod Distance Educ China, 2017, 15(16): 156-163. DOI: 10.3969/j.issn.1672-2779.2017.16.066.
- [3] Jang MG, Ji YL, Yang JY, et al. Intermittent PTH treatment can delay the transformation of mature osteoblasts into lining cells on the periosteal surfaces[J]. J Bone Miner Metab, 2016, 34(5):

- 532–539. DOI: 10.1007/s00774-015-0707-x.
- [4] Hall MP. Biotransformation and *in vivo* stability of protein biotherapeutics; impact on candidate selection and pharmacokinetic profiling[J]. Drug Metab Dispos, 2014, 42(11): 1873–1877. DOI: 10.1124/dmd.114.058347.
- [5] Vrahnas C, Buenzli PR, Pearson TA, *et al.* Differing effects of parathyroid hormone, alendronate, and odanacatib on bone formation and on the mineralization process in intracortical and endocortical bone of ovariectomized rabbits[J]. Calcif Tissue Int, 2018, 103(6): 625–637. DOI: 10.1007/s00223-018-0455-8.
- [6] Tabatabaeimalazy O, Salari P, Khashayar P, *et al.* New horizons in treatment of osteoporosis[J]. Daru, 2017, 25(1): 2. DOI: 10.1186/s40199-017-0167-z.
- [7] Phetfong J, Sanvoranart T, Nartprayut K, *et al.* Osteoporosis: the current status of mesenchymal stem cell-based therapy[J]. Cell Mol Biol Lett, 2016, 21: 12–17. DOI: 10.1186/s11658-016-0013-1.
- [8] Bonnet N, Conway SJ, Ferrari SL. Regulation of beta catenin signaling and parathyroid hormone anabolic effects in bone by the matricellular protein periostin[J]. Proc Natl Acad Sci USA, 2012, 109(37): 15048–15053. DOI: 10.1073/pnas.1203085109.
- [9] 郭鱼波, 马如风, 牛建昭, 等. 骨质疏松动物模型及其评价方法的研究进展[J]. 中国骨质疏松杂志, 2015(9): 1149–1154. DOI: 10.3969/j.issn.1006-7108.2015.09.027.
- Guo YB, Ma RF, Niu JZ, *et al.* The research progress in animal osteoporotic models and the evaluating methods [J]. Chin J Osteoporosis, 2015(9): 1149–1154. DOI: 10.3969/j.issn.1006-7108.2015.09.027.
- [10] Kalu DN. The ovariectomized rat model of postmenopausal bone loss[J]. Bone Miner, 1991, 15(3): 175–191.
- [11] Zhang LX, Balani YM, Trinh S, *et al.* Differential effects on bone and mesenchymal stem cells caused by intermittent and continuous PTH administration [J]. Natl Med J China, 2018, 98(10): 781–787. DOI: 10.3760/cma.j.issn.0376-2491.2018.10.014.
- [12] Vial IN, Peter Rubin J. Commentary on: isolation and differentiation potential of human mesenchymal stem cells from adipose tissue harvested by water jet-assisted liposuction [J]. Aesthet Surg J, 2015, 35(8): 1040–1041. DOI: 10.1093/asj/sjv176.
- [13] Moerman EJ, Teng K, Lipschitz, DA, *et al.* Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPAR- γ 2 transcription factor and TGF- β /BMP signaling pathways [J]. Aging Cell, 2004, 3(6): 379–389. DOI: 10.1111/j.1474-9728.2004.00127.x.
- [14] Wright EG, Lorimore SA, Lord BI. Effect of haemopoietic stem-cell proliferation regulators on early and late spleen colony-forming cells[J]. Cell Tissue Kinet, 1985, 18(2): 193–199.
- [15] Ocarino Nde M, Boeloni JN, Jorgetti V, *et al.* Intra-bone marrow injection of mesenchymal stem cells improves the femur bone mass of osteoporotic female rats[J]. Connect Tissue Res, 2010, 51(6): 426–433. DOI: 10.3109/03008201003597049.
- [16] Sang WK, Pajevic PD, Selig M, *et al.* Intermittent parathyroid hormone administration converts quiescent lining cells to active osteoblasts[J]. J Bone Miner Res, 2012, 27(10): 2075–2084. DOI: 10.1002/jbmr.1665.
- [17] Kushida T, Inaba M, Hisha H, *et al.* Intra-bone marrow injection of allogeneic bone marrow cells: a powerful new strategy for treatment of intractable autoimmune diseases in MRL/lpr mice [J]. Blood, 2001, 97(10): 3292–3299. DOI: 10.1182/blood.V97.10.3292.
- [18] Lotinun S, Sibonga JD, Turner RT. Evidence that the cells responsible for marrow fibrosis in a rat model for hyperparathyroidism are preosteoblasts [J]. Endocrinology, 2005, 146(9): 4074–4081. DOI: 10.1210/en.2005-0480.

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