·论著·

左、右归丸及其拆方含药血清对骨髓间充质干细胞增殖和成骨诱导的影响

何文智[1] 宋囡[2] 王智民[3] 任艳玲[4*]
(1. 辽宁中医药大学药学院,沈阳 110032; 2. 辽宁中医药大学基础医学院辽宁中医药大学附属第二医院;
3. 辽宁中医药大学第一临床学院;4. 辽宁中医药大学基础医学院)

中图分类号: R681 **文献标识码**: A **文章编号**: 1006-7108(2013)07-0697-06

摘要: **目的** 观察左、右归丸及其拆方含药血清对体外培养的大鼠骨髓间充质干细胞(bone marrow mesenchymal stem cells, BMSCs)增殖和向成骨细胞(osteoblast, OB)诱导的影响。**方法** 运用全骨髓贴壁法分离和培养大鼠BMSCs;分别以左归丸、右归丸、两方共同药、滋肾阴药、补肾阳药、阳性对照药补佳乐制备的大鼠含药血清加诱导剂(地塞米松、维生素 C、β-甘油磷酸钠)、诱导剂和空白含药血清组共 8 组对 BMSCs 进行干预,采用 MTT 法检测 BMSCs 增殖和左、右归丸及其拆方含药血清对 BMSCs 增殖的影响;流式细胞仪对 BMSCs 进行鉴定;用 PNPP 法检测左、右归丸及其拆方含药血清对 BMSCs 向 OB 诱导的影响。**结果** P4 代以后细胞细胞表型 CD90 呈阳性表达,CD11b/c、CD45 呈阴性表达。左、右归丸及其拆方含药血清对 BMSCs 增殖和成骨诱导都具有促进作用,左归丸、右归丸、滋肾阴药组与其他组比较效果显著($P<0.05$)。**结论** 左、右归丸及其拆方含药血清可以协同诱导剂对 BMSCs 增殖和成骨诱导具有显著的促进作用。

关键词: 左归丸;右归丸;骨髓间充质干细胞;细胞增殖;成骨诱导

Effect of Zuogui pill, Yougui pill, and their containing serum on the proliferation and osteogenic induction of bone marrow mesenchymal stem cells

HE Wenzhi[1], SONG Nan[2], WANG Zhimin[3], REN Yanling[4]
(1. College of Pharmacy; 2. College of Basic Medicine & the Second Affiliated Hospital; 3. The First Clinical Hospital;
4. College of Basic Medicine, Liaoning University of Traditional Chinese Medicine, Shenyang 110032, China)
Corresponding author: REN Yanling, Email: yanlingren@tom.com

Abstract: **Objective** To observe the effect of Zuogui pill, Yougui pill, and their containing serum on the proliferation of rat bone marrow mesenchymal stem cells (BMSCs) cultured in vitro and the induction of BMSCs differentiated into osteoblasts (OB). **Methods** Rat BMSCs were isolated using adherence method and then cultured with different medium. Serum containing Zuogui pill, Yougui pill, both of Zuogui and Yougui pill, nourishing the kidney drugs, tonifying the kidney drugs, and positive control drugs (Bujiale) was collected. Then, BMSCs were cultured with the drug containing-serum plus inductive agents (including dexamethasone, vitamin C, and β-sodium glycerophosphate), inductive agents, or drug-containing serum. The proliferation of BMSCs was detected using MTT method. BMSCs were identified using flow cytometry. The effect of Zuogui pill, Yougui pill, and their containing serum on inducing BMSCs differentiating into OB was detected using PNPP method. **Results** At the 4th passage, CD90 was positive, while CD11b/c and CD45 were negative at the surface of the cells. Zuogui pill, Yougui pill, and their containing serum could significantly promote the proliferation and osteogenic induction of BMSCs. Zuogui pill, Yougui pill, and nourishing the kidney drugs had significant effect when compared with others ($P<0.05$). **Conclusion** Zuogui pill, Yougui pill, and their containing serum, plus with inductive agents, all could significantly promote the proliferation and osteogenic induction of BMSCs.

基金项目: 辽宁省教育厅创新团队项目(LT2010068),辽宁省高等学校优秀人才支持计划(LR201025),辽宁省科技厅自然科学基金项目计划(201102148)

* 通讯作者: 任艳玲,Email: yanlingren@tom.com

Key words: Zuogui pill; Yougui pill; Bone marrow mesenchymal stem cells; Cell proliferation; Osteogenic induction

骨质疏松症(osteoporosis,OP)是一种以低骨量和骨组织微结构破坏为特征,导致骨骼脆性增加,出现腰背、四肢疼痛,脊柱畸形甚至骨折的全身性疾病,以原发性骨质疏松症最为常见[1]。左、右归丸均始载于明·张介宾《景岳全书》,左归丸能滋肾补阴,用于真阴不足证,症见腰酸膝软、盗汗、神疲口燥等;右归丸诸药配伍体现"阴中求阳"法则,阳得阴助,生化无穷,共具温阳益肾、填精补血以收培补肾中元阳之效。前期研究显示[2-4],左、右归丸皆可有效防治绝经后骨质疏松。左、右归丸均可干预骨髓间充质干细胞(bone marrow mesenchymal stem cells, BMSCs)[5-6],但尚未见二者的比较研究。本实验现就左、右归丸及其拆方含药血清对体外培养的大鼠BMSCs的作用展开实验研究,建立稳定的BMSCs培养体系,为研究中药干预BMSCs的疗效及作用机制提供实验依据。

1 材料与方法

1.1 实验动物

SPF级SD大鼠70只,2月龄,雌雄各半,体重210±10 g,购于辽宁长生生物技术有限公司,许可证号:SCXK(辽)2010-0001,用于制备含药血清。提取BMSCs采用上述雄性大鼠2只。

1.2 主要药物及试剂

中药饮片购买于辽宁中医药大学附属第一医院,左归丸(成分:熟地黄24 g、炒山药12 g、枸杞子12 g、山茱萸12 g、鹿胶炒珠12 g、制菟丝子12 g、川牛膝酒炙9 g、龟胶炒珠12 g)、右归丸(成分:熟地黄24 g、炒山药12 g、枸杞子12 g、山茱萸9 g、鹿角炒珠12 g、制菟丝子12 g、当归9 g、肉桂6 g、杜仲12 g、附子6 g)、共同药方(成分:熟地黄24 g、炒山药12 g、枸杞子12 g、山茱萸9 g、鹿胶炒珠12 g、制菟丝子12 g)、滋肾阴药方(成分:熟地黄24 g、炒山药12 g、枸杞子12 g、山茱萸12 g、龟板胶12 g)、补肾阳药方(成分:肉桂6 g、杜仲12 g、制附6 g、鹿角胶12 g、菟丝子12 g),以上五种药方自制成水煎剂,生药量为1 g·ml^{-1};补佳乐戊酸雌二醇片(法国DELPHARM Lille S. A. S. 批号国药准字J20080036);改良型α-MEM培养液、胎牛血清(FBS)(Hyclone);青链霉素混合液(100×)(Genview);CD45抗体、CD11b抗体、CD90抗体(Biolegend);PBS(北京鼎国昌盛);胰酶、MTT、Triton X-100、β-甘油磷酸钠、维生素C、地塞米松(Sigma)。

1.3 主要仪器

HF safe-1200型生物安全柜(上海力申);3111型CO_2培养箱(Thermo,美国);DFC320型倒置显微镜(Leica,美国);iMark型酶标仪(Bio-Rad,美国);FACSCailbur流式细胞仪(BD,美国)。

1.4 含药血清的制备与实验分组

将70只大鼠随机分为7组:左归丸组(ZGW)、右归丸组(YGW)、两方共同药组(GTY)、滋阴药组(ZYY)、补阳药组(BYY)、补佳乐组(BJL)、空白对照组(Control),每组10只,雌雄各半,按每kg体重大鼠的给药量为人的6.3倍计算,并且每天给大鼠灌胃量为正常人用药量的2倍,每日早晚各灌胃1次,空白对照组灌服蒸馏水,于第4 d给药2 h后,腹腔注射10%水合氯醛麻醉,采用大鼠腹主动脉取血,静置2 h后,离心(2500 rpm,4℃,20 min),收集血清,56℃水浴灭活30 min,分装后,-86℃保存。实验分为8组,ZGW+诱导剂(YDJ)组、YGW+YDJ组、GTY+YDJ组、ZYY+YDJ组、BYY+YDJ组、BJL+YDJ组,以上各组含有相应的10%药物血清及诱导剂(含10^{-7}mol·L^{-1}地塞米松、50 μmol·L^{-1}维生素C、10 mmol·L^{-1}β-甘油磷酸钠的α-MEM培养液)、YDJ组(含10% FBS的诱导剂)、Control组(含10% Control组含药血清的α-MEM培养液)。

1.5 大鼠BMSCs的分离与培养

取2月龄SPF级SD雄性大鼠脱颈处死,75%乙醇浸泡10 min后,无菌条件下取双侧股骨,去除骨上附着软组织后,转移至超净工作台。去除两侧骨骺端,用5 ml含1×10^{5}U·L^{-1}青霉素、100 mg·L^{-1}硫酸链霉素的改良型α-MEM培养液反复冲洗骨髓腔,至骨髓腔变白,反复吹打成单细胞悬液,将其移入15 ml无菌离心管中,1000 r·min^{-1}离心3 min,倾去上清液后,加入含体积分数10% FBS的基础培养液(含1×10^{5}U·L^{-1}青霉素、100 mg·L^{-1}硫酸链霉素的α-MEM培养液),再悬细胞,接种于25 cm^{2}培养瓶,置37℃、5% CO_2细胞培养箱中培养。接种后第4d首次换液,以后每3 d换液1次。培养10~14 d,待细胞长至80%融合时,用胰酶消化液进行消化、传代。

1.6 BMSCs鉴定

P4代细胞经PBS洗三遍,胰酶消化后,再用1

ml PBS 重悬后转入 1.5 ml EP 管中，离心 1500 rpm，5 min，1 ml PBS 重悬，细胞浓度为 1×10^6 个·ml^{-1}，分别加入 CD45 抗体、CD11b/c 抗体、CD90 抗体，避光，4℃，30 min 孵育后，再用 PBS 清洗一次，0.3 ml PBS 再次重悬，上流式细胞仪检测。

1.7 BMSCs 生长曲线绘制

第三代 BMSCs 经胰酶消化，用含体积分数 10% FBS 的基础培养液分别稀释成 1×10^5 个·ml^{-1}、5×10^4 个·ml^{-1}、3×10^4 个·ml^{-1}的细胞悬液，以每孔 200 μl 接种于 96 孔培养板上，调零孔不加细胞，每组 8 复孔，标记后置 37℃、5% CO_2 细胞培养箱中孵育，分别在第 1 d、2 d、3 d、4 d、5 d、6 d、7 d、8 d、9 d 每孔加入预先配制的 5 mg·ml^{-1} MTT 溶液 10 μl，于 37℃、5% CO_2 细胞培养箱中继续孵育 4 h 终止培养，弃去孵育液每孔加入 DMSO 100 μl，室温振荡混匀 15 min。待沉淀完全溶解后，酶标仪上以 490 nm 测定各孔光密度（optical density, OD），结果以 OD490 值表示细胞增殖水平。

1.8 MTT 法检测 BMSCs 增殖率

细胞接种同“1.7”。24 h 后大部分细胞贴壁，第 4 d 换用含体积分数 0.2% 的基础培养液饥饿培养，细胞同步化 24 h 后吸弃旧培养液，每组按照“1.4”的要求加入对应含药血清培养液 200 μl，每组 8 复孔，继续培养 6 d、9 d、12 d。每孔加入 5 mg·ml^{-1}的 MTT 溶液 10 μl，于 37℃、5% CO_2 细胞培养箱中孵育 4 h 后，弃去孵育液每孔加入 DMSO 100 μL，室温振荡混匀 15 min。待沉淀完全溶解后，酶标仪上以 490 nm 测定 OD 值。

1.9 PNPP 法检测成骨细胞碱性磷酸酶（ALP）活性

细胞接种同“1.7”。细胞饥饿处理同“1.8”，细胞同步化 24 h 后吸弃旧培养液，加入培养液 180 μl，预孵育细胞 30 min 后，加入 20 μl 各组含药血清，每组 8 复孔，继续培养 6 d、9 d、12 d。弃培养液，每孔用 4℃预冷的 PBS 洗涤 3 次，加入 100 μl 0.1% Triton X-100 裂解液，4℃过夜；在 20℃和 4℃间反复冻融 3 次，加入 pNPP-DEA 缓冲液（pNPP 液：DEA 液 = 1∶1，现配现用）100 μl，立即混匀后，37℃恒温振荡反应 30 min，立即加入 80 μl 0.5 M NaOH 溶液终止反应。酶标仪上 415 nm 测定 OD 值。

2 统计学分析

计量数据采用 SPSS 13.0 软件处理，用 One-Way Anova 进行分析，结果用均数 ± 标准差（$\bar{x} \pm s$）表示，组间比较采用方差分析，$P < 0.05$ 有统计学意义。

3 结果

3.1 BMSCs 的形态学特征

全骨髓贴壁法分离的 BMSCs 呈圆形，胞体透亮，折光性强，大小不一，不能辨认细胞核，细胞与周围的红细胞等血系细胞互相混杂。24 h 后即可见少数贴壁细胞，刚贴壁的细胞仍保持圆形，48 h 贴壁细胞分裂增殖，开始呈三角形、多角形，形成 3～10 个细胞的细胞团。随着培养时间的延长，细胞深处较多突起，有的突起相互连接。最初 2～5 d 细胞增殖慢，此后细胞不断分裂增殖，形成晕团状，含 30～50 个细胞，细胞向四周伸展，边缘的细胞呈长梭形，折光性强，第 6～8 d 时集落迅速增多，呈漩涡状或水草样，放射状向周围不断扩大，互相融合，但集落有大有小，有的集落细胞呈多层，如图 1 所示，12～15 d 细胞融合。此时细胞的排列尚未形成明显的方向性。

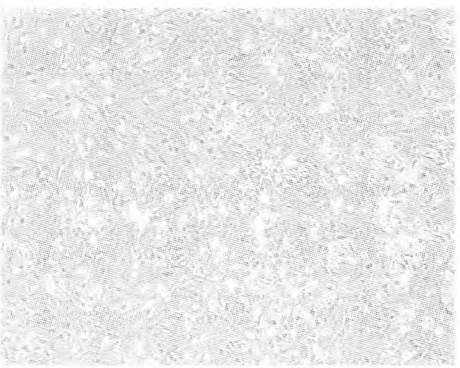


图 1 BMSCs 原代培养第 8 天（×100）

Fig. 1 The primary culture of BMSCs at the 8th day (×100)

3.2 BMSCs 流式细胞仪鉴定

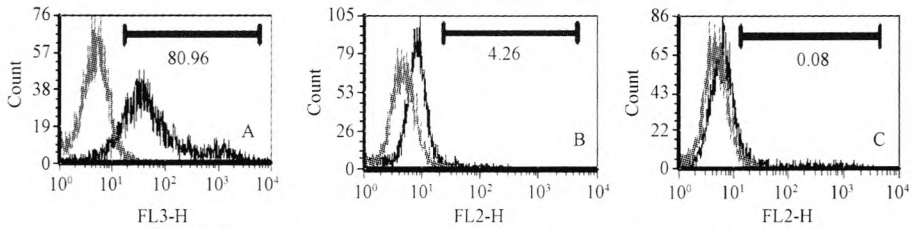
经流式细胞仪检测，CD90（80.96%）表达为阳性，CD11b/c（4.26%）、CD45（0.08%）表达为阴性，如图 2 所示。

3.3 BMSCs 的生长曲线

BMSCs 生长曲线呈 S 形，接种后第 1～2 d 为潜伏期，从第 3 d 开始细胞增殖加速进入对数生长期，第 8～9 d 达到高峰，以后进入平台期，如图 3 所示。

3.4 含药血清对 BMSCs 增殖的影响

如表 1 所示，6 d 时与 Control 组比较，ZG + YDJ、YG + YDJ、GT + YDJ、ZY + YDJ、BY + YDJ、BJ + YDJ 有显著性差异（$P < 0.05$）；与 YDJ 组比较，ZG + YDJ、YG + YDJ、GT + YDJ、ZY + YDJ、BY + YDJ、BJ + YDJ 有显著性差异（$P < 0.05$）；与 BJ + YDJ 组比



注：A为Per cp/cy5.5 anti-CD90；B为PE anti-CD11b/c；C为PE anti-CD45

图 2 BMSCs 表面标志物的流式鉴定

Fig. 2 The identification of BMSCs using flow cytometry

注：A 为 Per cp/cy 5.5 anti-CD90；B 为 PE anti-CD11b/c；C 为 PE anti-CD45

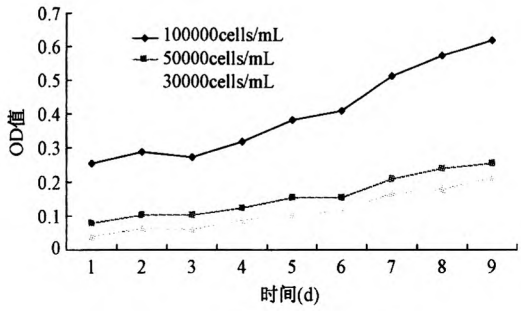


图 3 P4 代 BMSCs 生长曲线

Fig. 3 The growth curve of the 4th passage of BMSCs

表 1 左、右归丸及其拆方含药血清对 BMSCs 增殖的影响($\bar{x} \pm s$)

Table 1 The effect of Zuogui pill, Yougui pill, and their containing serum on the proliferation of BMSCs ($\bar{x} \pm s$)

组别	时间		
	6 d	9 d	12 d
ZG + YDJ	0.721 ± 0.093*☆■	0.549 ± 0.060*☆■	0.539 ± 0.042*☆■□
YG + YDJ	0.587 ± 0.095*☆■□	0.387 ± 0.041*☆■□	0.384 ± 0.044*☆□
GT + YDJ	0.492 ± 0.072*☆■□	0.359 ± 0.036*☆■□	0.288 ± 0.025*☆□
ZY + YDJ	0.579 ± 0.065*☆■□	0.509 ± 0.054*☆■	0.424 ± 0.008*☆■□
BY + YDJ	0.277 ± 0.036*☆□	0.284 ± 0.036*☆□	0.194 ± 0.033■□
BJ + YDJ	0.304 ± 0.048*☆	0.304 ± 0.048*☆	0.305 ± 0.044*☆
YDJ	0.194 ± 0.028	0.198 ± 0.023	0.141 ± 0.044
Control	0.190 ± 0.014	0.190 ± 0.014	0.119 ± 0.008

注：$n = 8$，“*”表示与 Control 组比较 $P < 0.05$；“☆”表示与 YDJ 组比较 $P < 0.05$；“■”表示与 BJ 组比较 $P < 0.05$；“□”表示与 ZG 组比较 $P < 0.05$

较，ZG + YDJ、YG + YDJ、GT + YDJ、ZY + YDJ 有显著性差异（$P < 0.05$）；与 ZG + YDJ 组比较，YG + YDJ、GT + YDJ、ZY + YDJ、BY + YDJ 有显著性差异（$P < 0.05$）。9 d 时与 Control 组比较，ZG + YDJ、YG + YDJ、GT + YDJ、ZY + YDJ、BY + YDJ、BJ + YDJ 有显著性差异（$P < 0.05$）；与 YDJ 组比较，ZG + YDJ、YG + YDJ、GT + YDJ、ZY + YDJ、BY + YDJ、BJ + YDJ 有显著性差异（$P < 0.05$）；与 BJ + YDJ 组比较，ZG + YDJ、YG + YDJ、GT + YDJ、ZY + YDJ 有显著性差异（$P < 0.05$）；与 ZG + YDJ 组比较，YG + YDJ、GT + YDJ、ZY + YDJ、BY + YDJ 有显著性差异（$P < 0.05$）。12 d 时与 Control 组比较，ZG + YDJ、YG + YDJ、GT + YDJ、ZY + YDJ、BJ + YDJ 有显著性差异（$P < 0.05$）；与 YDJ 组比较，ZG + YDJ、YG + YDJ、GT + YDJ、ZY + YDJ、BJ + YDJ 有显著性差异（$P < 0.05$）；与 BJ + YDJ 组比较，ZG + YDJ、ZY + YDJ、BY + YDJ 有显著性差异（$P < 0.05$）；与 ZG + YDJ 组比较，YG + YDJ、GT + YDJ、ZY + YDJ、BY + YDJ 有显著性差异（$P < 0.05$）。

3.5 含药血清对 BMSCs 成骨诱导的影响

如表 2 所示，6 d 时与 Control 组比较，ZG + YDJ、YG + YDJ、GT + YDJ、ZY + YDJ、BY + YDJ、BJ + YDJ 有显著性差异（$P < 0.05$）；与 YDJ 组比较，ZG + YDJ、YG + YDJ、GT + YDJ、ZY + YDJ、BY + YDJ、BJ + YDJ 有显著性差异（$P < 0.05$）；与 BJ + YDJ 组比较，ZG + YDJ、BY + YDJ 有显著性差异（$P < 0.05$）；与 ZG + YDJ 组比较，YG + YDJ、GT + YDJ、ZY + YDJ、BY + YDJ 有显著性差异（$P < 0.05$）。9 d 时与 Control 组比较，ZG + YDJ、YG + YDJ、GT + YDJ、ZY + YDJ、BJ + YDJ、YDJ 有显著性差异（$P < 0.05$）；与 YDJ 组比较，ZG + YDJ、YG + YDJ、GT + YDJ、ZY + YDJ、BY + YDJ、BJ + YDJ 有显著性差异（$P < 0.05$）；与 BJ + YDJ 组比较，ZG + YDJ、GT + YDJ、BY + YDJ 有显著性差异（$P < 0.05$）；与 ZG + YDJ 组比较，YG + YDJ、GT + YDJ、ZY + YDJ、BY + YDJ 有显著性差异（$P < 0.05$）。12 d 时与 Control 组比较，ZG + YDJ、YG + YDJ、GT + YDJ、ZY + YDJ、BJ + YDJ、YDJ 有显著性差异（$P < 0.05$）；与 YDJ 组比较，ZG + YDJ、YG + YDJ、GT + YDJ、ZY + YDJ、BY + YDJ、BJ + YDJ 有显著性差异（$P < 0.05$）；与 BJ + YDJ 组比较，ZG + YDJ、GT + YDJ、BY + YDJ 有显著性差异（$P < 0.05$）；与 ZG + YDJ 组比较，YG + YDJ、GT + YDJ、ZY + YDJ、BY + YDJ 有显著性差异（$P < 0.05$）。

表 2 左、右归丸及其拆方含药血清对 BMSCs 成骨诱导的影响($\bar{x} \pm s$)

Table 2 The effect of Zuogui pill, Yougui pill, and their containing serum on the osteogenic induction of BMSCs ($\bar{x} \pm s$)

组别	时间		
	6 d	9 d	12 d
ZG + YDJ	0.415 ± 0.066*☆■	0.456 ± 0.029*☆■	0.402 ± 0.014*☆■□
YG + YDJ	0.362 ± 0.031*☆□	0.340 ± 0.021*☆□	0.352 ± 0.010*☆□
GT + YDJ	0.287 ± 0.027*☆□	0.326 ± 0.026*☆■□	0.324 ± 0.020*☆■□
ZY + YDJ	0.356 ± 0.017*☆□	0.352 ± 0.018*☆□	0.368 ± 0.026*☆□
BY + YDJ	0.223 ± 0.043*☆■□	0.247 ± 0.021■□	0.153 ± 0.026*☆□
BJ + YDJ	0.329 ± 0.037*☆	0.360 ± 0.017*☆	0.361 ± 0.028*☆
YDJ	0.112 ± 0.016	0.267 ± 0.029*	0.431 ± 0.018*
Control	0.109 ± 0.010	0.226 ± 0.012	0.177 ± 0.019

注：$n = 8$，“*”表示与 Control 组比较 $P < 0.05$；“☆”表示与 YDJ 组比较 $P < 0.05$；“■”表示与 BJ 组比较 $P < 0.05$；“□”表示与 ZG 组比较 $P < 0.05$

4 讨论

在众多的干细胞中，BMSCs 以其来源充足、取材方便、易于体外扩增、不表达主要组织相容性复合体（MHC）Ⅱ类分子以及不牵涉伦理问题而成为干细胞移植和组织工程研究热点[7]。体外培养的 BMSCs 体积小，结构简单，成梭形，核质比较大，能以自我复制的方式增殖[8]。研究 BMSCs 的细胞周期发现，BMSCs 中有 90% 处于 G0/G1 期。说明 BMSCs 具有高分化潜能[9]。骨质疏松发病的主要原因是雌激素缺乏引发的成骨细胞（osteoblast，OB）的骨形成不足和破骨细胞（osteoclast，OC）的骨吸收亢进导致的骨重建失衡；OB 不仅决定骨的形成，同时也调节 OC 对骨的吸收，是骨代谢的主要功能细胞，OB 增殖和分化在很大程度上决定了最终形成的骨量[10]。而 BMSCs 能够诱导成为 OB，并且已经逐渐成为 OB 来源的重要途径，本实验通过研究左、右归丸及其拆方含药血清对 BMSCs 增殖和成骨诱导，以期进一步明确中医药对 BMSCs 的干预机制。

目前用于分离骨髓间充质干细胞的方法有[11-13]：①依据细胞的密度来分离的密度梯度离心法；②依据与底物粘附特性来分离细胞的贴壁筛选法；③依据细胞体积进行流式细胞仪分离法；④依据细胞表面特异性抗原进行免疫磁珠分离法。前两种分离方法是目前国内较为普遍采用的方法。后两种方法筛选出的细胞较纯，但对实验条件和设备要求高，程序复杂，容易对细胞造成损伤，因此应用受到很大限制。密度梯度离心法培养所需的时间较贴壁分离法长，这可能与离心过程中丢失了骨髓微环境中对 BMSCs 生长有利的细胞因子和促贴附物质有关。而全骨髓贴壁法是根据干细胞贴壁的特性，定期换液除去不贴壁细胞，如造血系细胞、内皮细胞等，从而达到纯化 BMSCs 的目的，自 Friedenstein[14] 等 1968 年利用自然贴壁法获得 BMSCs 后，该方法目前仍得到广泛应用。用此方法获得的 BMSCs 主要包含成纤维样间质干细胞、小的非粒性细胞（循环干细胞，RS-1）、小的粒性细胞（RS-2）以及内皮细胞、部分造血干细胞等多种细胞成分[15]，所以本实验采用全骨髓贴壁法分离大鼠 BMSCs。

研究认为[16-18]，BMSCs 表面抗原 CD105（SH2），CD73（SH3、SH4），STRO-1，α-肌动蛋白，CD29，CD44，CD90，CD106，CD120a，CD124，CD166 和 HLA-ABC 等呈阳性，而 CD11b，CD14，CD34 和 CD45 等造血谱系标志呈阴性。本实验通过流式细胞仪鉴定 CD90 表达阳性，CD11b/c、CD45 表达阴性，可确认本实验分离培养的细胞为 BMSCs。

体外培养 BMSCs 使其增殖并向 OB 分化，主要依赖于一定的培养条件。因此控制好培养条件就成为体外诱导分化为 OB 的关键。历年来，有学者[19]研究单纯中药对 BMSCs 增殖和成骨诱导的影响，也有学者[20]研究中药协同诱导剂对 BMSCs 增殖和成骨诱导的影响，但结果显示[21]中药协同诱导剂较单纯用中药对 BMSCs 增殖和成骨诱导可靠。本实验分别采用同种浓度左、右归丸及其拆方含药血清加诱导剂以及单纯用经典诱导剂诱导的方法，并以补佳乐戊酸雌二醇片为阳性对照药，比较左、右归丸及其拆方含药血清对 BMSCs 向 OB 诱导的影响。左、右归丸及其拆方组、诱导剂组分别在左、右归丸及其拆方含药血清、诱导剂的作用下大鼠 BMSCs 由单一的纤维状变成立方形和多角形等多种形态，细胞大量增殖、重叠，细胞之间界限模糊，逐渐形成了多个散在的细胞结节，胞质中出现钙盐结晶体，这些变化与 OB 有相似的形态和生长特点。地塞米松、β-甘油磷酸钠、维生素 C 是常用的 OB 诱导剂[22]，胚胎干细胞研究显示在地塞米松、β-甘油磷酸钠、维生素 C 的作用下它可向 OB 分化[23]。所以本实验研究所采用的阳性对照组 OB 条件培养液由诱导剂组成。通过本次实验研究发现，左、右归丸及其拆方含药血清可以协同诱导剂对 BMSCs 增殖和成骨诱导具有显著的促进作用。

近年来很多文献从病因病机、辨证论治、药物研究、动物模型研究方面对中医药治疗原发性骨质疏松症进行研究，特别是药物研究已经成为预防和治

疗原发性骨质疏松症的重要途径。《黄帝内经》说："肾主骨生髓""肝主筋藏血"，故骨之强弱与肝肾中精血盛衰关系密切，精血充盛则骨髓化生有源，反之肝肾亏虚，精亏血少骨髓化源不足，不能营养骨骼而致骨髓空虚，故方用左归丸加减，益肾滋阴壮骨。肾为先天之本，脾为后天之本，肾中精气为后天形体之基础，而肾中精气亦有赖于水谷精微不断化生与补充，脾气虚弱、水谷精微化生不足，不能滋养先天之精，则导致先后天之精俱虚，无以充养骨髓，故方用右归丸加减，温肾助阳补虚。中药对 BMSCs 成骨诱导的研究主要集中在补肾药，而对于补气、活血及其他类中药的研究相对较少[24]。治疗原发性骨质疏松症时，选用药物多以甘温补益、滋养肝肾、兼及五脏为基本特点，以补肾益精为主，辅以健脾养血、活血祛瘀、强筋壮骨，标本兼顾[25]。含药血清培养细胞可以直接观察中药的药物学效应，便于阐述药理机制，使现代科技与传统医学相结合，有利于推动中医、中药现代化[26]。本实验利用中药血清药理学方法，通过观察左、右归丸及其拆方含药血清对体外培养的 BMSCs 增殖和成骨诱导的影响，证实了左、右归丸及其拆方对 BMSCs 增殖和成骨诱导具有促进作用，特别是左归丸、右归丸、滋肾阴药组效果表现更佳。本实验研究从药效学的角度，阐释了中医学"肾主骨、生髓"理论的科学内涵，明确左、右归丸的配伍机制，为进一步探究中药干预 BMSCs 的发病机制提供实验依据。

【参考文献】

[1] Mao wenqing, Tian tian. The etiology and pathogenesis of primary osteoporosis. Chinese journal of osteoporosis, 2011, 17(10): 937-940.

[2] Lv haibo, Ren yanling, Wang ying, et al. Experimental research about the preventive and therapeutic effect of Zuogui pill on ovariectomized rats, Chinese journal of osteoporosis , 2010, 16(11): 847-850, 823.

[3] Wei jinqiang, Lv chaohui. Mechanism of kidney in traditional Chinese medicine treatment of primary osteoporosis. Massage and rehabilitation medicine magazine, 2012, 3(27): 51-52.

[4] Liang qiming, Xu xiaozhi, Pan guoquan, et al. Yougui pill of add and subtract in the treatment of kidney deficiency type osteoporosis study. Chinese medicine guide, 2011, 9(33): 5-7.

[5] Li yanfen, Sun lanjun, Zhao yingqiang, et al. Compound danshen dropping pill intervention between the bone marrow mesenchymal stem cell transplantation after the experimental research of myocardial regeneration. Chinese medicine modern distance education of China, 2009, 7(9): 81-83.

[6] Gong yuanyuan, Zhang lihua. Traditional Chinese medicine on bone marrow mesenchymal stem cell research advances in the effect of multidirectional differentiation. Journal of henan traditional Chinese medicine, 2002, 32(2): 252-254.

[7] Van Damme A, Vanden Driessche T, Collen D, et al. Bone marrow stromal cells as targets for gene therapy. Gurr Gene Ther 2002, 2(2): 195-209.

[8] Hua song, Wu hao. The research progress of bone marrow mesenchymal stem cells. Chinese journal of animal husbandry, 2004, 40(10): 38-41.

[9] Ye jingmei, Tan huo. The research progress of bone marrow mesenchymal stem cells. Journal of guangzhou medical college, 2006, 34(1): 69-71.

[10] Hao changying, Ren yanling, Zhao jinru. Zuigui pill containing medicine serum through ERK/Smads signal pathway intervention function of MC3T3-E1 cell gene expression. Chinese pharmacological bulletin, 2012, 28(6): 872-876.

[11] Mai xia, Chen li, Zha lagahu, et al. Rat bone marrow mesenchymal stem cell separation and cultivation and its biological characteristics were observed. Chinese tissue engineering research and clinical rehabilitation, 2007, 11(3): 463-466.

[12] Zhang bensi, Wang fan, Deng li, et al. Separate and cultivate rat bone marrow mesenchymal stem cells and its phenotypic and functional characteristics. Journal of Sichuan university(Medical sciences), 2003, 34(4): 738-741.

[13] Wang wen, Li jing, Wang zongren, et al. Compare under the condition of different isolation methods and cultivation of rat bone marrow mesenchymal stem cell proliferation situation. Chinese tissue engineering research and clinical rehabilitation, 2007, 11(3): 482-489.

[14] Friedenstein AJ, Chailakhyan RK, Gerasimov UV. Bone marrow osteogenic stem cells: in vitro cultivation and transplantation in diffusion chambers. Cell Tissue Kinet, 1987, 20(3): 263-272.

[15] Friedenstein AI, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenie and hematopoietic tissues. Transplantation, 1968, 6(2): 230-247.

[16] Hennrick KT, Keeton AG, Nanua S, et al. Lung cells from neonates show a mesenchymal stem cell phenotype. American Journal of Respiratory & Critical Care Medicine, 2007, 175(11): 1158-1164.

[17] Woodbury D, Schwarz EJ, Prockop DJ, et al. Adult rat and human bone marrow stromal cells differentiate into neurons. J Neurosci Res, 2000, 61(4): 364-370.

[18] Woodbury D, Reynolds K, Black IB. Adult bone marrow stromal stem cells express germ Line, ectodermal, endodermal and mesodermal genes prior to neurogenesis. J Neurosci Res, 2002, 69(6): 908-917.

（下转第 680 页）

kyphoplasty in the treatment of vertebral compression fractures: a systemic review. Eur Spine J, 2006, 15: 1050-1067

[16] Khanna AJ, Reinhardt MK, Togawa D, et al. Functional outcomes of kyphoplasty for the treatment of osteoporotic and osteolytic vertebral compression fractures. Osteoporos Int, 2006, 17: 817-826.

[17] Wang JC, Mummaneni PV, Haid RW. Current treatment strategies for the painful lumbar motion segment: posterolateral fusion versus interbody fusion. Spine, 2005, 30(Suppl 16): S33-43.

[18] Che Yanjun, Chen liang, Yang Huilin, et al. Surgical outcomes for recurrent lumbar disc herniation. Chinese Journal of Spine and Spinal Cord. 2010, 20(9): 730-735.

[19] Yang Huilin, Wang Genlin, Liu Jiayong. Nonunion osteoporotic vertebral fractures treated by balloon kyphoplasty. SAS, 2008, Miami, USA.

[20] Garfin SR, Buckly RA, Ledlie J. Balloon kyphoplasty for symptomatic vertebral body compression fractures results in rapid, significant, and sustained improvements in back pain, function, and quality of life for elderly patients. Spine, 2006, 31: 2213-2220.

[21] Ledlie JT, Renfro MB. Kyphoplasty treatment of vertebral fractures: 2-year outcomes show sustained benefits. Spine, 2006, 31: 57-64.

[22] Yang Huilin, Yuan HA, Chen Liang, et al. Balloon kyphoplasty for the treatment of osteoporotic vertebral compression fractures. Chinese Journal of Orthopaedics, 2003, 23: 262-265.

(收稿日期: 2012-07-25)

(上接第 702 页)

[19] Fan hongqi, Sun huisheng, Liu zhenqi, et al. The traditional Chinese medicine of compound bone setting on bone marrow mesenchymal stem cells to osteoblasts in vitro proliferation and differentiation. Chinese tissue engineering research and clinical rehabilitation, 2007, 11(10): 1818-1822.

[20] Cheng zhian, Song shaoyun, Wu yanfeng, et al. Jiangu two immortal pill mediated between osteoblast induced directional differentiation of mesenchymal stem cells and its osteogenetic activity. Chinese journal of traumatology, 2005, 13(1): 8-11.

[21] Ma xinlong, Sun xiaolei, Ma jianxiong, et al. Platelet rich plasma unite calcification induced on bone marrow mesenchymal stem cell proliferation and distribution of active effects. China combine traditional Chinese and western medicine surgical magazine, 2010, 16(2): 195-199.

[22] Dupree JL, Bigbee, JW. A cetylcholinesterase inhibitor treatment delays recovery axotomy in culture dorsal root ganglion neurons. JN eurocytol, 1996, 25(8): 439.

[23] Buttery LD, Bourne S, Xynos JD. Differentiation of osteoblasts and in vitro bone formation from murine embryonic stem cells. TissueEng, 2001, 7(1): 89.

[24] Yang feng, Tang dezhi, Bian qin, et al. Traditional Chinese medicine induced osteogenesis of mesenchymal stem cells differentiation between bone marrow. Chinese tissue engineering research and clinical rehabilitation, 2011, 15 (10): 1847-1850.

[25] Guo yang, Ma yong. Traditional Chinese medicine prescription analysis commonly used for the treatment of osteoporosis. Chinese journal of experimental formulas of Chinese medicine, 2010, 16 (17): 188-191.

[26] Li jing, Yin fei, Yao shukun, et al. Serum pharmacology were reviewed. Chinese journal of basic medicine in traditional Chinese medicine, 2009, 15(3): 234-236.

(收稿日期: 2012-09-27)

左、右归丸及其拆方含药血清对骨髓间充质干细胞增殖和成骨诱导的影响

作者： 何文智， 宋囡， 王智民， 任艳玲， HE Wenzhi， SONG Nan， WANG Zhimin， REN Yanling

作者单位： 何文智,HE Wenzhi(辽宁中医药大学药学院,沈阳,110032)， 宋囡,SONG Nan(辽宁中医药大学基础医学院辽宁中医药大学附属第二医院)， 王智民,WANG Zhimin(辽宁中医药大学第一临床学院)， 任艳玲,REN Yanling(辽宁中医药大学基础医学院)

刊名： 中国骨质疏松杂志ISTIC

英文刊名： Chinese Journal of Osteoporosis

年，卷(期)： 2013,19(7)

参考文献(26条)

1. Mao wenqing;Tian tian The etiology and pathogenesis of primary osteoporosis[期刊论文]-Chinese Journal of Osteoporosis 2011(10)

2. Lv haibo;Ren yanling;Wang ying Experimental research about the preventive and therapeutic effect of Zuogui pill on ovariectomized rats[期刊论文]-Chinese Journal of Osteoporosis 2010(11)

3. Wei jinqiang;Lv chaohui Mechanism of kidney in traditional Chinese medicine treatment of primary osteoporosis 2012(27)

4. Liang qiming;Xu xiaozhi;Pan guoquan Yougui pill of add and subtract in the treatment of kidney deficiency type osteoporosis study 2011(33)

5. Li yanfen;Sun lanjun;Zhao yingqiang Compound danshen dropping pill intervention between the bone marrow mesenchymal stem cell transplantation after the experimental research of myocardial regeneration[期刊论文]-Chinese medicine modern distance education of China 2009(09)

6. Gong yuanyuan;Zhang lihua Traditional Chinese medicine on bone marrow mesenchymal stem cell research advances in the effect of multidirectional differentiation 2002(02)

7. Van Damme A;Vanden Driessche T;Collen D Bone marrow stromal cells as targets for gene therapy 2002(02)

8. Hua song;Wu hao The research progress of bone marrow mesenchymal stem cells 2004(10)

9. Ye jingmei;Tan huo The research progress of bone marrow mesenchymal stem cells[期刊论文]-Journal of guangzhou medical college 2006(01)

10. Hao changying;Ren yanling;Zhao jinru Zuigui pill containing medicine serum through ERK/Smads signal pathway intervention function of MC3T3-E1 cell gene expression 2012(06)

11. Mai xia;Chen li;Zha lagahu Rat bone marrow mesenchymal stem cell separation and cultivation and its biological characteristics were observed 2007(03)

12. Zhang bensi;Wang fan;Deng li Separate and cultivate rat bone marrow mesenchymal stem cells and its phenotypic and functional characteristics[期刊论文]-Journal of Sichuan university(Medical sciences) 2003(04)

13. Wang wen;Li jing;Wang zongren Compare under the condition of different isolation methods and cultivation of rat bone marrow mesenchymal stem cell proliferation situation 2007(03)

14. Friedenstein AJ;Chailakhyan RK;Gerasimov UV Bone marrow osteogenic stem cells:in vitro cultivation and transplantation in diffusion chambers 1987(03)

15. Friedenstein AI;Petrakova KV;Kurolesova AI;Frolova GP Heterotopic of bone marrow.Analysis of precursor cells for osteogenie and hematopoietic tissues 1968(02)

16. Hennrick KT;Keeton AG;Nanua S Lung cells from neonates show a mesenchymal stem cell phenotype 2007(11)

17. Woodbury D;Schwarz E J;Prockop DJ Adult rat and human bone marrow stromal cells differentiate into neurons[外文期刊] 2000(04)

18. Woodbury D;Reynolds K;Black IB Adult bone marrow stromal stem cells express germ Line,ectodermal,endodermal and mesodermal genes prior to neurogenesis 2002(06)

19. Fan hongqi;Sun huisheng;Liu zhenqi The traditional Chinese medicine of compound bone setting on bone marrow mesenchymal stem cells to osteoblasts in vitro proliferation and differentiation 2007(10)

20. Cheng zhian;Song shaoyun;Wu yanfeng Jiangu two immortal pill mediated between osteoblast induced directional differentiation of mesenchymal stem cells and its osteogenetic activity[期刊论文]-Chinese Journal of Traumatology 2005(01)

21. Ma xinlong;Sun xiaolei;Ma jianxiong Platelet rich plasma unite calcification induced on bone marrow mesenchymal stem cell proliferation and distribution of active effects[期刊论文]-China combine traditional Chinese and western medicine surgical magazine 2010(02)

22. Dupree JL;Bigbee.JW A cetylcholinesterase inhibitor treatment delays recovery axotomy in culture dorsal root ganglion neurons 1996(08)

23. Buttery LD;Bourne S;Xynos JD Differentiation of osteoblasts and in vitro bone formation from murine embryonic stem cells 2001(01)

24. Yang feng;Tang dezhi;Bian qin Traditional Chinese medicine induced osteogenesis of mesenchymal stem cells differentiation between bone marrow 2011(10)

25. Guo yang;Ma yong Traditional Chinese medicine prescription analysis commonly used for the treatment of osteoporosis 2010(17)

26. Li jing;Yin fei;Yao shukun Serum pharmacology were reviewed 2009(03)