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·论著·

补肾活血方对去势骨质疏松大鼠循环 Serotonin 表达的影响

梁冬波 王力平 覃智斌* 唐福宇 王润生 关威
柳州市中医院,广西 柳州 545001

中图分类号:R274 **文献标识码**:A **文章编号**:1006-7108(2014)05-0508-05

摘要:**目的** 探讨补肾活血方对去势大鼠循环 5-羟色胺(Serotonin)表达水平的影响。**方法** 30 只 6 月龄 SD 雌性大鼠去卵巢制作骨质疏松模型,另取 10 只行假手术,建立假手术组。3 个月后,卵巢切除的大鼠随机分为三组,然后分别予补肾活血方(中药组),阿仑膦酸钠(西药组)和生理盐水(模型组)治疗,假手术组给予等量生理盐水。经过 8 周的治疗后处死大鼠,检测左侧股骨骨密度,ELISA 检测血清 Serotonin、核因子 κ B 受体活化因子配体(RANKL)和骨转换指标 P1NP、CTX 水平。**结果** 模型组骨密度低于假手术组($P<0.001$);中药组和西药组全段股骨骨密度高于模型组($P<0.01$ 和 $P<0.001$)。中药组和西药组血清 P1NP 低于模型组(P 均 <0.001),而假手术组、中药组与西药组之间无统计学差异。中药组和西药组血清 CTX 低于模型组(P 均 <0.001),高于假手术组(P 均 <0.001)。中药组血清 Serotonin 水平低于西药组和模型组(P 均 <0.001),假手术组与中药组之间无统计学差异。中药组和西药组血清 RANKL 低于模型组(P 均 <0.001),假手术组、中药组与西药组之间无统计学差异。**结论** 补肾活血方可能是通过降低去势大鼠循环 Serotonin 表达水平,进而抑制 RANKL 表达水平,从而提高去势大鼠骨密度。

关键词:中药;去势大鼠;骨质疏松;Serotonin

Effect of nourishing the kidney and invigorating the blood recipe on circulating serotonin level in ovariectomized rats

LIANG Dongbo, WANG Liping, QIN Zhibin, TANG Fuyu, WANG Runsheng, GUAN Wei
Department of Orthopedics, Liuzhou Hospital of Traditional Chinese Medicine, Liuzhou 545001, China
Corresponding author: QIN Zhibin, Email: nick201@163.com

Abstract: **Objective** To investigate the effect of nourishing the kidney and invigorating the blood recipe on the circulating serotonin level in ovariectomized rats. **Methods** Thirty 6-month female Sprague Dawley rats were ovariectomized to induce osteoporosis, and another 10 rats underwent sham operation to establish sham-operated (SHAM) group. After 3 months, the ovariectomized rats were randomly divided into 3 groups: herb group, alendronate group, and OVX group. Rats in herb group were treated with nourishing the kidney and invigorating the blood recipe; while rats in alendronate group were treated with alendronate. Rats in OVX group and SHAM group were treated with normal saline. After 8-week treatment, all the rats were sacrificed. The bone mineral density (BMD) of the left femur was detected. The serum levels of serotonin, RANKL, and bone turnover markers, including P1NP and CTX, were detected using ELISA. **Results** BMD in OVX group was lower than that in SHAM group ($P<0.001$), while BMD in the intact femur in herb group and alendronate group was higher than that in OVX group ($P<0.01$ and $P<0.001$). The serum P1NP level in herb group and alendronate group was significantly lower than that in OVX group ($P<0.001$), while no significant difference among SHAM group, herb group, and alendronate group was observed. The serum CTX level in herb group and alendronate group was lower than that in OVX group ($P<0.001$), while it was higher than that in SHAM group ($P<0.001$). The serum serotonin level in herb group was significantly lower than that in OVX group and alendronate group ($P<0.001$). But no significant difference between SHAM group and herb group was observed. The serum RANKL level in herb group and alendronate group decreased significantly compared with that in OVX group ($P<0.001$), and no significant difference among SHAM group, herb group, and alendronate group was observed. **Conclusion** Nourishing the kidney and invigorating blood recipe can enhance BMD in OVX rats most likely by reducing the expression of serum serotonin and further by inhibiting the expression of RANKL.

*通讯作者:覃智斌,Email:nick201@163.com

Key words: Herb; Ovariectomized rat; Osteoporosis; Serotonin

绝经后骨质疏松症 (Postmenopausal osteoporosis, PMO) 是一种因卵巢激素缺乏导致骨吸收超过骨形成引起的全身性骨骼障碍性疾病[1,2],骨吸收能力提高是通过增强破骨细胞活性实现的,因此,抑制破骨细胞活性是 PMO 的主要治疗原则。细胞和动物实验研究表明肠源性 Serotonin (也称循环 Serotonin) 可抑制成骨细胞增殖和骨形成[3,4],促进破骨细胞分化和骨吸收[5]。最近的一项关于人体的研究证明较高的循环 Serotonin 水平可能增加骨转换,抑制骨形成[6]。因此,肠源性 Serotonin 是负性调节骨代谢的重要因子,抑制其合成可降低骨转换水平,并阻止破骨细胞分化。本研究通过建立去势骨质疏松大鼠模型,观察补肾活血方对循环 Serotonin 表达水平的影响,探讨补肾活血方治疗骨质疏松症的可能机理。

1 材料和方法

1.1 动物

选用 6 月龄清洁级健康 SD 雌性大鼠 40 只,体质量为 (275.52 ± 33.36) g,购于南方医科大学实验动物中心 (许可证号:SCXK-粤-2011-0025)。所有大鼠均在恒温、恒湿的清洁环境中标准饲料喂养,室温为 23 ~ 25 ℃,湿度 50% 左右,自由摄食饮水。

1.2 药物

补肾活血方[7] 由熟地黄、山茱萸、牡丹皮、山药、茯苓、泽泻、杜仲、淫羊藿、黄芪、丹参、红花、三七粉等 12 味中药组成,由柳州市中医院药剂科提供。阿伦磷酸钠片,由杭州默沙东制药有限公司提供 (国药准字 J20080069)。

1.3 造模与分组

适应喂养 1 周后,将 40 只大鼠随机分成 2 组,即切除双侧卵巢组 (30 只) 和假手术组 (10 只)。卵巢切除组用 10% 水合氯醛溶液按 0.3 ml/100 g 行腹腔注射麻醉后,取背侧改良切口,于深部脂肪层中可发现粉红色卵巢,子宫角处结扎后切除卵巢。假手术组仅切除周围少量脂肪组织。术后分笼饲养。术后 3 个月,将卵巢切除组大鼠随机分为模型组、阿仑膦酸钠组 (西药组) 和中药组,每组 10 只。

1.4 药物干预

造模 3 个月后,经人-大鼠体表面积比值折算大鼠等效给药量,中药组灌胃给予补肾活血方 2.5 mg 生药/kg,给药浓度为 0.25 mg 生药/ml,1 次/d。西药组灌胃给予阿伦磷酸钠 7 mg 生药/kg,给药浓度为 0.7 mg 生药/ml,1 次/周。假手术组和模型组灌服 10 mL/kg 生理盐水。给药后每周称量体重 1 次,根据体重调整给予的受试药物剂量。干预措施连用 8 周,8 周后全部处死取材。

1.5 样本收集

最后一次灌胃后 2 h,麻醉后酒精消毒下半身。使用一次性采血器在腹主动脉采血,1000 × g 离心 15min,取上层血清储存于 -80 ℃。处死大鼠后在工作台上仔细分离左侧股骨,彻底剔除软组织后用 37 ℃生理盐水纱布包裹后放入 EP 管中,-20 ℃冰箱保存,骨密度检测前取出解冻。

1.6 指标检测

1.6.1 离体区域骨密度测量:应用小动物专用双能 X 线骨密度仪 (HOLOGIC) 对各组大鼠左侧股骨各部位进行离体骨密度 (BMD) 测量,仪器由广东省人民医院核医学科提供[Discovery A (S/N 82239)]。通过软件进行股骨区域划分,扫描时无介质。

1.6.2 血清 Serotonin, RANKL 和骨转换指标 (PINP 和 CTX) 的含量检测:采用酶联免疫吸附试验 (ELISA) 试剂盒 (武汉华美生物工程有限公司提供)。Serotonin 的灵敏度为 0.4 ng/ml,批内和批间差均为 < 15%;RANKL, PINP 和 CTX 的灵敏度分别为 15.63 pg/ml, 15.6 pg/ml 和 3.9 pg/ml,批内差均为 < 8%,批间差均为 < 15%。

1.7 统计学方法

采用 SPSS13.0 统计软件进行分析,采用均数 ± 标准差 ($\bar{x} \pm s$) 表示,多组均数比较采用单因素方差分析和 Tukey 检验。

2 结果

2.1 各组大鼠左侧股骨离体骨密度比较

治疗 8 周后,模型组的股骨区域 BMD 值均低于假手术组 (均为 $P < 0.001$),说明骨质疏松症造模成功。模型组全段股骨 BMD 低于中药组和西药组 ($P = 0.002$ 和 $P < 0.001$),中药组全段股骨 BMD 低于假手术组 ($P = 0.024$)。中药组和西药组近端股骨 BMD 均高于模型组 ($P = 0.002$ 和 $P < 0.001$),低于假手术组 ($P = 0.004$ 和 $P = 0.030$)。中药组和西药组股骨干 BMD 均高于模型组 (均为 $P < 0.001$),低于假手术组 (均为 $P < 0.001$)。中药组和西药组远端股骨 BMD 高于模型组 ($P = 0.001$ 和 $P < 0.001$),

低于假手术组($P<0.001$ 和 $P=0.001$)。见表 1。

表 1 各组大鼠左侧股骨区域性骨密度(BMD)比较

Table 1 Comparison of the regional bone mineral density (BMD) of the left femur of rats in each group

组别 group	假手术组 Sham group	模型组 model group	中药组 herb group	西药组 Alendronate group	F 值 F	P 值 P
全段股骨 Intact femur	0.329 ± 0.011#	0.267 ± 0.033	0.302 ± 0.010#*	0.312 ± 0.012#	17.181	<0.001
近端股骨 Proximal femur	0.310 ± 0.016#	0.257 ± 0.018	0.284 ± 0.009#*	0.290 ± 0.009#*	20.356	<0.001
股骨干 Femoral shaft	0.357 ± 0.013#	0.284 ± 0.012	0.328 ± 0.009#*	0.319 ± 0.012#*	54.386	<0.001
远端股骨 Distal femoral	0.328 ± 0.011#	0.278 ± 0.010	0.300 ± 0.008#*	0.307 ± 0.012#*	34.269	<0.001

注:与模型组相比,#$P<0.05$;与假手术组相比,*$P<0.05$

Note: Compared with the model group, #representative $P<0.05$; compared with the sham operation group, *representative $P<0.05$

2.2 各组大鼠血清 Serotonin, RANKL, PINP 和 CTX 的比较

假手术组和中药组血清 Serotonin 水平明显低于模型组(均为 $P<0.001$)和西药组(均为 $P<0.001$),假手术组与中药组($P=0.267$)以及模型组与西药组($P=0.381$)之间无统计学差异。假手术组、中药组和西药组血清 RANKL 水平明显低于模型组(均为 $P<0.001$),前三组比较无统计学差异($P>0.05$)。假手术组、中药组和西药组血清 P1NP 水平明显低于模型组(均为 $P<0.001$),前三组比较无统计学差异($P>0.05$)。假手术组、中药组和西药组血清 CTX 水平明显低于模型组(均为 $P<0.001$),中药组和西药组明显高于假手术组(均为 $P<0.001$),中药组和西药组无统计学差异($P=0.972$)。见表 2。

表 2 ELISA 检测各组大鼠血清 Serotonin, RANKL, PINP 和 CTX 的表达水平

Table 2 The expression levels of serum serotonin, RANKL, PINP, and CTX using ELISA test in each group

组别 group	假手术组 Sham group	模型组 model group	中药组 herb group	西药组 Alendronate group	F 值 F	P 值 P
Serotonin	2.410 ± 0.216#	3.280 ± 0.134	2.556 ± 0.155#	3.152 ± 0.098*△	60.319	<0.001
RANKL	21.815 ± 2.352#	40.405 ± 6.408	23.880 ± 3.321#	22.353 ± 3.026#	38.333	<0.001
P1NP	22.388 ± 1.776#	45.000 ± 10.745	23.163 ± 3.674#	20.338 ± 1.872#	33.019	<0.001
CTX	108.800 ± 8.094#	188.250 ± 17.284	156.250 ± 5.211#*	153.300 ± 17.993#*	47.723	<0.001

注:与模型组相比,#$P<0.05$;与假手术组相比,*$P<0.05$;与中药组相比,△$P<0.05$

Note: Compared with model group, # representatives $P<0.05$; compared with the sham group, * representative $P<0.05$; compared with the herb group, △representative $P<0.05$

3 讨论

肾虚是骨质疏松发病的主要病机,此外,血瘀也是骨质疏松的一个重要病机[7]。临床应用补肾活血方治疗肾虚血瘀型老年性骨质疏松患者可有效减轻疼痛,改善肾虚血瘀证候积分,提高生存质量。在近期止痛效果、证候积分改善程度和提高生存质量方面优于阿仑膦酸钠组(固邦片)。在体外干预成骨细胞实验中,补肾活血方含药血清可促进成骨细胞的增殖,提高 ALP 活性和增加矿化结节数量[7]。然而,补肾活血方是否影响 PMO 的骨代谢状态,目前还不清楚。因此,在补肾活血方有效改善老年骨质疏松患者症状以及提高生活质量的良好疗效基础上,进一步探索其对 PMO 骨代谢的影响以及可能机制,为临床防治 PMO 的合理用药提供科学的实验依据。本研究的数据表明,补肾活血方可通过提高去卵巢大鼠的骨密度,降低骨转换水平从而部分缓解骨量丢失,这个过程可能与血清 Serotonin 和 RANKL 水平下降有关。

绝经后妇女雌激素水平下降,骨转换率增加,骨吸收多于骨形成,导致骨量减少,骨强度降低,发生骨质疏松[8],其中破骨细胞活动性占主导地位,核

因子 κ B 受体活化因子配体(RANKL)是破骨细胞形成和发挥功能的关键调节因子[9,10]。绝经期妇女雌激素缺乏可上调 RANKL 的表达,表明它可能是促进骨吸收、增加骨量丢失的关键调控因子[11]。雌激素和 RANKL 抑制剂通过直接阻断 RANKL 介导的破骨细胞生成来调节破骨细胞发育[12,13]。因此,RANKL 在破骨细胞形成中起关键作用,已成为治疗骨量过度丢失性骨疾病的潜在靶标。

循环 Serotonin 是目前讨论的热点问题之一[5,6],TPH1 是限速酶色氨酸羟化酶 1,在十二指肠的肠嗜铬细胞内参与循环 Serotonin 的合成,与野生型小鼠相比,骨表型 TPH1 基因敲除小鼠的血循环中几乎没有 Serotonin 的表达[14]。最近的一项研究表明,抑制循环 Serotonin 生物合成对增加去势大鼠骨量的作用效果基本上等同于抗骨吸收药物[15]。Chabbi-Achengli 等[5] 经体内外研究发现循环 Serotonin 在破骨细胞分化和功能上具有正向调节作用,抑制循环 Serotonin 合成减少了骨吸收,是因为 TPH1 基因敲除小鼠破骨细胞数量减少。另外,破骨细胞前体在 RANKL 刺激下可以表达 TPH1 并合成 Serotonin,而在 TPH1 基因敲除小鼠的皮细胞和骨髓巨噬细胞内,即使在外源性 RANKL 刺激下,破骨细胞数量明显下降,但加入外源性 Serotonin 后,破骨细胞形成数量增多。也就是说,循环 Serotonin 对 RANKL 介导的破骨细胞形成过程中起到正向调节作用。此外,Modder 等[6] 发现在女性中高水平循环 Serotonin 浓度,同时伴有骨转换水平升高,骨形成能力下降。因此,循环 Serotonin 水平可以作为评估 PMO 骨代谢状态的一个分子标记。上述文献报道循环 Serotonin 和 RANKL 的关系,在我们的研究结果中也得到了证实。我们发现,中药组和假手术组血清 Serotonin 和 RANKL 浓度均显著低于模型组,而西药组只有血清 RANKL 浓度低于模型组。

本研究选择抗骨吸收西药阿伦磷酸钠做对照,因为阿伦磷酸钠能有效增加 PMO 妇女的骨密度并降低骨转换水平,预防髋部骨折的发生,已被确立为一线治疗药物[16]。然而,近期的研究表明,非典型股骨骨折发生与长期口服阿仑膦酸钠密切相关,因此,阿仑膦酸钠不能常规应用于骨质疏松患者[17,18]。本研究发现,中药组血清 Serotonin 浓度显著低于西药组,而中药组血清 RANKL 浓度虽高于西药组,但二者无统计学差异。表明西药组增加去势大鼠骨密度可能是通过直接下调 RANKL 表达来实现的,这与 Eslami 等[19] 的研究结果一致。

综上所述,本实验在蛋白水平观察了补肾活血方对去势大鼠骨代谢调控的影响,发现其可能是通过下调血清 Serotonin 和 RANKL 浓度从而起到抑制破骨细胞活动性的目的,而阿伦磷酸钠是通过直接降低血清 RANKL 的表达来抑制破骨细胞的生成,从而达到治疗 PMO 的目的。

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补肾活血方对去势骨质疏松大鼠循环 Serotonin 表达的影响

作者： 梁冬波， 王力平， 覃智斌， 唐福宇， 王润生， 关威， LIANG Dongbo， WANG Liping， QIN Zhibin， TANG Fuyu， WANG Runsheng， GUAN Wei

作者单位： 柳州市中医院,广西柳州,545001

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