·基础研究·

二至丸抑制绝经后骨质疏松大鼠骨代谢紊乱的作用机制研究

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摘　要　**目的:**探讨二至丸抑制绝经后骨质疏松大鼠骨代谢紊乱的作用机制。**方法:**2 月龄雌性清洁级 SD 大鼠 60 只,采用随机数字表随机分为假手术组 15 只与手术组 45 只。假手术组在双侧卵巢周围切除少许脂肪组织,手术组摘除双侧卵巢建立绝经后骨质疏松症大鼠模型。造模后 2 周,再将手术组大鼠随机分为模型组、二至丸组与雌二醇组,每组 15 只。二至丸组和雌二醇组大鼠,分别用二至丸混悬液和戊酸雌二醇片混悬液灌胃;假手术组和模型组大鼠,用生理盐水灌胃;每日 1 次,连续灌胃 12 周。药物干预结束后,采用 ELISA 法检测大鼠血清中骨代谢标记物骨特异性碱性磷酸酶(bone alkaline phosphotase,BALP)、抗酒石酸酸性磷酸酶－5b(tartrate－resistant acid phosphatase－5b,TRACP－5b)、基质金属蛋白酶－9(matrix metalloproteinase－9,MMP－9)、组织蛋白酶 K(cathepsin K,Cath－K)含量;并每组随机取 3 只大鼠的 L_5 椎体,以 4% 多聚甲醛固定,行骨密度(bone mineral density,BMD)及组织学检测;每组其余 12 只大鼠 L_5 腰椎以液氮保存,行骨代谢标记物 mRNA 表达的检测。并对各组检测结果进行比较。**结果:**①BMD 检测结果。药物干预 12 周后,4 组间腰椎 BMD 检测结果比较,差异有统计学意义[(0.16±0.02)g·cm^{-2},(0.10±0.03)g·cm^{-2},(0.13±0.02)g·cm^{-2},(0.14±0.02)g·cm^{-2};$F=5.800$,$P=0.005$];假手术组、二至丸组与雌二醇组高于模型组($P=0.001$,$P=0.024$,$P=0.010$);假手术组与二至丸组、雌二醇组比较,差异无统计学意义($P=0.123$,$P=0.236$);二至丸组与雌二醇组比较,差异无统计学意义($P=0.701$)。②组织学检测结果。药物干预 12 周后,假手术组腰椎骨小梁致密,分布均匀,排列规则,表面光滑,交织成网状,骨小梁间隙均匀。模型组腰椎骨小梁变细、分布稀疏、散乱、不连续,表面粗糙,完整性差,出现碎片、断裂等。二至丸组与雌二醇组腰椎骨小梁变细,数量减少,分布稀疏,排列较规则。4 组间骨小梁面积百分比比较,差异有统计学意义[(23.25±5.32)%,(11.82±3.87)%,(17.10±3.83)%,(18.08±2.59)%;$F=8.144$,$P=0.001$];模型组、二至丸组与雌二醇组低于假手术组($P=0.000$,$P=0.015$,$P=0.038$);二至丸组与雌二醇组高于模型组($P=0.034$,$P=0.014$);二至丸组与雌二醇组比较,差异无统计学意义($P=0.677$)。③骨代谢标记物血清含量检测结果。药物干预 12 周后,4 组间血清 BALP 含量比较,差异有统计学意义[(58.14±9.04)单位·L^{-1},(40.91±6.47)单位·L^{-1},(51.13±7.88)单位·L^{-1},(52.23±7.41)单位·L^{-1};$F=5.239$,$P=0.008$];假手术组、二至丸组与雌二醇组高于模型组($P=0.001$,$P=0.034$,$P=0.012$);假手术组与二至丸组、雌二醇组比较,差异无统计学意义($P=0.133$,$P=0.286$);二至丸组与雌二醇组比较,差异无统计学意义($P=0.645$)。4 组间血清 TRACP－5b 含量比较,差异有统计学意义[(0.71±0.13)pg·mL^{-1},(0.98±0.22)pg·mL^{-1},(0.79±0.09)pg·mL^{-1},(0.77±0.07)pg·mL^{-1};$F=3.874$,$P=0.025$];假手术组、二至丸组与雌二醇组低于模型组($P=0.004$,$P=0.038$,$P=0.022$);假手术组与二至丸组、雌二醇组比较,差异无统计学意义($P=0.320$,$P=0.459$);二至丸组与雌二醇组比较,差异无统计学意义($P=0.795$)。4 组间血清 MMP－9 含量比较,差异有统计学意义[(1.63±0.23)pg·mL^{-1},(2.01±0.35)pg·mL^{-1},(1.64±0.25)pg·mL^{-1},(1.58±0.18)pg·mL^{-1};$F=3.507$,$P=0.034$];假手术组、二至丸组与雌二醇组低于模型组($P=0.021$,$P=0.023$,$P=0.009$);假手术组与二至丸组、雌二醇组比较,差异无统计学意义($P=0.972$,$P=0.699$);二至丸组与雌二醇组比较,差异无统计学意义($P=0.673$)。4 组间血清 Cath－K 含量比较,差异有统计学意义[(2.55±0.40)pg·mL^{-1},(4.06±0.87)pg·mL^{-1},(3.07±0.77)pg·mL^{-1},(2.73±0.69)pg·mL^{-1};$F=5.539$,$P=0.006$];假手术组、二至丸组与雌二醇组低于模型组($P=0.001$,$P=0.024$,$P=0.004$);假手术组与二至丸组、雌二醇组比较,差异无统计学意义($P=0.217$,$P=0.671$);二至丸组与雌二醇组比较,差异无统计学意义($P=0.408$)。④骨代谢标记物 mRNA 表达检测结果。药物干预 12 周后,4 组间 BALPmRNA 表达比较,差异有统计学意义[(1.01±0.04),(0.62±0.10),(0.78±0.13),(0.83±0.13);$F=14.482$,$P=0.000$];模型组、二至丸组与雌二醇组低于假手术组($P=0.000$,$P=0.001$,$P=0.007$);二至丸组与雌二醇组高于模型组($P=0.015$,$P=0.002$),二至丸组与雌二醇组比较,差异无统计学意义($P=0.385$)。4 组间 TRACP－5bmRNA 表达比较,差异

基金项目:国家自然科学基金重点项目(81230087),福建省自然科学基金项目(2015J01339),福建省卫生系统中青年骨干人才培养项目(2015－ZQN－JC－32),福建省高校新世纪优秀人才支持计划项目(闽科教[2015]54 号)

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有统计学意义[(0.98±0.04),(2.05±0.41),(1.67±0.26),(1.50±0.21);$F=16.574$,$P=0.000$];模型组、二至丸组与雌二醇组高于假手术组($P=0.000$,$P=0.000$,$P=0.003$);二至丸组与雌二醇组低于模型组($P=0.024$,$P=0.002$);二至丸组与雌二醇组比较,差异无统计学意义($P=0.282$)。4组间MMP-9mRNA表达比较,差异有统计学意义[(1.00±0.06),(1.86±0.17),(1.51±0.32),(1.45±0.35);$F=11.684$,$P=0.000$];模型组、二至丸组与雌二醇组高于假手术组($P=0.000$,$P=0.002$,$P=0.007$);二至丸组与雌二醇组低于模型组($P=0.028$,$P=0.010$);二至丸组与雌二醇组比较,差异无统计学意义($P=0.643$)。4组间Cath-KmRNA表达比较,差异有统计学意义[(0.99±0.03),(2.52±0.57),(1.85±0.27),(1.74±0.54);$F=13.543$,$P=0.000$];模型组、二至丸组与雌二醇组高于假手术组($P=0.000$,$P=0.002$,$P=0.005$);二至丸组与雌二醇组低于模型组($P=0.011$,$P=0.004$);二至丸组与雌二醇组比较,差异无统计学意义($P=0.663$)。**结论**:对于绝经后骨质疏松模型大鼠,二至丸和戊酸雌二醇片均可增加其骨密度和骨小梁数量;作用机制可能是通过促进BALP表达、抑制TRACP-5b、MMP-9与Cath-K表达,从而抑制骨代谢紊乱;两种药物的作用相当。

关键词　骨质疏松,绝经后;大鼠;二至丸;骨密度;骨特异性碱性磷酸酶;抗酒石酸酸性磷酸酶5b;基质金属蛋白酶9;组织蛋白酶K

Study on mechanism of action of Erzhi Wan(二至丸) in inhibiting bone metabolism disorder in rats with postmenopausal osteoporosis

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ABSTRACT　**Objective**: To explore the mechanism of action of Erzhi Wan(二至丸, EZW) in inhibiting bone metabolism disorder in rats with postmenopausal osteoporosis(PMOP). **Methods**: Sixty 2-month-old clean-grade female SD rats were randomly divided into sham-operated group(15) and operation group(45) by using random digits table. The resection of fat around bilateral ovaries were performed on rats in sham-operated group, and the bilateral ovariectomy were performed on rats in operation group to build the PMOP rat models. At 2 weeks after the modeling, the rats in operation group were randomly subdivided into model group, EZW group and estradiol group, 15 cases in each group. The rats in EZW group and estradiol group were intragastric administrated with EZW suspension and estradiol valerate suspension respectively, while the others in sham-operated group and model group were intragastric administrated with normal saline(NS), once a day for consecutive 12 weeks. After the end of drug intervention, the serum contents of bone alkaline phosphotase(BALP), tartrate-resistant acid phosphatase-5b(TRACP-5b), matrix metalloproteinase-9(MMP-9) and cathepsin K(Cath-K) were measured by using ELISA method. Three rats were randomly selected from each group and their L_5 vertebraes were fetched out and fixed with 4% paraformaldehyde, and then the bone mineral density(BMD) detection and histological detection were performed. The L_5 vertebraes of the other 12 rats in each group were preserved in liquid nitrogen, and the mRNA expression of bone metabolism marker were detected. The detection results were compared between the 4 groups. **Results**: After 12-week drug intervention, there was statistical difference in the detection result of lumbar vertebra BMD between the 4 groups(0.16+/-0.02, 0.10+/-0.03, 0.13+/-0.02, 0.14+/-0.02 g/cm(2); $F=5.800$, $P=0.005$). The BMD were higher in sham-operated group, EZW group and estradiol group compared to model group($P=0.001$, $P=0.024$, $P=0.010$). There was no statistical difference in the BMD between sham-operated group and EZW group and between sham-operated group and estradiol group($P=0.123$, $P=0.236$), and there was no statistical difference in the BMD between EZW group and estradiol group($P=0.701$). After 12-week drug intervention, the lumbar bone trabeculas of rats of sham-operated group were in a compact state and were uniformly distributed and regularly arranged, and they interlaced to form a netty structure with smooth surface and uniform interspace. The bone trabeculas of rats of model group became thin and were characterized by sparse, scattered and discontinuous distribution, and they presented with debris, rupture, rough surface and poor integrity. The bone trabeculas became thin and declined in number and characterized by sparse distribution and relatively regular arrangement in rats of EZW group and estradiol group. There was statistical difference in the area percentage of bone trabecula between the 4 groups(23.25+/-5.32, 11.82+/-3.87, 17.10+/-3.83, 18.08+/-2.59%; $F=8.144$, $P=0.001$). The area percentage of bone trabecula was lower in model group, EZW group and estradiol group compared to sham-operated group($P=0.000$, $P=0.015$, $P=0.038$), and was higher in EZW group and estradiol group compared to model group($P=0.034$, $P=0.014$), and there was no statistical difference in the area percentage of bone trabecula between EZW group and estradiol group($P=0.677$). After 12-week drug intervention, there was statistical difference in the serum contents of BALP between the 4 groups

(58.14 +/- 9.04,40.91 +/- 6.47,51.13 +/- 7.88,52.23 +/- 7.41 unit/l; $F = 5.239$, $P = 0.008$). The serum contents of BALP were higher in sham-operated group, EZW group and estradiol group compared to model group ($P = 0.001$, $P = 0.034$, $P = 0.012$). There was no statistical difference in the serum contents of BALP between sham-operated group and EZW group ($P = 0.133$) and between sham-operated group and estradiol group ($P = 0.286$) and between EZW group and estradiol group ($P = 0.645$). There was statistical difference in the serum contents of TRACP - 5b between the 4 groups (0.71 +/- 0.13,0.98 +/- 0.22,0.79 +/- 0.09,0.77 +/- 0.07 pg/ml; $F = 3.874$, $P = 0.025$). The serum contents of TRACP - 5b were lower in sham-operated group, EZW group and estradiol group compared to model group ($P = 0.004$, $P = 0.038$, $P = 0.022$), and there was no statistical difference in the serum contents of TRACP - 5b between sham-operated group and EZW group ($P = 0.320$) and between sham-operated group and estradiol group ($P = 0.459$) and between EZW group and estradiol group ($P = 0.795$). There was statistical difference in the serum contents of MMP - 9 between the 4 groups (1.63 +/- 0.23,2.01 +/- 0.35,1.64 +/- 0.25,1.58 +/- 0.18 pg/ml; $F = 3.507$, $P = 0.034$). The serum contents of MMP - 9 were lower in sham-operated group, EZW group and estradiol group compared to model group ($P = 0.021$, $P = 0.023$, $P = 0.009$). There was no statistical difference in the serum contents of MMP - 9 between sham-operated group and EZW group ($P = 0.972$) and between sham-operated group and estradiol group ($P = 0.699$) and between EZW group and estradiol group ($P = 0.673$). There was statistical difference in the serum contents of Cath - K between the 4 groups (2.55 +/- 0.40,4.06 +/- 0.87,3.07 +/- 0.77,2.73 +/- 0.69 pg/ml; $F = 5.539$, $P = 0.006$). The serum contents of Cath - K were lower in sham-operated group, EZW group and estradiol group compared to model group ($P = 0.001$, $P = 0.024$, $P = 0.004$). There was no statistical difference in the serum contents of Cath - K between sham-operated group and EZW group ($P = 0.217$) and between sham-operated group and estradiol group ($P = 0.671$) and between EZW group and estradiol group ($P = 0.408$). After 12 - week drug intervention, there was statistical difference in BALP mRNA expression between the 4 groups (1.01 +/- 0.04,0.62 +/- 0.10,0.78 +/- 0.13, 0.83 +/- 0.13; $F = 14.482$, $P = 0.000$). The BALP mRNA expressions were lower in model group, EZW group and estradiol group compared to sham-operated group ($P = 0.000$, $P = 0.001$, $P = 0.007$), and were higher in EZW group and estradiol group compared to model group ($P = 0.015$, $P = 0.002$), and there was no statistical difference in the BALP mRNA expression between EZW group and estradiol group ($P = 0.385$). There was statistical difference in the TRACP - 5b mRNA expression between the 4 groups (0.98 +/- 0.04,2.05 +/- 0.41,1.67 +/- 0.26,1.50 +/- 0.21; $F = 16.574$, $P = 0.000$). The TRACP - 5b mRNA expressions were higher in model group, EZW group and estradiol group compared to sham-operated group ($P = 0.000$, $P = 0.000$, $P = 0.003$), and were lower in EZW group and estradiol group compared to model group ($P = 0.024$, $P = 0.002$), and there was no statistical difference in the TRACP - 5b mRNA expression between EZW group and estradiol group ($P = 0.282$). There was statistical difference in the MMP - 9 mRNA expression between the 4 groups (1.00 +/- 0.06,1.86 +/- 0.17,1.51 +/- 0.32,1.45 +/- 0.35; $F = 11.684$, $P = 0.000$). The MMP - 9 mRNA expressions were higher in model group, EZW group and estradiol group compared to sham-operated group ($P = 0.000$, $P = 0.002$, $P = 0.007$), and were lower in EZW group and estradiol group compared to model group ($P = 0.028$, $P = 0.010$), and there was no statistical difference in the MMP - 9 mRNA expression between EZW group and estradiol group ($P = 0.643$). There was statistical difference in Cath - K mRNA expression between the 4 groups (0.99 +/- 0.03,2.52 +/- 0.57,1.85 +/- 0.27,1.74 +/- 0.54; $F = 13.543$, $P = 0.000$). The Cath - K mRNA expressions were higher in model group, EZW group and estradiol group compared to sham-operated group ($P = 0.000$, $P = 0.002$, $P = 0.005$), and were lower in EZW group and estradiol group compared to model group ($P = 0.011$, $P = 0.004$), and there was no statistical difference in the Cath - K mRNA expression between EZW group and estradiol group ($P = 0.663$). **Conclusion**: For PMOP rat models, both EZW and estradiol valerate tablets can inhibit bone metabolism disorder through promoting the expression of BALP and inhibiting the expression of TRACP - 5b, MMP - 9 and Cath - K, which may be the mechanisms of action in increasing the BMD and the number of bone trabecula. The two drugs are similar to each other in curative effect.

Key words osteoporosis, postmenopausal; rats; Erzhi Pill; bone density; bone alkaline phosphotase; tartrate - resistant acid phosphatase 5b; matrix metalloproteinase 9; cathepsin K

绝经后骨质疏松症(postmenopausal osteoporosis, PMOP)是绝经后女性因体内卵巢功能下降,骨吸收 - 骨形成偶联过程失衡,导致骨量丢失、骨强度下降、骨脆性增加的代谢性骨病[1-2]。近年来,随着人口的老龄化,PMOP 的发病率呈逐渐上升趋势,PMOP 的防治已成为医学界关注的焦点问题之一[3]。PMOP 属中医学"骨痿"的范畴,主要病因病机为肾虚髓亏、骨髓生化乏源[4-5]。补肾经典方二至丸(女贞子、墨旱莲)具有滋阴补肾、益精凉血之效,可抑制骨量减少、改善骨小梁三维结构、增加骨结构强度与力学强度[6]。笔

者建立绝经后骨质疏松大鼠模型,观察二至丸对骨代谢的影响,探讨二至丸抑制骨代谢紊乱的作用机制,现报告如下。

1　材料与仪器

1.1　实验动物　清洁级SD大鼠60只,雌性,2月龄,体质量140~160 g,购自上海斯莱克实验动物有限责任公司,合格证号:SCXK(沪)2012-0002。在福建中医药大学实验动物中心饲养。饲养条件:温度20~26 ℃,相对湿度40%~70%,12 h明暗循环,自由饮水。实验方案由福建中医药大学医学伦理委员会审查批准。

1.2　药物与试剂　二至丸(江西国药有限责任公司,批准文号:国药准字Z36020794),戊酸雌二醇片(拜耳医药保健有限公司广州分公司,批准文号:国药准字J20130009),苏木素-伊红(hematoxylin and eosin,HE)染色试剂盒(北京索莱宝科技有限公司),乙二胺四乙酸二钠(国药集团化学试剂有限公司),甘油醛-3-磷酸脱氢酶(glyceraldehyde-3-phosphate dehydrogenase,GAPDH)、骨特异性碱性磷酸酶(bone alkaline phosphotase,BALP)、抗酒石酸酸性磷酸酶-5b(tartrate-resistant acid phosphatase-5b,TRACP-5b)、基质金属蛋白酶-9(matrix metalloproteinase-9,MMP-9)、组织蛋白酶K(cathepsin K,Cath-K)ELISA试剂盒(上海西唐生物科技有限公司),TRizol试剂(Invitrogen公司),反转录试剂盒、荧光定量试剂盒(TaKaRa公司)。

1.3　实验仪器　Discovery Wi双能X线骨密度仪(Hologic公司),光学显微镜(Olympus公司),RM2235石蜡切片机(Leica公司),7900HT-TLDA实时荧光定量PCR仪(ABI公司)。

2　方　法

2.1　分组与造模　适应性喂养1周后,60只大鼠采用随机数字表随机分为假手术组15只与手术组45只。10%水合氯醛溶液0.3 mL·100 g^{-1}腹腔注射麻醉后,假手术组在双侧卵巢周围切除少许脂肪组织,手术组摘除双侧卵巢建立PMOP动物模型[7]。造模后2周,再采用随机数字表将手术组大鼠随机分为模型组、二至丸组与雌二醇组,每组15只。

2.2　药物干预　造模后2周,用人和动物药物等效剂量换算公式,以成人临床用药剂量计算动物用药剂量[8]。用生理盐水将二至丸配制成90 mg·mL^{-1}的混悬液,将戊酸雌二醇片配制成10 μg·mL^{-1}的混悬液。按照1 mL·100 g^{-1}的剂量,二至丸组和雌二醇组大鼠,分别用二至丸混悬液和戊酸雌二醇片混悬液灌胃;假手术组和模型组大鼠,用生理盐水灌胃;每日1次,连续灌胃12周。

2.3　实验指标检测　药物干预结束后,10%水合氯醛溶液0.3 mL·100 g^{-1}腹腔注射麻醉,每只大鼠用含1%肝素钠的抗凝管腹主动脉采血10 mL,低温离心15min(离心半径15 cm,转速3000 r·min^{-1}),取上层血清,-20 ℃保存,进行血清骨代谢标记物含量检测。采血后,取出大鼠L_5腰椎。每组随机取3只大鼠的L_5腰椎以4%多聚甲醛固定,进行骨密度(bone mineral density,BMD)及组织学检测;每组其余12只大鼠L_5腰椎以液氮保存,进行骨代谢标记物mRNA表达的检测。

2.3.1　骨密度检测　采用DiscoveryWi双能X线骨密度仪,检测大鼠L_5腰椎骨密度。

2.3.2　组织学检测　L_5腰椎常规固定、脱水、包埋、切片,HE染色,光学显微镜下观察骨组织形态结构的变化。并采用Motic Med 6.0数码医学图像系统,计算骨小梁面积百分比(骨小梁面积百分比=测量框内骨小梁面积÷测量框总面积×100%)。

2.3.3　骨代谢标记物血清含量检测　采用ELISA法检测血清中BALP、TRACP-5b、MMP-9、Cath-K含量,具体检测方法按照Elisa试剂盒说明进行。

2.3.4　骨代谢标记物mRNA表达检测　采用实时荧光定量PCR检测。液氮研磨腰椎骨组织,TRIzol法提取总RNA,定量后取1 μg RNA逆转录成cDNA。根据GenBank基因序列设计引物(上海生物工程技术服务有限公司)。BALP Forward:5′-CAT CCT GTA TGG CAA TGG G-3′,Reverse:5′-CATC CTG TAT GGC AAT GGG-3′,216 bp;TRAP-5b Forward:5′-AAC TTG CGA CCA TTG TTA-3′,Reverse:5′-GGG GAC CTT TCG TTG ATG T-3′,245 bp;MMP-9 Forward:5′-TCC CCA GAG CGT TAC TCG CT-3′,Reverse:5′-ACC TGG TTC ACC CGG TTG TG-3′,144 bp;Cath-K Forward:5′-GCA GCA GAA TGG AGG CAT TG-3′,Reverse:5′-TTC AGG GCT TTC TCG TTC CC-3′,141 bp;GAPDH Forward:5′-ATT GTC AGC AAT GCA TCC TG-3′,Reverse:5′-ATG GAC TGT GGT CAT GAG CC-3′,102 bp。SYBR GREEN

PCR 反应体系 20 μL,SYBR Premix Ex TaqTM Ⅱ(2×)10 μL,ROX Reference Dye Ⅱ(50×)0.4 μL,cDNA 模板 2 μL,焦碳酸二乙酯水 6 μL,上下游引物各 0.8 μL。采用 2 - △△CT 计算法,以 GAPDH 为内参计算目的基因的表达。

2.4　数据统计学处理　采用 SPSS19.0 统计软件对所得数据进行处理。4 组腰椎 BMD、骨小梁面积百分比、血清骨代谢标记物含量、腰椎骨代谢标记物 mRNA 表达的组间总体比较均采用单因素方差分析,组间两两比较均采用 LSD - t 检验。检验水准 $\alpha = 0.05$。

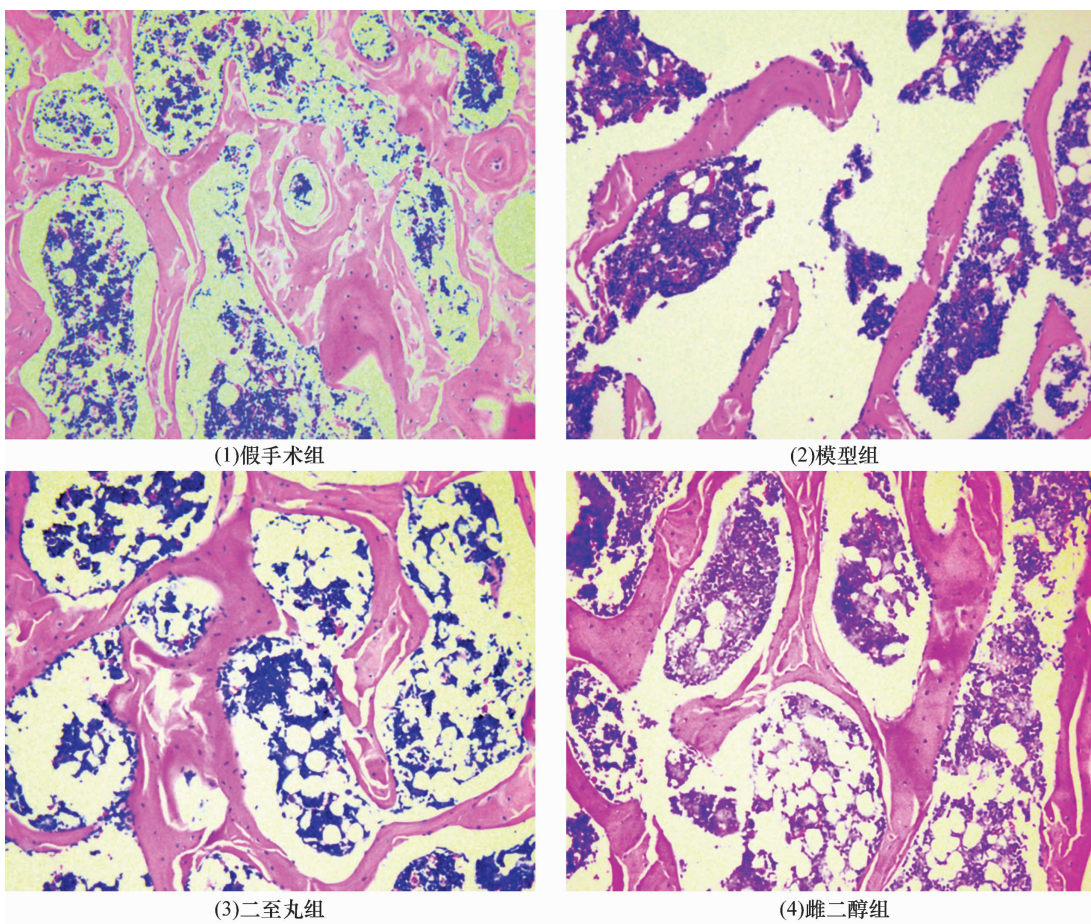
3　结　果

3.1　BMD 检测结果　药物干预 12 周后,4 组间腰椎 BMD 检测结果比较,差异有统计学意义;假手术组、二至丸组与雌二醇组高于模型组($P = 0.001$,$P = 0.024$,$P = 0.010$);假手术组与二至丸组、雌二醇组比较,差异无统计学意义($P = 0.123$,$P = 0.236$);二至丸组与雌二醇组比较,差异无统计学意义($P = 0.701$)。见表 1。

表 1　4 组去卵巢骨质疏松模型大鼠腰椎骨密度

组别	样本量(只)	骨密度($\bar{x} \pm s$,g · cm^{-2})
假手术组	3	0.16 ± 0.02
模型组	3	0.10 ± 0.03
二至丸组	3	0.13 ± 0.02
雌二醇组	3	0.14 ± 0.02
F 值		5.800
P 值		0.005

3.2　组织学检测结果　药物干预 12 周后,假手术组腰椎骨小梁致密,分布均匀,排列规则,表面光滑,交织成网状,骨小梁间隙均匀[图 1(1)]。模型组腰椎骨小梁变细、分布稀疏、散乱、不连续,表面粗糙,完整性差,出现碎片、断裂等[图 1(2)]。二至丸组与雌二醇组腰椎骨小梁变细,数量减少,分布稀疏,排列较规则[图 1(3)、图 1(4)]。4 组间骨小梁面积百分比比较,差异有统计学意义;模型组、二至丸组与雌二醇组低于假手术组($P = 0.000$,$P = 0.015$,$P = 0.038$);二至丸组与雌二醇组高于模型组($P = 0.034$,$P = 0.014$);二至丸组与雌二醇组比较,差异无统计学意义($P = 0.677$)。见表 2。



(1)假手术组　(2)模型组　(3)二至丸组　(4)雌二醇组

图 1　4 组绝经后骨质疏松模型大鼠腰椎骨小梁形态(HE 染色　×100)

表 2　4 组绝经后骨质疏松模型大鼠腰椎骨小梁面积百分比

组别	样本量（只）	腰椎骨小梁面积百分比（$\bar{x}\pm s$）
假手术组	3	（23.25 ± 5.32）%
模型组	3	（11.82 ± 3.87）%
二至丸组	3	（17.10 ± 3.83）%
雌二醇组	3	（18.08 ± 2.59）%
F 值		8.144
P 值		0.001

3.3　骨代谢标记物血清含量检测结果　药物干预 12 周后，4 组间血清 BALP 含量比较，差异有统计学意义；假手术组、二至丸组与雌二醇组高于模型组（$P=0.001$，$P=0.034$，$P=0.012$）；假手术组与二至丸组、雌二醇组比较，差异无统计学意义（$P=0.133$，$P=0.286$）；二至丸组与雌二醇组比较，差异无统计学意义（$P=0.645$）。4 组间血清 TRACP－5b 含量比较，差异有统计学意义；假手术组、二至丸组与雌二醇组低于模型组（$P=0.004$，$P=0.038$，$P=0.022$）；假手术组与二至丸组、雌二醇组比较，差异无统计学意义（$P=0.320$，$P=0.459$）；二至丸组与雌二醇组比较，差异无统计学意义（$P=0.795$）。4 组间血清 MMP－9 含量比较，差异有统计学意义；假手术组、二至丸组与雌二醇组低于模型组（$P=0.021$，$P=0.023$，$P=0.009$）；假手术组与二至丸组、雌二醇组比较，差异无统计学意义（$P=0.972$，$P=0.699$）；二至丸组与雌二醇组比较，差异无统计学意义（$P=0.673$）。4 组间血清 Cath－K 含量比较，差异有统计学意义；假手术组、二至丸组与雌二醇组低于模型组（$P=0.001$，$P=0.024$，$P=0.004$）；假手术组与二至丸组、雌二醇组比较，差异无统计学意义（$P=0.217$，$P=0.671$）；二至丸组与雌二醇组比较，差异无统计学意义（$P=0.408$）。见表 3。

3.4　骨代谢标记物 mRNA 表达检测结果　药物干预 12 周后，4 组间 BALPmRNA 表达比较，差异有统计学意义；模型组、二至丸组与雌二醇组低于假手术组（$P=0.000$，$P=0.001$，$P=0.007$）；二至丸组与雌二醇组高于模型组（$P=0.015$，$P=0.002$），二至丸组与雌二醇组比较，差异无统计学意义（$P=0.385$）。4 组间 TRACP－5bmRNA 表达比较，差异有统计学意义；模型组、二至丸组与雌二醇组高于假手术组（$P=0.000$，$P=0.000$，$P=0.003$）；二至丸组与雌二醇组低于模型组（$P=0.024$，$P=0.002$）；二至丸组与雌二醇组比较，差异无统计学意义（$P=0.282$）。4 组间 MMP－9mRNA 表达比较，差异有统计学意义；模型组、二至丸组与雌二醇组高于假手术组（$P=0.000$，$P=0.002$，$P=0.007$）；二至丸组与雌二醇组低于模型组（$P=0.028$，$P=0.010$）；二至丸组与雌二醇组比较，差异无统计学意义（$P=0.643$）。4 组间 Cath－KmRNA 表达比较，差异有统计学意义；模型组、二至丸组与雌二醇组高于假手术组（$P=0.000$，$P=0.002$，$P=0.005$）；二至丸组与雌二醇组低于模型组（$P=0.011$，$P=0.004$）；二至丸组与雌二醇组比较，差异无统计学意义（$P=0.663$）。见表 4。

表 3　4 组绝经后骨质疏松模型大鼠血清骨代谢标记物含量

组别	样本量（只）	骨特异性碱性磷酸酶（$\bar{x}\pm s$，单位·L^{-1}）	抗酒石酸酸性磷酸酶－5b（$\bar{x}\pm s$，pg·mL^{-1}）	基质金属蛋白酶－9（$\bar{x}\pm s$，pg·mL^{-1}）	组织蛋白酶 K（$\bar{x}\pm s$，pg·mL^{-1}）
假手术组	15	58.14 ± 9.04	0.71 ± 0.13	1.63 ± 0.23	2.55 ± 0.40
模型组	15	40.91 ± 6.47	0.98 ± 0.22	2.01 ± 0.35	4.06 ± 0.87
二至丸组	15	51.13 ± 7.88	0.79 ± 0.09	1.64 ± 0.25	3.07 ± 0.77
雌二醇组	15	53.23 ± 7.41	0.77 ± 0.07	1.58 ± 0.18	2.73 ± 0.69
F 值		5.239	3.874	3.507	5.539
P 值		0.008	0.025	0.034	0.006

表 4　4 组绝经后骨质疏松模型大鼠腰椎骨代谢标记物 mRNA 表达　$\bar{x}\pm s$

组别	样本量（只）	骨特异性碱性磷酸酶	抗酒石酸酸性磷酸酶－5b	基质金属蛋白酶－9	组织蛋白酶 K
假手术组	12	1.01 ± 0.04	0.98 ± 0.04	1.00 ± 0.06	0.99 ± 0.03
模型组	12	0.62 ± 0.10	2.05 ± 0.41	1.86 ± 0.17	2.52 ± 0.57
二至丸组	12	0.78 ± 0.13	1.67 ± 0.26	1.51 ± 0.32	1.85 ± 0.27
雌二醇组	12	0.83 ± 0.13	1.50 ± 0.21	1.45 ± 0.35	1.74 ± 0.54
F 值		14.482	16.574	11.684	13.543
P 值		0.000	0.000	0.000	0.000

表中数据为以甘油醛－3－磷酸脱氢酶为参照，将其表达量当作 1，计算出的各检测指标的相对表达量

4 讨 论

生理情况下,骨代谢周期反映了骨吸收与骨形成的动态平衡状态[9]。骨吸收是骨基质降解的过程,主要受破骨细胞调控;骨形成是骨基质合成与矿化的过程,主要受成骨细胞调控[10]。骨重建的稳态主要依赖于破骨细胞骨吸收-成骨细胞骨形成之间偶联的动态平衡,从而维持骨量的稳态[11]。若破骨细胞骨吸收相对增强或成骨细胞骨形成相对减弱,则出现骨吸收大于骨形成的病理现象,引发骨量的丢失[12]。

女性绝经后,卵巢功能衰退引起内源性雌激素分泌减少与骨细胞表面的雌激素受体数量下降,导致骨吸收-骨形成偶联失衡,出现破骨细胞活性增强,骨小梁吸收加快,吸收陷窝加深;成骨细胞活性相对减弱,骨形成速度减慢,导致不可逆的骨丢失,并出现骨小梁变薄与间隙增宽、骨体积减少、骨小梁连接破坏等病理现象[13-14]。骨质量与 BMD 是综合决定骨强度的关键因素,骨质量由骨的显微结构、代谢转换、矿化程度与骨基质的特性决定;BMD 是以单位面积或单位体积的骨量来表示[15]。

骨代谢标志物体现骨代谢的水平与特点,也是评估骨质量的关键指标,骨吸收与骨形成标志物的比率变化可以间接体现 BMD 变化。BALP 反映成骨细胞的功能,来源于成骨细胞,是评价骨形成与骨转换的常用代谢指标[16]。成骨细胞和前成骨细胞的活性,与血清 BALP 水平呈线性关系,提示 BALP 可作为骨形成的特异性指标。骨吸收过程中,破骨细胞附着于骨表面,形成相对封闭的骨吸收微环境,释放 TRACP、MMPs 与半胱氨酸蛋白酶等基质降解酶,吸收骨质。TRACP 分为 TRACP-5a 和 TRACP-5b 两种亚型,骨吸收过程中破骨细胞释放的 TRACP-5b,是破骨细胞数量及功能活性的有效标志物[17]。在骨吸收过程中,破骨细胞释放大量 TRACP-5b,血清 TRACP-5b 含量变化,可间接体现破骨细胞活性与骨吸收状态,提示 TRACP-5b 是破骨细胞活性与骨吸收状态的特异性指标之一。MMP-9 是 MMPs 基因家族中的一员,主要在破骨细胞与骨髓单核细胞中表达,参与破骨细胞的发育和募集过程[18]。MMP-9 在破骨细胞中可呈特异性高表达,具有降解细胞外基质的功能,在破骨细胞迁移、侵蚀与锚着过程中发挥重要的调控作用[19]。Cath-K 是一种半胱氨酸蛋白酶,选择性的大量表达于破骨细胞,具有较强的溶骨活性,其生理作用底物是骨基质中 I 型胶原蛋白,Cath-K还能降解骨基质中骨桥接素与骨连接素,是一个调控骨吸收的关键降解酶[20-21]。

本研究结果表明,对于绝经后骨质疏松模型大鼠,二至丸和戊酸雌二醇片均可增加其骨密度和骨小梁数量;作用机制可能是通过促进 BALP 表达、抑制 TRACP-5b、MMP-9 与 Cath-K 表达,从而抑制骨代谢紊乱;两种药物的作用相当。

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(2017-09-05 收稿　2017-09-12 修回)