镓盐对骨质疏松症大鼠骨组织中羟基磷灰石及胶原含量的影响*☆

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Effects of gallium nitrate on the content of hydroxyapatite and collagen in osseous tissues of rats with osteoporosis

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Abstract

BACKGROUND: In recent years, it is discovered that gallium nitrate can decrease bone conversion.

OBJECTIVE: To observe the effects of gallium nitrate on the content of hydroxyapatite and collagen in rats with osteoporosis.

METHODS: The rats were divided into control group, osteoporosis group and gallium nitrate group. Osteoporosis rat models were made by cutting off the bilateral ovaries in the latter two groups. The rats in the gallium nitrate therapy group were treated with 1 mg/kg gallium nitrate, three times a week through abdominal cavity; those in the control and osteoporosis groups were administrated with normal saline. The rats in the three groups had free access to water and standard food. All the contents of hydroxypatite and collagen in osseous tissue were determined and analyzed.

RESULTS AND CONCLUSION: The content of hydroxyaptite between the osteoporosis group and normal control group was different significantly ($P < 0.05$); there were no significant differences among gallium nitrate therapy group, control group and osteoporosis group ($P > 0.05$), but the content of hydroxyaptite in the gallium nitrate group had increasing trend compared with that in the normal group. The level of collagen in osteoporosis group was obviously lower than that in normal control group and gallium nitrate therapy group ($P < 0.05$). When osteoporosis happened, the level of collagen in osteoporosis rats decreased and the content of hydroxyapatite was in the trend of increasing, and gallium nitrate could inhibit the process.

Pang W, Kang L, Fu YL, Wang QY, Jia HB.Effects of gallium nitrate on the content of hydroxyapatite and collagen in osseous tissues of rats with osteoporosis. Zhongguo Zuzhi Gongcheng Yanjiu yu Linchuang Kangfu. 2011;15(34): 6289-6291. [http://www.crter.cn http://en.zglckf.com]

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Supported by: Medical Research Fund of Lanzhou Military Area Command of Chinese PLA, No. LXH20-11*

Received: 2011-05-31
Accepted: 2011-07-01

摘要

背景：硝酸镓是近10多年来发现的一种能降低骨转换的药物。

目的：观察硝酸镓对骨质疏松大鼠骨羟基磷灰石及胶原含量的影响。

方法：实验分为3组。正常组大鼠开腹切除1小块脂肪，其余大鼠切除双侧卵巢制作大鼠骨质疏松症动物模型。正常组及骨质疏松组腹腔注射生理盐水，硝酸镓组腹腔注射硝酸镓，3次/周，常规饲料喂养12周后取大鼠双侧股骨。

结果与结论：骨质疏松组大鼠骨组织中羟基磷灰石含量明显高于正常组($P < 0.05$)；硝酸镓组与正常组相比有升高趋势($P > 0.05$)，与骨质疏松组间差异无显著性意义($P > 0.05$)，骨质疏松组骨中胶原较正常组及硝酸镓组明显降低($P < 0.05$)。结果表明，发生骨质疏松时骨中胶原含量明显降低，羟基磷灰石含量有升高趋势，硝酸镓可以抑制这一过程。

关键词：硝酸镓；骨质疏松症；羟基磷灰石；胶原；大鼠
doi:10.3969/j.issn.1673-8225.2011.34.005

庞炜，康乐，付友兰，王倩云，贾红兵. 镓盐对骨质疏松症大鼠骨组织中羟基磷灰石及胶原含量的影响[J].中国组织工程研究与临床康复，2011，15(34):6289-6291. [http://www.crter.org http://cn.zglckf.com]

0 引言

骨代谢的过程是成骨细胞形成新骨和破骨细胞吸收旧骨的过程，骨量的多少取决于同一骨重建单位中骨形成与骨吸收的平衡。骨质疏松症是由于此平衡的破坏，骨吸收大于骨形成而造成的[1-2]。主要表现为单位体积骨组织的骨基质和骨矿物质等比例减少，最终导致骨的结构改变，骨的体积不变而骨量减少，骨密度降低，骨强度减弱。大量文献所涉及的研究是全身用药控制骨质疏松，主要集中在雌激素[3-9]、二膦酸盐[10-18]、甲状旁腺素等方面[19-20]。

近年来镓盐对骨代谢的影响受到重视，镓在体内主要蓄积于骨组织、皮下或静脉，静脉注射镓盐后，迅速进入软组织和骨骼中，并抑制骨代谢。镓盐对骨质疏松骨钙、镁、磷的影响有很多的报道[21-23]，而对骨矿物质中的羟基磷灰石及骨基质中的胶原含量的作用研究的很少，实验观察硝酸镓对骨质疏松大鼠骨羟基磷灰石及胶原含量的影响。

1 材料和方法

设计：随机对照动物实验。

时间及地点：实验于2004-09/2007-05在西安交通大学医学院骨病研究所完成。

材料：清洁级3月龄雌性SD大鼠66只，由西安交通大学医学院实验动物中心提供。实验过程中对待动物处置符合2006年科学技术部发

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中图分类号:R318
文献标识码:A
文章编号:1673-8225(2011)34-06289-03

收稿日期: 2011-05-31
修回日期: 2011-07-01
(20110531003/W•W)

布的《关于善待实验动物的指导意见》[24]。

试剂：硝酸镓，由北京有色金属研究总院合成，主要成分为$Ga(NO)_3$，用生理盐水配制成1 g/L的硝酸镓溶液备用。

实验方法：

实验动物分组、造模：大鼠在适应性饲养1周后，随机分为3组，即正常组16只、骨质疏松组25只 、硝酸镓组25只。全部大鼠均用乌拉坦腹腔注射麻醉，打开腹腔。正常组开腹后切一小块脂肪后缝合，而骨质疏松组及硝酸镓组开腹后切除双侧卵巢后缝合，手术后保暖，4 d后大鼠恢复，进入实验。

干预方法与取材：正常组及骨质疏松组，腹腔注射1 mL/kg生理盐水，3次/周；硝酸镓组大鼠腹腔注射1 mL/kg硝酸镓，3次/周。所有实验动物以常规饲料喂养12周后用200 g/L乌拉坦腹腔注射麻醉，取动物双侧股骨，骨组织在低温冰箱(−70 ℃)保存。

骨中羟基磷灰石的测定：将骨组织灰化成粉末状，用X射线粉末衍射仪分析骨盐中羟基磷灰石的含量。测定角度范围5°~60°，发射狭缝0.5 deg，散射狭缝为0.5 deg，接受狭缝为0.3 mm，扫描速度为10.0 deg/min。

骨中胶原的测定：骨去骨膜后低温干燥，研成骨粉，丙酮脱脂后自然干燥，按1 g组织加100 g木瓜蛋白酶于100 mL pH 6.5磷酸缓冲液中，67 ℃水浴8 h消化。取骨消化液0.5 mL加浓HCl 0.5 mL，100℃消化8 h，后用NaOH中和(pH=7)加水至50 mL，备用，取样1 mL，加入水2 mL，加入0.05 mol/L氨胺T 1 mL混匀，静置20 min，再加埃氏试剂1 mL，混匀，60 ℃水浴20 min，冷却，560 nm比色测定羟脯氨酸含量。

胶原含量=羟脯氨酸×7.69

主要观察指标：各组大鼠骨组织中羟基磷灰石和羟脯氨酸的水平。

统计学分析：所有实验数据采用SPSS 14.0软件包进行统计分析，实验结果计量均以$\bar{x}\pm s$表示，样本均数间比较使用t 检验，以双侧α=0.05为显著性检验水准。

2 结果

2.1 实验动物数量分析 实验选用大鼠66只，分为3组，骨质疏松组大鼠死亡3只，硝酸镓组大鼠死亡2只，进入结果分析61只。

2.2 各组大鼠骨组织中羟基磷灰石的含量 正常组的骨中羟基磷灰石量为(50.27±8.69)%，骨质疏松组羟基磷灰石量为(72.83±12.19)%，与正常组相比有显著性差异。硝酸镓组骨中羟基磷灰石量为(63.39±10.06)%，与正常组相比有升高趋势，与骨质疏松组相比有降低趋势。见表1。

表 1 各组大鼠骨组织中羟基磷灰石及胶原水平比较
Table 1 Comparison of hydroxypatite and collagen content in bone tissues of different groups ($\bar{x}\pm s$, n=10)

Group	n	Hydroxyaptite (%)	Collagen (g/L)
Normal	16	50.27±8.69	69.78±4.53
Osteoporosis	22	72.83±12.19[a]	48.34±6.76[a]
Gallium nitrate	23	63.39±10.06	61.52±5.37[b]

[a]P < 0.05, *vs.* normal group; [b]P < 0.05, *vs.* osteoporosis group

2.3 各组大鼠骨组织中胶原的含量 正常组的骨中胶原水平为(69.78±4.53) g/L，骨质疏松组胶原水平为(48.34±6.76) g/L，硝酸镓组胶原水平为(61.52±5.37) g/L。骨质疏松组与正常组及硝酸镓组比较，胶原明显减少，差异有显著性意义(P < 0.05)，硝酸镓组与正常组比较差异无显著性意义(P > 0.05)。

3 讨论

骨矿物质主要由无定形钙磷混合物(磷酸钙)和结晶钙磷羟基磷灰石(纳米晶羟基磷灰石)构成[25-26]。磷酸钙是钙盐的初级矿化成分，他进一步钙化结晶形成纳米晶羟基磷灰石[27]。当骨组织老化时，骨中羟基磷灰石与磷酸钙相比增加。

实验结果显示骨质疏松组羟基磷灰石含量百分比有增高的趋势。这有可能是由于成骨细胞分泌的骨钙素不能充分羧化，影响骨矿沉积的结果。骨质疏松骨中羟基磷灰石含量增高，是单位体积中磷酸钙吸收较快、沉积减少，羟基磷灰石相对分解吸收缓慢的结果，使骨单位体积中羟基磷灰石含量百分比的增高。

骨基质由胶原、脂类、糖蛋白等构成，其中胶原占90%以上。雌激素可刺激赖氨酸酰化酶的活性，并且加速胶原分泌和成熟，使骨骼胶原含量增加[28]。

实验对胶原检测的结果显示，骨质疏松时，同等质量的骨中胶原含量较正常对照有明显降低。这里需重点指出的是，骨质疏松时，骨有机质与矿物质是等比减少的，矿物质中的羟基磷灰石因相对分解吸收缓慢而呈相对过剩。

大量实验研究表明，镓不仅能降低骨溶解作用[29]，而且还能降低骨胶原分解速率[30]。本结果显示应用硝酸镓预防治疗骨质疏松后，骨组织中羟基磷灰石百分比含量有降低趋势，胶原含量明显增高。这与镓降低骨溶解，相对减少磷酸钙的吸收，和降低骨胶原分解速率有密不可分的关系。

综上所述，骨质疏松时，骨组织中胶原减少，羟基磷灰石相对过剩，矿物质与胶原纤维的有机结合结构发生改变，骨组织脆性加大，这可能就是骨质疏松患者易

发生骨折的机制之一。而镓盐能对这一过程有明显的改善作用。

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基金资助: 兰州军区医药卫生科研基金项目（LXH20-11）

作者贡献: 实验的设计及评估由第一、二作者共同完成，实施由全体作者共同完成，所有作者均受过动物实验培训。

利益冲突: 课题未涉及任何厂家及相关雇主或其他经济组织直接或间接的经济或利益的赞助。

伦理批准: 实验过程中对动物处置符合2006年科学技术部发布的«关于善待实验动物的指导意见»。

创新性说明: 骨质疏松症与钙、镁、磷的关系有很多的报道，而与羟基磷灰石及胶原含量的变化关系研究较少。课题将镓盐引入对骨质疏松的研究中，旨在观察应用镓盐对骨质疏松症骨矿物质中的羟基磷灰石及骨基质中的胶原含量变化的影响。结果表明骨质疏松时，骨组织中胶原减少，羟基磷灰石相对过剩，矿物质与胶原纤维的有机结合结构发生改变，骨组织脆性加大，而镓盐能对这一过程有明显的改善作用。