·基础研究·

去势大鼠骨质疏松发病过程中股骨骨小梁的微焦点CT研究*

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摘要

目的：应用微焦点计算机断层扫描(Micro-CT)研究大鼠去势后股骨松质骨骨微结构的改变及其改变规律。

方法：24只10周龄健康雌性SD大鼠随机分为两组：去势组与对照组，每组12只，去势组大鼠行去势即双侧卵巢切除术，对照组不作任何处理。去势后3月，经Micro-CT扫描股骨后，行三维重建，并进行股骨骨松质骨微结构相关参数定量检测。

结果：与对照组相比，去势组大鼠骨体积分数(BV/TV)、骨小梁厚度(Tb.Th)及骨小梁数量(Tb.N)分别减小72.6%、39.0%和56.4%($P<0.05$)；骨表面积和骨体积比(BS/BV)、骨小梁分离度(Tb.Sp)及骨小梁模式因子(Tb.Pf)分别增加63.7%、354.2%和72.6%($P<0.05$)；而骨小梁平均骨密度(Tb.Mean)分别为(443.92±39.07)HU和(428.67±50.82)HU，无明显变化($P>0.05$)。

结论：去势大鼠股骨骨松质骨质量明显降低，骨小梁数量减少，骨小梁分离度增加，骨小梁平均骨密度无明显改变，提示大鼠去势后骨质疏松发病过程中，股骨松质骨微结构的改变是以骨小梁的破坏为基本单位进行的。

关键词　大鼠；去势；股骨；骨小梁；微焦点计算机断层扫描

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Abstract

Objective: To investigate the changes of bone microstructure in femoral cancellous bone and the possible change law by micro-computed tomography (Micro-CT) in ovariectomized rats.

Method: Twenty-four healthy 10 week-old female Sprague Dawley (SD) rats were randomly divided into two groups: ovariectomized group (ovx group) and control group (control group), each group with 12 rats. The ovx group rats were ovariectomized with bilateral ovarian resection, and the control group rats were not made any processing. Three months after ovariectomy, the bone microstructure related parameters of femur cancellous bone were quantitatively detected after micro-CT scan and 3-D reconstruction.

Result: Compared with control group, the bone volume fraction (bone volume/total volume, BV/TV), trabecular thickness (Tb. Th) and trabeculae number (Tb.N) decreased in ovx group rats, and decreased respectively by 72.6%, 39.0% and 56.4% ($P<0.05$); The ratio of bone surface area and bone volume (BS/BV), trabecular spacing (Tb.Sp) and trabeculae pattern factor (Tb.Pf) increased in ovx group, and increased respectively by 63.7%, 354.2% and 72.6% ($P<0.05$); And there was no difference in the mean of trabeculae bone (Tb.mean) between the two groups, and were (443.92±39.07) HU, (428.67±50.82) HU ($P>0.05$).

Conclusion: There was significant reduction of bone quality in femoral cancellous bone in ovariectomized rats; the decreased trabeculae number (Tb.N) and no change of mean of trabeculae bone (Tb.mean) in ovariectomized

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rats indicated that the destruction of bone trabeculae was the basic unit for the changes of femoral cancellous bone microstructures in the process of osteoporosis of rats after ovariectomy.

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Key word rat; ovaricetomy; femur; bone trabecular; micro-computed tomography

绝经后骨质疏松主要由雌激素缺乏所致,是以骨量丢失和骨组织微细结构破坏为主要特征的全身性骨代谢疾病[1]。其骨量丢失和微结构的退变以富含松质骨的股骨最为明显[2]。微焦点计算机断层扫描(micro-CT)是空间分辨率在100μm以下的CT,是一种可以全面立体的测量骨微结构、评价骨质量的新技术[3—4]。Martin-Badosa等[5]和David等[6]发现,应用micro-CT对小鼠骨骼进行研究,可以通过三维立体重建,分析测量骨小梁厚度、骨小梁体积及骨小梁分离度等,进而全面、立体、实时的检测骨质量的变化。本研究采用micro-CT技术,研究大鼠去势后股骨骨松质骨微结构的变化,同时探讨雌激素缺乏所致骨微结构改变的可能规律。

1 材料与方法

1.1 动物分组

24只10周龄健康雌性SD大鼠(第四军医大学实验动物中心提供),体重(210±20)g,随机分为去势(ovx)组与对照(control)组,每组12只。去势组切除大鼠双侧卵巢,对照组不作任何处理。两组大鼠均在(24—28)℃,通风良好的相同条件下饲养,自由摄水食。

1.2 去势术

去势组大鼠在1%戊巴比妥钠全麻条件下切除双侧卵巢,0.35ml/100g大鼠体重,腹腔注射,行双侧背部切口,切除卵巢后,分层缝合肌肉层与皮肤。

1.3 组织获取

去势3月后,脱颈处死大鼠,去除软组织,剥离右侧股骨,4%多聚甲醛固定6h,micro-CT扫描。

1.4 micro-CT扫描

本实验采用德国西门子公司Siemens Inveon micro型显微CT,扫描层厚度可达10μm。将处理好的股骨沿长轴平行于扫描床长轴放置,以透明胶布固定,行micro-CT扫描。扫描参数:电压80kV,电流500μA,扫描方式360°旋转,曝光时间3000ms,图像平面分辨率1024×1024,像素点尺寸10μm×10μm,层间距10μm。扫描完成后,进行组织重建。重建完成后,在重建图像内股骨最大投影面选择兴趣区(region of interest, ROI),ROI局限于距股骨干骺端侧1mm,宽2mm,厚1mm范围内。在兴趣区进行3D(three-dimensional)模式构建,以计算机自动生成阈值,提取图像信息,完成图像二值化。以micro-CT自带Inveon Reserch Workplace V2.2.0(SIEMENS)软件进行定量分析,获得大鼠股骨松质骨骨微结构参数:骨体积分数(bone volume/total volume, BV/TV,%)、骨表面积和体积比(bone surface area/bone volume,BS/BV, mm^{-1})、骨小梁厚度(trabecular thickness,Tb.Th, μm)、骨小梁数量(trabeculae number,Tb.N, mm^{-1})、骨小梁分离度(trabecular spacing, Tb.Sp, μm)、骨小梁模式因子(trabeculae pattern factor,Tb.Pf, mm^{-1})及骨小梁平均骨密度(mean of trabeculae bone,Tb.mean, HU)。

1.5 统计学分析

所有实验均重复3次以上,所有数据均采用SPSS 13.0软件进行分析,结果均以均数±标准差表示,两组间比较采用独立样本t检验。

2 结果

根据前述方法,得到清晰的去势大鼠与正常大鼠股骨micro-CT骨扫描的二维切片图和3D结构模式图(图1—4),去势后,小梁间隔减少,大鼠骨质量降低;去势后,大鼠Tb.N减少,Tb.Sp增加,Tb.Th降低,骨小梁形态结构杆状化。

正常大鼠与去势大鼠股骨松质骨骨微结构3D结构参数(表1)。研究结果表明,与正常大鼠相比,去势大鼠的BV/TV、Tb.Th及Tb.N分别减小72.6%、39.0%和56.4%($P<0.05$);BS/BV、Tb.Sp及Tb.Pf分别增加63.7%、354.2%和72.6%($P<0.05$);而两组大鼠Tb.mean分别为(443.92 ± 39.07)HU和(428.67±50.82)HU,无明显变化($P>0.05$)。

图1 control组股骨2D切片图

图2 ovx组股骨2D切片图

图3 control组骨小梁3D结构示意图

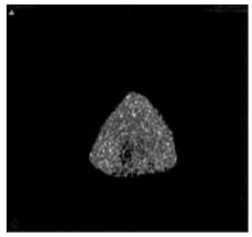

图4 ovx组骨小梁3D结构示意图

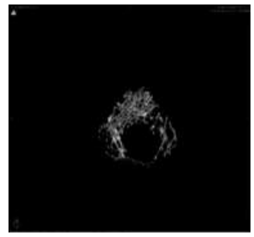

表1 ovx组大鼠去势后3月与control组大鼠股骨骨松质骨小梁参数比较 ($\bar{x}\pm s$)

组别	鼠数	BV/TV(%)	BS/BV(mm^{-1})	Tb.Th(μm)	Tb.N(mm^{-1})	Tb.Sp(μm)	Tb.Pf(mm^{-1})	Tb.mean(HU)
ovx组	12	0.15 ± 0.07	30.43 ± 5.21	0.07 ± 0.01	2.11 ± 0.64	0.44 ± 0.16	8.64 ± 3.72	428.67 ± 50.82
control组	12	0.54 ± 0.13	18.59 ± 3.26	0.11 ± 0.02	4.84 ± 0.22	0.1 ± 0.03	5.01 ± 4.16	443.92 ± 39.07
P		0.0038	0.0055	0.0081	0.0039	0.0287	0.0025	0.1336

3 讨论

随着人口老龄化社会的到来，骨质疏松越来越成为世界性的公共卫生问题，加重了社会卫生资源压力。在骨质疏松的防治中，除外骨密度，骨质量，如骨有机基质、矿物成分及骨微结构等，与骨强度亦具有密切的关系。小梁骨骨微结构参数，已经被应用在年老个体的骨质量评估中，用来进一步研究年龄对骨质量的影响作用[7—8]。研究发现，在绝经后早期，只有骨小梁最先发生明显的骨量丢失[9]。

micro-CT即μCT，又称显微CT，已经广泛用于皮质骨及小梁骨的病理生理研究方面，用于形成高分辨率图像和骨微结构参数[10]，通过micro-CT获得的骨小梁结构参数与传统组织学方法具有良好相关性[11—12]。本实验所用micro-CT扫描厚度达10μm，甚至可精确观察骨小梁，在操作时，可精确定位所观察区域，并进行局部自动放大、分析，在扫描同时，可进行图像重建，几分钟即可完成。

本实验采用去势大鼠作为绝经所致骨质疏松的动物模型。实验结果显示，去势后大鼠股骨松质骨骨小梁BV/TV、Tb.N明显减少，Tb.Sp、Tb.Pf明显增加，与文献报道一致[13—15]，表明去势后骨质量有明显降低。但有研究显示，大鼠去势后，Tb.N减少，由于受力增加，残余骨小梁发生代偿性肥厚，Tb.Th较去势前有明显增加[16—17]，与本实验Tb.Th明显减少的结果不同。有学者发现，扫描仪器分辨率较低时，存在部分容积效应，可致实验值Tb.Th与实际值相比有明显偏高[18—19]；也有学者发现，老年人股骨Tb.Th随年龄增加而明显降低，但在受压应力区，Tb.Th有相对升高表现[20]。本研究中Tb.mean未有明显变化，我们考虑可能是由于去势后，骨小梁骨量和骨小梁体积成等比例丢失和降低，使Tb.mean未发生明显变化有关；也有文献报道，去势后大鼠Tb.mean较去势前增加[14]，我们分析造成差异的原因在于扫描时间，扫描部位或者是扫描仪器分辨率的不同。

骨质疏松病理特点除外骨量丢失，也包括骨质量的改变，骨微体系的研究已经成为骨质量研究的最大组成部分[3—4,21]。微结构体系参数已作为一些抗骨质疏松研究疗效评价的直接生物指标[22—24]。micro-CT作为研究小梁骨形态学特征的重要工具[25]，在对骨微结构特征的研究方面，已经独立于传统的骨密度的检测，在骨强度的预测方面发挥着重要作用。micro-CT不仅可以对特定骨组织区域内的病理刺激和疗效反应提供独特的高分辨率信息[26]，也可无创性的，对皮质骨和小梁骨的微创伤进行3D检测[12,27]，相比组织学方法，同时降低了检测时间和工作量[28]。

micro-CT以其无创性、连续性、全面立体及高效性的特点，通过对骨质量的检测及骨强度预测，在骨质疏松病理学诊断方面发挥着越来越重要的作用。但是，由于其检测空间的限制，micro-CT目前仅能检测小型动物及小块组织，在活体检测时，过多或过频繁的暴露在其X线辐射剂量条件下，可能会

导致骨骼系统生长停滞、骨量丢失、骨骼畸形及血液异常等的不良反应[26]。尽管解决这些问题的难度很大，但是我们相信，随着医学的发展，micro-CT在骨质疏松的早期防治中的优势会越来越突出，并且发挥出其应有的作用。

综上所述，去势后大鼠骨松质骨微结构发生明显变化，尤其Tb.N明显降低及Tb.Sp明显增加的结果，表明在去势后骨质疏松发病过程中，骨小梁在骨微结构改变过程中发挥着重要作用；同时Tb.mean无明显变化，提示在去势后骨微结构的破坏是以单个骨小梁的整体丧失为基本单位进行的。

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