

基础研究

阿伦磷酸钠联合肾骨安对去势小鼠终板结构和椎间盘退变的影响

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【摘要】目的:观察阿伦磷酸钠(alendronate, ALN)联合补肾中药——肾骨安(herbs, HB)对去势小鼠椎体终板、椎间盘退变(intervertebral disc degeneration, IVDD)的影响,初步探讨 ALN+HB 干预 IVDD 的作用机制。**方法:**32 只 12 周龄健康雌性 C57BL/6J 小鼠,随机分为正常对照组(对照组, $n=8$)、去卵巢组(OVX 组, $n=8$)、ALN 组($n=8$)及 ALN+HB 组($n=8$)。对照组行假手术,其余各组行双侧卵巢摘除。术后 3d 开始给药,连续给药 2 个月后取材,HE 及番红 O-固绿染色观察椎间盘组织形态学改变并进行病理评分,显微计算机断层扫描(μ -CT)检测终板表面孔隙变化及附近骨赘增生情况,免疫组织化学法检测椎间盘中 II 型胶原(Collagen-type II, Col2)的表达。**结果:**实验过程中无小鼠死亡。HE 及番红 O-固绿染色对照组椎间盘结构和形态基本正常;OVX 组椎间盘终板发生严重骨化重塑,以下位终板更为明显,同时出现终板与髓核的边界结构紊乱,椎间盘基质的丢失及裂隙形成;ALN 组终板骨化重塑较 OVX 组轻,髓核结构形态改善,软骨终板内仍可见一些小的骨化区;ALN+HB 组终板骨化重塑不明显,番红 O 固绿染色染色可见软骨终板着色加深。IVDD 病理评分、终板表面孔隙率(%)和 Col2 积分光密度(OD)值分别为 1.20 ± 0.84 、 28.60 ± 4.04 和 27764.7 ± 1958.2 , OVX 组为 4.00 ± 1.58 、 56.80 ± 9.39 和 17213.6 ± 1021.3 , ALN 组为 2.40 ± 0.89 、 36.20 ± 3.42 和 18921.4 ± 888.1 , ALN+HB 组为 2.00 ± 0.71 、 29.60 ± 6.19 和 23420.1 ± 2439.6 。OVX 组与对照组比较 IVDD 病理评分、终板孔隙率均显著性升高($P<0.05$);ALN 组、ALN+HB 组与 OVX 组比较二者均显著性降低($P<0.05$);ALN 组、ALN+HB 组与对照组比较,及 ALN+HB 组与 ALN 组比较二者均无统计学差异($P>0.05$)。OVX 组、ALN 组和 ALN+HB 组与对照组比较 Col2 OD 值均显著性降低($P<0.05$),ALN 组与 OVX 组比较无统计学差异($P>0.05$),ALN+HB 组与 OVX 组、ALN 组比较均显著性升高($P<0.05$)。**结论:**OVX 小鼠雌激素缺乏可引起椎体终板的骨化重塑及孔隙率增加,导致 IVDD。ALN 及 ALN+HB 均可以抑制椎体终板的重塑,降低终板孔隙率,保证终板结构和功能完整,从而能延缓雌激素缺乏相关 IVDD, ALN+HB 效果更为明显。

【关键词】椎间盘退变;卵巢切除;终板;阿伦磷酸钠;肾骨安;小鼠

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【Abstract】 Objectives: To observe the effects of alendronate (ALN) combined with tonifying-kidney herbs (herbs, HB) on the endplate lesion and intervertebral disc degeneration (IVDD) in ovariectomized mice, and to investigate the mechanism of ALN+HB in the treatment of IVDD. **Methods:** Thirty-two 12-week-old healthy female C57BL/6J mice were randomly divided into four groups as follows: control group (CT, $n=8$), the ovariectomized group (OVX, $n=8$), the alendronate treated group (ALN, $n=8$) and the group treated with alendronate combining with tonifying-kidney herbs (ALN+HB, $n=8$). The mice in CT group underwent sham operation, and the mice in the other groups were bilateral ovariectomized. Animals were dosed with medicine three days after surgery and sacrificed at two months later. HE and safranin O-fast green staining were used to detect

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the morphological changes of intervertebral disc, and the pathological score was recorded. The u-CT scan was used to examine the surface porosity of endplate and the osteophyte formation nearby. The expression of collagen-type II (Col2) in the intervertebral disc was detected by immunohistochemistry. **Results:** No mice died during the experiment. The structure and morphology of intervertebral disc in CT group showed normal in Hematoxylin-Eosin and Safranin O-Fast green staining. In OVX group, severe ossification occurred on the endplate which was more obvious in the caudal side. Besides, the border between the endplate and the nucleus pulposus was disordered with matrix degradation and crack formation. However, the ossification remodeling of the endplate and structural morphology of the nucleus pulposus in ALN group were obviously improved when compared with OVX group, although small ossification lesions in endplates were still existed. In ALN+HB group, there was hardly no ossification remodeling in endplates, and safranin O staining showed deep dyeing in cartilaginous endplate. The IVDD score, endplate surface porosity(%) and Col2 integral optical density (IOD) were 1.20 ± 0.84 , 28.60 ± 4.04 and 27764.7 ± 1958.2 in CT group, 4.00 ± 1.58 , 56.80 ± 9.39 and 17213.6 ± 1021.3 in OVX group, 2.40 ± 0.89 , 36.20 ± 3.42 and 18921.4 ± 888.1 in ALN group, and 2.00 ± 0.71 , 29.60 ± 6.19 and 23420.1 ± 2439.6 in ALN+HB group, respectively. Both the pathological score of IVDD and the porosity of the endplate were significantly higher in OVX group than in tCT group, ALN group and ALN+HB group ($P < 0.05$). There was no significant difference among ALN group, ALN+HB group and CT group ($P > 0.05$). The Col2 IOD in OVX, ALN and ALN+HB groups was significantly lower than that in CT group ($P < 0.05$), but had no significant difference between ALN group and OVX group ($P > 0.05$). However, the Col2 IOD in ALN+HB group was significantly higher than that in OVX group and ALN group ($P < 0.05$). **Conclusions:** Estrogen deficiency in OVX mice can induce ossification remodeling and increasing permeability of endplate, which affects the disc metabolism and results in IVDD. Meanwhile, ALN and ALN+HB can inhibit the ossification remodeling, reduce the porosity, maintain the structural and the functional integrity of endplate, which delays the menopausal related IVDD. Furthermore, ALN+HB has a better effect on IVDD.

[Key words] Intervertebral disc degeneration; Ovariectomy; Endplate; Alendronate; Herb; Mice

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椎间盘退变(intervertebral disc degeneration, IVDD)是导致脊柱失稳,引起慢性腰痛和神经压迫性疼痛的主要原因,严重时致残。有研究显示,65岁以上的老年人群中70%左右患有不同程度的IVDD^[1],带来了巨大的社会经济负担。近年来,骨与软骨间代谢转换的相互影响越来越受到重视。作为椎间关节,椎间盘通过终板与椎体进行物质交换,且椎体终板Modic改变可在早期IVDD中被发现^[2],说明椎体及终板的退变与IVDD密切相关。一些研究发现女性绝经后的椎体骨质疏松与IVDD具有相关性^[3],雌激素下降造成的椎体骨质代谢异常及骨性终板的结构紊乱可导致IVDD加重^[4,5],说明椎体异常的骨代谢可能是促使IVDD发生的潜在危险因素。阿伦磷酸钠(ALN)能有效抑制骨质破坏,临床用于治疗骨质疏松,并且可以改善软骨下骨结构,减轻早期实验性骨关节炎的软骨退变^[6]。临床研究显示ALN可以延缓IVDD进程中椎间隙狭窄^[7],但也有研究显示ALN并不能阻止IVDD、骨关节炎等退变性疾病

病的病情进展,其远期治疗效果仍有争议^[8]。中医学理论认为“肾主骨生髓”,而骨质疏松、IVDD多发于以“肾虚”为主的中老年人群,通过补肾中药干预可能既可以强骨又可以壮髓,同时起到改善椎体骨质与IVDD的作用。将ALN与补肾中药联合应用或许能弥补ALN的一些不足,对IVDD具有更好的治疗作用。肾骨安(HB)是广东省中医院邓晋丰教授治疗腰腿痛的经验方,由熟附子、淫羊藿、仙茅、巴戟天、丹参等组成,有补肾益髓、强筋壮骨之效,临床疗效显著^[9]。为进一步探讨椎体、终板等对椎间盘的影响及ALN联合HB是否能改善骨质代谢及椎体终板结构,从而延缓IVDD,本研究制作去卵巢小鼠模型观察椎体结构和代谢改变与IVDD的关系,比较ALN与ALN+HB联合用药对骨质疏松相关性IVDD的干预效果,初步探讨其干预机制。

1 材料与方法

1.1 实验动物及造模

12 周龄健康 SPF 级 C57BL/6J 雌性小鼠 32 只 (18.3±1.2g)[购于中山大学实验动物中心, SCXK9 (粤)2011-0029], 4 只/笼饲养于广州中医药大学实验动物中心 SPF 动物房内 (22~25℃, 40%~60%相对湿度, 昼夜交替), 自由饮食。适应性喂养 3d 后随机分为 4 组: 对照组、OVX 组、ALN 组和 ALN+HB 组, 每组 8 只。对照组行假手术, 只暴露卵巢, 不做切除。其余 3 组行双侧卵巢摘除术: 腹腔注射 5%水合氯醛(4mg/10g 体重)麻醉, 腰背部剃毛, 70%酒精消毒备皮。小鼠俯卧位, 两侧髂棘最高点连线定位 L5 棘突, 依次向上扪及 L3 棘突, 做好标记, 后正中中线作 1.5cm 左右切口, 镊子钝性分离皮下筋膜, 将皮肤拉向腹侧, 可透过腹壁看见白色脂肪粒, 卵巢即包藏在脂肪中。手术刀于侧腹壁脂肪粒上方做 0.5cm 大小切口, 小心拉出脂肪及卵巢, 缝线结扎, 摘除卵巢, 余组织重新纳入腹腔, 生理盐水冲洗, 缝合腹壁, 同样的方法切除对侧卵巢, 缝合背部皮肤。本实验经广州中医药大学实验动物中心伦理批准。

1.2 动物给药

术后 3d 开始给药, 对照组与 OVX 组给予生理盐水灌胃, 0.2ml/10g, 1 次/日。ALN 组给予 ALN (Sigma, A -4978)400μg/kg 颈部皮下注射, 2 次/周。ALN+HB 组在给予 ALN 的基础上按照人与小鼠体表面积等效剂量比值换算 HB 汤剂 (广东省康美药业生产), 0.2ml/10g(含生药 1.44g/ml)灌胃, 1 次/日。连续给药 2 个月。

1.3 动物取材及标本处理

给药 2 个月后, 过量水合氯醛麻醉处死小鼠。获取 L1~L6 脊柱节段, 小心剔除周围肌肉等软组织, 手术刀截取 L4~L5 节段, 于 4%中性福尔马林中固定 48h, PBS 漂洗 3 次, 每次 15min。每组选取 3 个样本用于显微 CT(u-CT)检测, 其余样本于

10%的乙二胺四乙酸(EDTA)中脱钙 14d, 之后进行脱水、石蜡包埋和切片。

1.4 椎间盘组织病理染色及评分

组织 4μm 切片后行 HE 及番红 O-固绿染色。染色步骤: 二甲苯脱蜡 10min×2 次, 无水乙醇 5min×2 次, 95%乙醇 5min, 85%乙醇 5min, 70%乙醇 5min, 蒸馏水 5min, 苏木素 10min(固绿 5min), 盐酸酒精 2~5s (1%乙酸 10~15s), 流水冲洗 10min, 伊红复染 5min (番红 O 5min), 85%乙醇 5min, 95%乙醇 5min, 无水乙醇 5 min×2 次, 二甲苯透明 5min×3 次。中性树胶封片。光学显微镜 (Olympus, DP80, Japan)下观察椎体、终板及椎间盘病理改变。并根据 Luo 等^[10]的方法对 IVDD 程度进行病理评分(表 1)。

1.5 免疫组织化学方法检测 Col2 表达

切片脱蜡至水后, PBS 漂洗 5min×2 次, EDTA 抗原修复液 (pH 值 8.0) 中微波加热沸腾修复 20min, 蒸馏水漂洗 2 次, 3% H_2O_2 浸泡 10min 以阻断内源性过氧化氢酶, PBS 漂洗 5min×3 次, Col2 (Abcam, 1:400) 一抗 4℃孵育过夜, PBS 漂洗 10min×3 次。其余步骤按中杉金桥免疫组化二部检测法说明进行 (北京中杉金桥公司, PV6001)。DAB 显色 3min。苏木素对比染核 30s, 梯度脱水, 中性树胶封片。显微镜观察, 黄色或棕色颗粒代表阳性。所有样本图片使用 Image Pro Plus 6.0 软件进行 Col2 光密度的半定量分析。

1.6 u-CT 扫描及分析

采用 u-CT(Skyscan1172)扫描 L4/5 节段。扫描电压 59kV, 电流 100mA, 层厚 9μm。扫描完成后使用 NRecon v1.6 软件对原始图像进行重建, 使用 CTAAn v1.9 软件对终板感兴趣区进行参数分析, 使用 CTvox v3.0 软件进行图像的 3D 重建。

1.7 统计学方法

表 1 椎间盘退变的评分系统
Table 1 Intervertebral disc degeneration assessment scoring system

得分 Scores	髓核 Nucleus pulposus	纤维环 Annulus fibrosus	骨赘 Osteophyte
0	丰富的髓核胶质和大量脊索细胞 Bulging gel with abundant notochodal cells	致密的纤维层 Compact fibrous lamellas	无 Absence
1	脊髓细胞减少, 出现软骨样细胞 Notochodal cells loss; chondrocyte-like cells emergence	纤维软骨组织增生, 髓核纤维环 边界不清 Proliferation of fibrocartilaginous tissue and loss of nuclear-annular border	出现 Appearance
2	局灶性粘液样变性, 裂缝 Focal mucoid degeneration; clefts	纤维环裂隙 Fissures in annulus fibrosis	过度生长 Overgrowth
3	弥漫性粘液样变性, 裂缝贯穿髓核 Diffuse mucoid degeneration and clefts throughout nucleus	—	—

采用 SPSS 20.0 软件进行数据的统计分析, 所有数据进行正态性检验, 不同组别之间对比采用单因素方差分析(One-way Anova), 结果以均值 $\pm$ 标准差($\bar{x}\pm s$)表示。 $P<0.05$ 为具有统计学差异。

2 结果

2.1 椎间盘病理染色及评分

实验过程中无小鼠死亡。椎间盘组织石蜡切片 HE 及番红 O-固绿染色可见对照组椎间盘结构和形态基本正常;OVX 组椎间盘终板发生严重的骨化重塑,以下位终板更为明显,可见软骨终板中大量骨髓腔隙,充满骨髓细胞,同时出现终板与髓核的边界结构紊乱,椎间盘基质的丢失及裂隙形成;ALN 组终板的骨化重塑明显被抑制,髓核的结构形态也有改善,但软骨终板内仍可见一些小的骨化区;ALN+HB 组可见终板骨化重塑几乎被完全抑制,同时番红 O-固绿染色可见软骨终板着色加深(图 1,2)。对照组 IVDD 病理评分为 1.2 ± 0.84 ,OVX 组为 4.0 ± 1.58 ,ALN 组为 $2.4\pm$

0.89 ,ALN+HB 组为 2.0 ± 0.71 。OVX 组与对照组比较病理评分显著性升高($P<0.05$);ALN 组与 ALN+HB 组与 OVX 比较均显著性降低($P<0.05$);ALN+HB 组与 ALN 组比较无统计学差异,但相对于 ALN 组,ALN+HB 组评分有下降趋势。

2.2 椎间盘 u-CT 扫描和终板表面孔隙率

对照组 L4/5 椎间盘下位终板表面孔隙均一,大小适中,孔隙率(%)为 28.6 ± 4.04 。OVX 组终板表面孔隙率明显增加,为 56.8 ± 9.39 ,分布较为混乱,以后侧增加明显,与对照组比较有显著性差异($P<0.05$);同时,L5 椎体前缘靠近终板处可见少量骨赘形成。ALN 组终板表面孔隙率较 OVX 组显著性下降,为 36.2 ± 3.42 ,外周下降明显,终板中央髓核区仍存在小范围高孔隙区,与 OVX 组比较有显著性差异($P<0.05$);靠近终板椎体前缘可见明显的骨赘形成。ALN+HB 组终板表面孔隙较 ALN 组下降更为明显,为 29.6 ± 6.19 ,与 OVX 组和 ALN 组比较有统计学差异($P<0.05$),与对照组相近($P>0.05$);椎体前缘骨赘较 ALN 组有一定程度

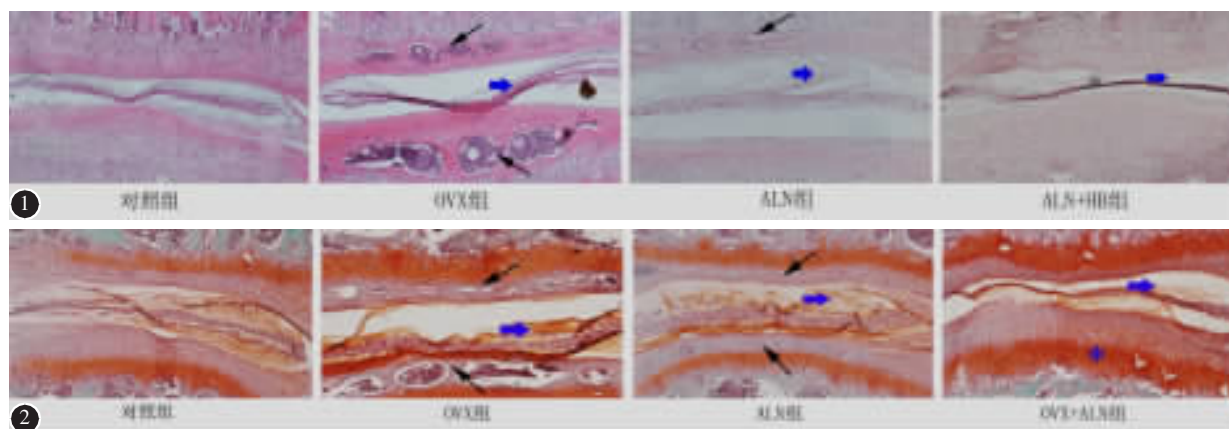


图 1 椎间盘 HE 染色:对照组椎间盘组织形态结构正常;OVX 组椎间盘终板骨化明显,髓核基质降解,边界裂隙形成;ALN 组终板可见少量骨化灶,髓核结构改善;ALN+HB 组终板几乎未见骨化,髓核结构改善(蓝色箭头指示髓核终板边界,黑色箭头指示终板骨化,×10) **图 2** 椎间盘番红 O-固绿染色:各组椎间盘组织病理情况与 HE 染色相似,结构分辨更为清楚;ALN+HB 组中生长板及软骨终板中番红 O 染色明显加深,表面蛋白多糖丰富(蓝色粗箭头指示髓核终板边界,黑色箭头指示终板的骨化,蓝色星号指示番红 O 深染,×10)

Figure 1 Hematoxylin-eosin(HE) staining of discs. The morphology of intervertebral disc in control group was normal. In OVX group, the ossification of endplate was obvious and matrix degradation with border fissure appeared. A few of ossification was seen in endplate of ALN group, and the structure of nucleus pulposus was improved. There was hardly no ossification of endplate of ALN+HB group (Blue arrows indicate the nuclear-annular border and black arrows indicate the ossification of endplate, ×10) **Figure 2** Safranin O-fast green staining of discs. The histopathological changes of intervertebral disc in each group were similar to those of HE staining, and the structure was more clearly distinguished. The staining of safranin O in the growth plate and cartilage endplate in ALN+HB group was significantly deeper indicated the proteoglycan was abundant (Blue arrows indicate the nuclear-annular border and black arrows indicate the ossification of endplate. Blue asterisk indicates deep staining of Safranin O, ×10)

减轻(图 3)。

2.3 椎间盘组织中 Col2 的表达

免疫组化结果显示, 对照组小鼠椎间盘软骨终板呈均匀深棕色, Col2 均匀分布于细胞间质中, 交织成网状, 排列规整, Col2 积分光密度值为 27764.7 ± 1958.2 。OVX 组终板呈浅黄棕色, 尤其在终板的骨化重塑区, 几乎未见阳性表达, Col2 光密度值为 17213.6 ± 1021.3 , 与对照组比较显著性减少 ($P < 0.05$)。ALN 组小鼠终板无 Col2 表达的区域减少, 但无骨化重塑区域的 Col2 表达未见明显增加, 光密度值为 18921.4 ± 888.1 , 与 OVX 组比较无显著性差异 ($P > 0.05$)。ALN+HB 组终板呈黄棕色, 且胶原的排列结构清晰可见, Col2 光密度值为 23420.1 ± 2439.6 , 与 OVX 组和 ALN 组比较显著性增加 ($P < 0.05$) (图 4)。

3 讨论

IVDD 是中老年人群的常见病多发病变, 近年来 IVDD 被认为是一种涉及髓核、纤维环、软骨终板、软骨下骨、周围韧带和软组织的多组织参与的退变^[1]。研究发现遗传、免疫、营养、炎症、机械应力等多种因素参与 IVDD 的发生发展^[1], 而异常机械应力和代谢因素之间的恶性循环可能是 IVDD 发生发展的中心环节^[12]。

软骨下骨病变在骨关节炎等退行性疾病中的作用逐渐成为研究热点, 异常机械应力导致软骨下骨加速重塑引起的微结构损伤可加重实验性骨关节炎^[13], 且在早期的骨关节炎中软骨下骨板的变薄与软骨损伤直接相关^[14]。与关节类似, 终板是影响椎间盘营养代谢与机械应力传导的关键结构, 其位于椎间盘与椎体的交界过渡区, 是椎间盘与相邻上下椎体力学传导的枢纽, 同时终板作为一种半透性屏障是椎间盘获取营养和排泄废物的主要通道^[15, 16]。终板结构和功能紊乱不但直接影

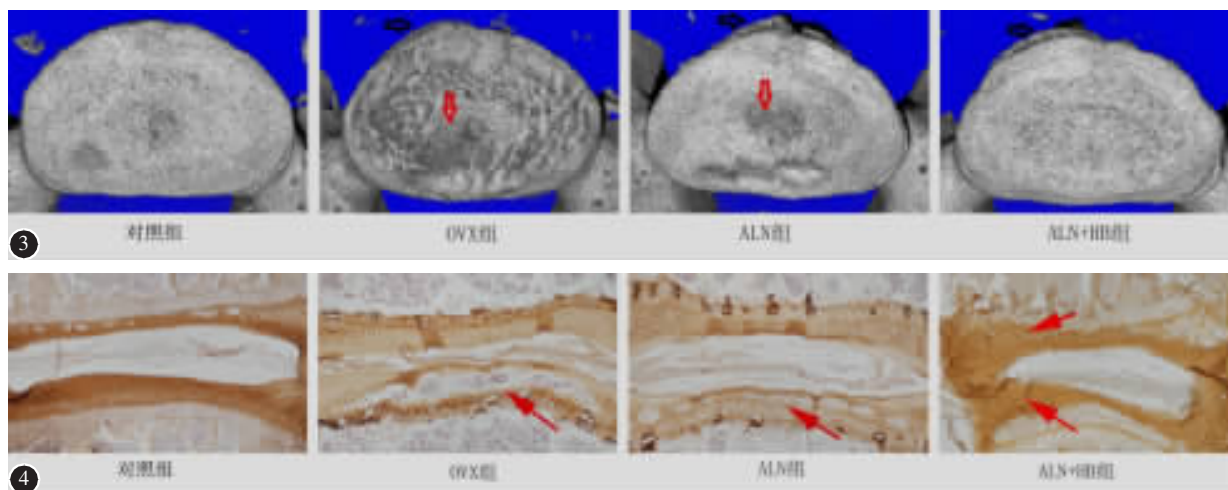


图 3 L4/5 椎间盘下位终板的表面孔隙率: 对照组终板表面孔隙正常, 终板边缘未见骨赘; OVX 可见表面孔隙明显增加, 终板附近少量骨赘形成; ALN 终板表面孔隙明显较少, 中央存在部分高孔隙区, 边缘可见明显骨赘; ALN+HB 组终板表面显著减少, 与对照组相似, 边缘可见少量骨赘 (红色箭头指示孔隙, 黑色箭头指示骨赘) **图 4** 椎间盘 Col2 的表达: 对照组 Col2 表达丰富, 胶原纤维排列有序; OVX 组 Col2 明显减少, 尤其在终板骨化区几乎未见 Col2 表达; ALN 组终板骨化区较 OVX 组减少, 但 Col2 表达未见改善; ALN+HB 组可见 Col2 表达明显增加 (红色箭头指示 Col2 表达, $\times 10$)

Figure 3 The porosity of the caudal endplate of L4/5 intervertebral discs. The surface porosity of endplate in control group was normal, no osteophyte was found in the edge of the endplate; OVX group showed a marked increase in surface porosity and mild osteophyte formation. ALN group's endplate had fewer pores on the surface except the central area and had severe osteophytes; The surface pores of the endplate of ALN+HB group were significantly reduced, a few of osteophytes can be seen at the edge (Red arrows indicate the pores on the endplate and black arrows indicate osteophyte) **Figure 4** The expression of Col2 in intervertebral discs. The expression of Col2 in control group was abundant and collagen fiber was arranged in order. The Col2 expression in OVX group was significantly decreased, especially in the ossification area of endplate. ossification area in ALN group was decreased compared with OVX group, but Col2 expression was not improved. The expression of Col2 in ALN+HB group was significantly increased (Red arrows indicate the expression of Col2, $\times 10$)

响椎间盘与椎体间的应力传导,而且其通透性的改变会扰乱正常的椎间盘代谢导致 IVDD。异常的机械刺激可通过机械传导导致力学敏感的骨、软骨细胞发生重塑^[17],同时椎体骨质代谢改变(如骨质疏松)会造成终板下骨结构的重塑改变。研究发现脊柱失稳会引起终板骨-软骨重塑导致 IVDD 的发生^[18],而绝经后骨质疏松与 IVDD 具有相关性,骨质疏松患者终板中更易发生许莫氏结节^[19],卵巢切除大鼠会出现终板损伤与 IVDD^[5]。这些都预示着终板是导致椎间盘机械应力与代谢失常恶性循环的承载者。椎体终板结构和功能异常对椎间盘力学传递和代谢造成不利影响可能是导致 IVDD 的关键因素。

椎间盘软骨终板和髓核中含有丰富的 Col2 和蛋白多糖,它们是构成椎间盘基质的主要成分,起到维持椎间盘水分、渗透压和缓冲传导负荷的关键作用。终板和纤维环 Col2 的降解导致基质结构的紊乱直接影响到椎间盘的力学缓冲和传递,同时髓核更易于从薄弱的纤维环或者终板突出进入椎管或者椎体。Col2 和蛋白多糖丢失引起髓核内压下降一方面导致椎间隙塌陷,此外缺乏循环载荷下的流体扩散使椎间盘与椎体物质交换出现异常^[20]。在 IVDD 和骨关节炎进展过程中会出现多种基质蛋白酶(matrix metalloproteinase, MMPs)和胶原酶的表达^[10],胶原基质结构破坏紊乱、抗原暴露、免疫炎症酶解反应会扰乱椎间盘内环境,诱发血管神经新生,促使椎间盘纤维化加速 IVDD 的进程。研究显示终板 Col2 降解促使基质的硬化将导致终板软骨细胞钙化,而这与 IVDD 程度有正相关关系^[21]。

ALN 是治疗骨质疏松的常用药物之一,效果显著。最近一些研究发现其能延缓 IVDD^[7],但临床远期疗效存在争议。动物研究结果显示,ALN 能抑制软骨下骨病变延缓骨关节炎的进展^[22]。此外,研究显示 ALN 抑制骨破坏可以改善椎间盘病变程度^[10],其主要通过抑制终板软骨下骨重塑和软骨终板的骨化以及椎间盘中 MMP-1、MMP-3、MMP-13 的表达起到延缓 IVDD 的作用,反映出通过 ALN 干预椎体骨质代谢和抑制椎体终板重塑可能是延缓 IVDD 的有效方法。基于此,ALN 可能是通过改善软骨下骨结构和渗透性,改善其表面软骨应力及代谢而起到治疗作用的,而保证骨-软骨复合体结构功能完整可能是延缓 IVDD

的关键。已有的一些研究显示,补肾中药可以很好地改善骨质疏松和椎间盘代谢^[23-26]。肾骨安是邓晋丰教授治疗 IVDD 及骨质疏松引起腰腿痛的常用方,有补肾益髓、强筋壮骨之效,临床效果显著,现作为广东省中医院院内制剂^[9]。联合应用 ALN+HB 对骨质疏松相关性 IVDD 可能具有更为理想的防治效果。

本研究结果显示,小鼠卵巢切除可导致终板的骨化重塑,同时终板表面孔隙显著增加,椎间盘 Col2 表达显著降低,从而发生 IVDD,说明椎体终板孔隙的增加并不能为椎间盘提供更多的营养而使椎间盘修复,反而扰乱了椎间盘的内环境稳态,促使 IVDD 发生和进展,其可能与无血管椎间盘的抗原暴露引起的免疫炎症反应以及终板病变导致的椎间盘生物力学改变有关。ALN 能抑制椎板重塑,降低孔隙率,延缓 IVDD,但同时促使了终板附近骨赘的形成,其可能是 ALN 抑制终板下骨破骨,椎间盘与椎体间负荷传导改变而引起的机体适应性改变。结果显示,ALN+HB 较 ALN 具有更好保持终板完整性和功能的作用趋势,其显著抑制了终板重塑,保证了合适的终板表面孔隙,同时较 ALN 组在一定程度上抑制了骨赘形成。Col2 在软骨中含量最为丰富,OVX 小鼠软骨终板 Col2 的表达显著下降,ALN 可很好地抑制了终板骨化重塑,但是并没有能明显促使 Col2 的表达。与 ALN 相比,ALN+HB 显著促进了软骨终板中 Col2 的表达,保护软骨终板基质,说明 HB 具有促使 Col2 合成代谢的作用。

综上所述,OVX 导致雌激素缺失促使软骨终板骨化重塑及孔隙增加导致 IVDD,ALN+HB 能更好地改善终板结构、功能和椎间盘代谢,促使 Col2 合成,从而延缓 IVDD,通过改善临近椎体骨质代谢和终板结构可能是促使 IVDD 修复的有效方法,但需要进行更多的动物实验以及临床研究加以证实,深入的机制仍有待更进一步的研究。

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