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论著

骨桥蛋白基因反义寡核苷酸对被动吸烟大鼠骨组织的影响

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摘要 目的 探讨骨桥蛋白（OPN）在被动吸烟所致大鼠骨吸收中的作用，了解OPN基因反义寡核苷酸（AS-OPN）对大鼠被动吸烟所致骨质疏松的影响，寻求治疗骨质疏松症的可能有效途径。方法 2月龄SD大鼠40只，随机分4组：对照组、被动吸烟组、AS-OPN组和有义OPN（S-OPN）组，每组10只。按密室熏烟法给大鼠被动吸烟，同时AS-OPN及S-OPN组大鼠每3 d分别iv给予AS-OPN（10 μg•L^{-1}）或S-OPN（10 μg•L^{-1}）6 μL•g^{-1}，吸烟组和对照组给同等剂量的生理盐水，实验持续4个月，然后进行指标的测定。（1）骨代谢生化指标测定：血Ca、血清骨钙素（BGP）和尿Ca/肌酐（Cr）。（2）骨密度测定：测量L_3～L_6各腰椎骨密度、双侧股骨和肱骨的整体骨密度及其7个感兴趣区（ROI）的骨密度。（3）骨形态计量学测定：① 静态参数包括骨小梁面积百分数、骨小梁厚、骨小梁数和骨小梁分离度；② 动态参数包括荧光周长百分率和破骨细胞计数。（4）骨生物力学测定：① L_4椎体压缩试验测量指标包括弹性模量、最大载荷、骨最大应变和能量吸收。② 右股骨三点弯曲试验测量指标包括最大载荷、弹性载荷、最大挠度和弯曲能量；弯曲弹性模量、最大弯曲应力、弯曲刚性系数和弯曲韧性系数。结果 与正常对照组比较，吸烟后大鼠骨密度降低、骨量减少、骨强度降低，破骨细胞数和骨吸收增加。与吸烟对照组比较，给予AS-OPN后大鼠的尿Ca/Cr比值降低(0.08±0.01 *vs* 0.11±0.02)；L_3，L_4，L_5，L_6各腰椎骨密度升高〔27.77±1.38 *vs* 25.20±1.94；26.80±1.66 *vs* 24.25±1.48；27.55±1.61 *vs* 24.20±2.13；26.63±1.17 *vs*（22.58±1.69） mg•cm^{-2}〕，左、右侧股骨骨密度升高〔25.39±1.34 *vs* 23.26±1.16，26.28±0. 92 *vs* (23.30±1.38)mg•cm^{-2}〕；左、右侧肱骨骨密度及其7个ROI的骨密度升高；骨小梁面积百分数、骨小梁厚度、骨小梁数升高〔6.29±0.67 *vs* (5.13±0.54)%，55.82±2.78 *vs* (49.10±4.36)μm，0.73±0.05 *vs* (0.64±0.07) mm〕；骨小梁分离度、破骨细胞计数和荧光周长百分率降低〔22.48±0.93 *vs* (23.58±0.59)mm，25.33±0.85 *vs* (16.90±0.84)mm^{-2}，38.56±1.63 *vs* (40.32±0.79)%〕；L_4椎体的弹性模量、最大载荷、骨最大应变和能量吸收升高〔951.1±6.6 *vs* (935.4±10.3)MPa，178.9±4.2 *vs* (174.3±2.5)N，(1.68±0.09)×10^{-2} *vs* (1.57±0.06)×10^{-2}；201.46±1.03 *vs* (199.25±1.47)N•mm〕；右股骨的最大载荷、弹性模量、最大挠度和弯曲能量升高〔100.59±1.35 *vs* (98.44±1.21)N，70.43±0.61 *vs* (69.26±0.94)N，1.66±0.06 *vs* (1.56±0.08) mm，80.06±1.07 *vs* (78.54±1.36)N•mm〕；右股骨的弯曲弹性模量、最大弯曲应力、弯曲刚性系数和弯曲韧性系数升高〔5.67±0.12 *vs* (5.52±0.12)GPa，168.24±1.00 *vs* (166.08±1.12)MPa，26.14±1.07 *vs* (24.88±1.13)kN•mm^2；17.4±0.9 *vs* (15.6±1.0)μm•N^{-1}。给予S-OPN对这些指标改变无明显影响。结论 OPN基因反义寡核苷酸可以抑制吸烟所致骨骼的骨密度、骨量、骨转换、骨结构、骨强度的改变。

关键词 烟草烟污染 骨桥蛋白 寡核苷酸类, 反义 骨和骨组织
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Influence of osteopontin gene antisense oligonucleotides on bone and bones of passive smoking rats

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Abstract

AIM To investigate effects of osteopontin (OPN) in rat bone resorption caused by passive smoking and to understand the influence of osteopontin gene antisense oligonucleotides (AS-OPN) on rat osteoporosis caused by passive smoking and to search for potential effective ways to treat osteoporosis. **METHODS** Forty Sprague-Dawley(SD) rats (2-month-old) were randomly divided into 4 groups: control, smoking, antisense oligonucleotides (AS-OPN) and sense oligonucleotides (S-OPN) groups. Apart from control group, other group rats were exposed to passive cigarette smoking daily. The rats in AS-OPN group were given 6 μL·g^{-1} AS-OPN 10 μg·L^{-1} every 3 d by intravenous injection. The rats in S-OPN group were given 6 μL·g^{-1} S-OPN 10 μg·L^{-1} every 3 d by intravenous injection. The rats in control group and smoking group were given the same dose normal saline every 3 d by intravenous injection. The time had been lasted for 4 months and then they were proceeded to the following measurements: (1) Bone metabolic biochemical indicators were measured including serum calcium(Ca), serum bone-gla-protein(BGP) and urine calcium/creatinine(Ca/Cr). (2) Bone mineral density (BMD) was measured including the BMD of 3rd lumbar, 4th lumbar,5th lumbar and 6th lumbar vertebrae (L_3-L_6) and the BMD of two sides overall femoral and humeral bones and their 7 regions of interest(ROI). (3) Bone histomorphometry was measured: ① Static parameters were including bone trabecula areas percentage, bone trabecula thickness, bone trabecula number and bone trabecula separation degrees.② Dynamic parameters were including fluorescence circumference percentage and osteoclast number. (4) Bone biomechanics was measured: ① The indicators of L_4 body of vertebra compression testing including elastic modulus, maximal loading, maximum strain of bone and energy absorbability. ② The indicators of right femur three point bending test including the structural mechanics indicators(maximal loading, elastic loading, maximum deformation and bending energy) and the materials mechanics indicators (bending modulus of elasticity, maximal bending stress, coefficient of bending stiffness and coefficient of bending ductility). **RESULTS** Compared to normal controls, smoking exposure decreased bone mineral density, bone mass, bone strength and increased osteoclast number and bone absorption. Compared with smoking exposure group, AS-OPN decreased urine Ca/Cr (0.08±0.01 *vs* 0.11±0.02), increased BMD of L_3-L_6 〔27.77±1.38 *vs* 25.20±1.94；26.80±1.66 *vs* 24.25±1.48；27.55±1.61 *vs* 24.20±2.13；26.63±1.17 *vs* (22.58±1.69) $mg\cdot cm^{-2}$〕, increased BMD of left femur and right femur 〔25.39±1.34 *vs* 23.26±1.16, 26.28±0. 92 *vs* (23.30±1.38)$mg\cdot cm^{-2}$〕; increased BMD of left humerus and right humerus and their seven ROI. AS-OPN increased percent trabecular bone, trabecular number and trabecular thickness 〔6.29±0.67 *vs* (5.13±0.54)%, 55.82±2.78 *vs* (49.10±4.36)μm, 0.73±0.05 *vs* (0.64±0.07)mm〕. AS-OPN decreased trabecular separation, fluorescence circumference percentage and osteoclast number 〔22.48±0.93 *vs* (23.58±0.59)mm, 25.33±0.85 *vs* (16.90±0.84)mm^{-2}, 38.56±1.63 *vs* (40.32±0.79)%〕. AS-OPN increased elastic modulus, maximal loading, maximum strain of bone and energy absorbability of L_4 〔951.1±6.6 *vs* (935.4±10.3) MPa, 178.9±4.2 *vs* (174.3±2.5)N, $(1.68±0.09)\times 10^{-2}$ *vs* $(1.57±0.06)\times 10^{-2}$；201.46±1.03 *vs* (199.25±1.47)N·mm〕. AS-OPN increased maximal loading, elastic loading, maximum deformation and bending energy of right femur 〔100.59±1.35 *vs* (98.44±1.21)N, 70.43±0.61 *vs* (69.26±0.94)N, 1.66±0.06 *vs* (1.56±0.08)mm, 80.06±1.07 *vs* (78.54±1.36)N·mm〕. AS-OPN increased bending modulus of elasticity, maximal bending stress, coefficient of bending stiffness and coefficient of bending ductility of right femur 〔5.67±0.12 *vs* (5.52±0.12)GPa, 168.24±1.00 *vs* (166.08±1.12)MPa, 26.14±1.07 *vs* (24.88±1.13)$kN\cdot mm^2$；17.4±0.9 *vs* (15.6±1.0)$\mu m\cdot N^{-1}$. While S-OPN could not prevent the adverse effects of smoking exposure on bone tissues. **CONCLUSION** AS-OPN is found to prevent the adverse effects of smoking exposure on bone mineral density, bone mass, bone turnover, bone structure and bone strength.

Key words tobacco smoke pollution osteopontin oligonucleotides antisense bone and bones

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