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158-160

尼莫地平对血小板聚集和血栓形成的抑制作用

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关键词 尼莫地平；血小板聚集；血栓形成；血栓素 B_2；6-酮前列腺素 $F_{1\alpha}$；腺苷二磷酸；颈动脉

目的：研究尼莫地平（Nim）对大鼠体内血小板聚集和动脉血栓形成的影响。**方法**：比浊法测定血小板的聚集率和抑制率；电刺激法测定 Nim 对体内动脉血栓形成的影响，放免法测定 Nim 对血浆 6-酮前列腺素 $F_{1\alpha}$ 和血栓素 B_2（6-keto-$PGF_{1\alpha}$/TXB_2）含量的影响。**结果**：Nim 4.5，9，18 和 36 $mg\cdot kg^{-1}\cdot d^{-1}$ ig 4 d 可显著抑制血小板的聚集。IC_{50}（95 % 可信限）为 26（9 - 44）$mg\cdot kg^{-1}$。Nim 4.5，9，18 $mg\cdot kg^{-1}\cdot d^{-1}$ ig 4 d 可显著延长电刺激诱导的颈动脉血栓形成时间。Nim 9 和 18 $mg\cdot kg^{-1}$ 可明显改善血浆中 6-keto-$PGF_{1\alpha}$/TXB_2 的比值。**结论**：Nim 抗血栓作用部分与改善 6-keto-$PGF_{1\alpha}$/TXB_2 比值有关。

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Skeletal effects of constant and terminated use of sodium risedronate in ovariectomized rats[1]

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KEY WORDS diphosphonates; ovariectomy; osteoporosis; sodium risedronate; tibia

AIM: To study the skeletal effects of constant and terminated use of sodium risedronate (Ris) treatment in the ovariectomized (Ova) rats. **METHODS**: Ris 5 $\mu g\cdot kg^{-1}$, sc, twice a wk. The proximal tibial metaphysis (PTM) were processed undecalcified for quantitive bone histomorphometry. **RESULTS**: (1) Placebo-treated (normal saline) Ova rats were characterized by decreased trabecular area (TA) on d 60, d 81, and d 150 compared with aging controls, and bone resorption was over formation with high bone turnover. (2) Ova rats were treated with Ris for 60, 81, and 150 d (Ris-on) increased (TA 217 %, 108 %, and 101 %) respectively, *vs* Ova rats and depressed bone turnover indices to aging control level, but bone mass did not maintain at high level in 150-d group as in the early stage. (3) Ova rats were pretreated with Ris for 60 d and then terminated (Ris-on/off), followed by sequential sacrifice of rats on 21 and 90 d. Withdrawal on 21 d showed the same results as the match-age Ris-on group. Withdrawal on 90 d still maintained cancellous bone mass at a high level *vs* 150 d Ris-on groups (+ 26 %) and aging control group (+ 27 %). **CONCLUSION**: Regimen of Ris 60 d on then 90 d off prevented the development of osteoporosis in Ova rats.

Sodium risedronate (Ris) shows a very strong antiresorption effect and low turnover associated with decreasing bone formation and lowering activation in animals[1-5] and humans[6]. Remodeling is very important to repair the microdamage. Prolonged depression of bone remodeling increased the risk of fracture. The purpose of this study was to determine

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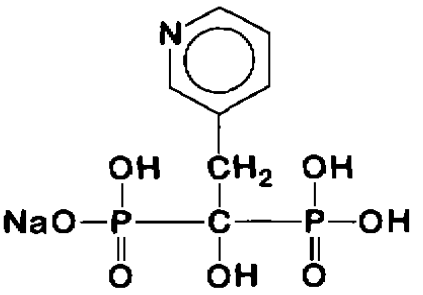
the difference of the effects of Ris administration and withdrawal in trabecular bone site.



Sodium risedronate

MATERIALS AND METHODS

Rats Sprague-Dawley rats, ♀, $n = 83$, 3-month-old, weighing 218 ± 18 g, (Guangdong Animal Experimental Center, China) were acclimated to the milieu for 2 wk. Rats were housed in 69 cm × 30 cm × 20 cm cages with water and food pellets (Animal Center of Guangdong Medical College) *ad lib*. Fifty-six rats were ovariectomized and 21 rats were sham operated from a dorsal approach[7]. Rats were divided into 12 groups.

Experimental design Basal control were killed on d 1 without treatment. Ovariectomy and sham operated controls (Ova and aging controls) received vehicle (normal saline) injections and were killed on 60, 81, or 150 d after operation. Ris-on groups received Ris (sc 5 $\mu g \cdot kg^{-1}$ twice a week) injections and killed on 60, 81, or 150 d after treatment. Ris-on/off groups received Ris for 60 d followed by vehicle injections for 21 or 90 d and were killed on d 81 or d 150. Ris was provided by Prof Webster SS Jee (Radiobiology Division, Bldg 586, School of Medicine, University of Utah, Salt Lake City UT 84112).

Labeling administraton All rats received subcutaneous injections of tetracycline 30 $mg \cdot kg^{-1}$ (Lederle Laboratory, Pearl River NY USA) on 14 and 13 d and calcein 5 $mg \cdot kg^{-1}$ (Sigma Chemical Co. St Louis MO. USA) 4 and 3 d before sacrifice.[8]

Autopsy and sample preparation Rats were killed by heart puncture under sodium pentobarbitital (40 $mg \cdot kg^{-1}$) anesthesia. The left tibia was cut into 3 equal parts which were then prestained and cut into sections[8].

Bone histomorphometry Measurements were made with a digitizing system consisting of a light and epifluorescent microscope, coupled to an Apple Macintosh computer with a morphometry program "Stereology" (KSS Computer Engineers, Magna UT)[9]. Total tissue area, trabecular bone area, and perimeter were measured to calculate the % of trabecular bone area (TbAr%), and trabecular width (TbWi), number (TbN) and separation (TbSp)[4]. Dynamic parameters included single- and double-labeled perimeters, interlabeled width, osteoid perimeter, eroded perimeter, and trabecular wall width. These parameters were used to calculate the % of labeled perimeters (LPm%), mineral apposition rate (MAR), bone formation rates (BFR), the % of osteoid perimeter (OPm%), the % of eroded perimeter (ErPm%), and activation frequency (ActF)[3,5].

Statistic analysis Data were presented as $\bar{x} \pm s$. One-way ANOVA with Fisher PLSD test of ANOVA was used for multiple comparisons between different groups[10].

RESULTS

Aging skeletal changes No significant change in cancellous bone mass of proximal tibial metaphysis (PTM) in sham-Ova rats was seen in the 150-d experiment (3- to 8-month-old). Decrease in bone formation indices (% LPm, BFR/BV) and increase in % eroded perimeter were observed between 60 and 150 d after sham operation (ie, between 5 and 8 months of age) (Tab 1).

Ova skeletal changes Decreased in TbAr and TbN with no change in TbWi was found during the first 60 d post-Ova. There was no further change in bone mass seen on d 81 and 150. Ova increased both bone formation parameter and eroded perimeters when compared to the sham-operated controls. ActF was elevated in 81 and 150 d (Tab 1).

Skeletal effects of Ris-on groups Treatment with Ris for 60 d markedly increased the TbAr and TbN in PTM of Ova rats. For dynamic data: Treatment with Ris for 60 d decreased bone formation indices (MAR, BFR/BV, and %OPm) and percent eroded perimeter with a marked reduction in ActF in Ova rats. Extended the Ris treatment to 81 d produced almost the same results as those of 60 d, but these bone mass did not maintain the same high level at 150 d as in the early stages (Tab 1).

Skeletal effects of Ris-on/off groups After cessation of Ris administration, the bone mass was unchanged on d 81 and maintained higher level than Ris-on and sham group on d 150 (Tab 1).

DISCUSSION

The diphosphonates are as effective as estrogen in inhibiting bone resorption and turnover, preventing Ova-induced cancellous bone loss, and maintaining bone mass for certain period[1-2]. Our experiment

Tab 1. Proximal tibia after Ris treatment and withdrawal in OVX rats. $^bP < 0.05$ *vs* sham-Ova; $^cP < 0.05$ *vs* Ova; $^bP < 0.05$ *vs* Ris-treated. % derived from formula $X_2 \div X_1 \times 100 - 100$.

Group	*n*	TbAr (%)	TbWi (μm)	TbN (#/mm)	TbSp (μm)	LPm (%)	MAR (μm/d)	BFR/BV (%/a)	OPm (%)	ErPm −(%)	ActF (cycle/a)
Basal	4	21 ± 10	57 ± 4	3.7 ± 0.7	224 ± 58	22 ± 4	0.75 ± 0.02	178 ± 35	12 ± 3	0.31 ± 0.18	2.2 ± 0.6
60-Sham	6	26 ± 7	57 ± 4	5.0 ± 1.0	174 ± 65	29 ± 4	0.80 ± 0.13	250 ± 69	4.8 ± 2.6	0.32 ± 0.17	0.9 ± 0.5
81-Sham	6	23 ± 2	61 ± 6	3.8 ± 0.6	211 ± 44	27 ± 5	0.73 ± 0.11	197 ± 45	5.6 ± 4.4	0.42 ± 0.23	0.8 ± 0.7
150-Sham	7	20 ± 8	59 ± 5	3.3 ± 1.3	308 ± 206	21 ± 3	0.72 ± 0.06	157 ± 37	5.2 ± 3.4	0.8 ± 0.3	0.9 ± 0.6
60-Ova	6	10 ± 6	58 ± 5	1.7 ± 0.9	695 ± 409	36 ± 7	0.95 ± 0.28	362 ± 152	15 ± 12	0.9 ± 0.9	3.6 ± 3.4
%60-Sham		−61^b	2	−63^b	300^b	23	19	45^b	217^b	178^b	299
81-Ova	6	16 ± 4	63 ± 7	2.5 ± 0.5	353 ± 85	29 ± 3	0.99 ± 0.16	282 ± 78	17 ± 4	0.86 ± 0.24	4.4 ± 1.5
%81-Sham		−31	3	−34^b	67	7	36^b	43^b	204^b	105^b	428^b
150-Ova	7	10 ± 3	67 ± 10	1.5 ± 0.3	644 ± 176	27 ± 3	0.99 ± 0.12	220 ± 41	11.0 ± 2.3	0.88 ± 0.21	3.4 ± 2.8
%150-Sham		−50^b	13	−56^b	109^b	27	25^b	40^b	104^b	14	290^b
60-Ova + Ris	6	32 ± 4	52 ± 5	6.0 ± 0.7	110 ± 199	24 ± 7	0.62 ± 0.10	177 ± 55	2.5 ± 2.2	0.24 ± 0.18	0.34 ± 0.29
%60-Sham		23	−9	38	−37	−16	−23	−29	−49	−25	−63
%60-Ova		218^c	−11	268^c	−84^c	−32^c	−35^c	−51^c	−84^c	−73^c	−91^c
81-Ova + Ris	6	33 ± 7	59 ± 6	5.6 ± 1.2	129 ± 49	27 ± 13	0.57 ± 0.06	153 ± 59	2.5 ± 1.7	0.30 ± 0.18	0.35 ± 0.28
%81-Sham		43^b	−4	48	−39	−2	−22	−22	−54	−29	−58
%81-Ova		108^c	−6	124^c	−63^c	−8	−42^c	−46^c	−85^c	−65^c	−92^c
150-Ova + Ris	7	20 ± 3	50 ± 5	4.0 ± 0.4	203 ± 24	16 ± 3	0.52 ± 0.09	104 ± 23	1.7 ± 0.6	0.66 ± 0.24	0.23 ± 0.06
%150-Sham		1	−15	21	−34	−22	−28	−34	−67	−14	−74
%150-Ova		101^c	−25	173^c	−69^c	−39^c	−42^c	−53^c	−84^c	−25	−93^c
Ova + Ris/V21	6	32 ± 11	53 ± 6	5.9 ± 1.7	125 ± 47	25 ± 7	0.63 ± 0.14	180 ± 66	2.2 ± 1.7	0.29 ± 0.09	0.41 ± 0.47
%81-Sham		40^b	−14	58	−41	−9	−14	−9	−61	−31	−51
%81-Ova + Ris		103^c	−16	139^c	−65^c	−15	−36^c	−36^c	−87^c	−66^c	−91^c
%81-Ova + Ris		−2	−10	7	−3	−7	11	17	−13	−3	17
Ova + Ris/V90	7	25 ± 4	56 ± 8	4.5 ± 0.23	169 ± 15	17 ± 3	0.72 ± 0.19	132 ± 34	4.7 ± 3.7	0.43 ± 0.17	0.85 ± 0.89
%150-Sham		27^b	−5	35	−45	−19	0	−16	−10	−44^b	−3
%150-Ova + Ris		153^c	−16	205^c	−74^c	−36^c	−20	−40^c	−56^c	−51^c	−75^c
%150-Ova + Ris		26^h	13	12	−17	4	38	27	176	−35	270^h
%Ova + Ris/V21		−21	6	−25	35	−31	14	−27	113	48	107

showed the same results in this study. Since bone formation couples to bone resorption during bone remodeling, depressing bone turnover will have the potential to hamper the repair of microdamage and increase the fragility of bone. Ris has strong chemical affinity for the surfaces of calcium hydroxyapatite, the major inorganic component of bone; there is less bone surface reaction[11]. For these reasons, the current study emphasizes that after the 60 d Ris administration then withdrawal of Ris 21 and 90 d to determine skeletal effect in this regimen, compared with consistant Ris-treatment of 150 d, the extra cancellous bone mass induced by 60 d of Ris treatment was maintained in 90 d withdrawal Ris group, not maintained in 90 d Ris-on group. This results indicate that the regimen of this study is a good one according to bone remodeling pattern and Ris characteristics. These results is consistent with another authors' results[2,12], although their regimen is a different time schedule.

In summary, the skeletal effects of withdrawal of Ris treatment were found to be protective against bone loss in Ova rats, with a mechanism similar to that of anti-resorptive agents, ie suppression of bone turnover. The prolonged skeletal effects of Ris after withdrawal of the drug are probably a consequence of the long retention of Ris in the bone, even after short time Ris treatment (60 d). These findings suggest that Ris compound used in a regimen of 60 d on and 90 d off may be sufficient for long-term prevention of bone loss in postmenopausal and oophorectomized women.

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160 – 163

利塞膦酸钠使用和撤药后对去卵巢大鼠骨骼的影响[1]

R681.

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关键词 二磷酸盐类; 卵巢切除术; 骨质疏松; 利塞膦酸钠; 胫骨

目的: 探讨利塞膦酸钠(Ris)使用和撤药对去卵巢大鼠骨骼的影响. **方法**: Ris 5 $\mu g \cdot kg^{-1}$, 皮下注射一周两次. 胫骨上段不脱钙骨制片测量. **结果**: (1) 去卵巢喂水组胫骨骨小梁面积三组都减少, 出现骨吸收大于骨形成的骨高转化率. (2) Ris 治疗 (Ris-on) 的去卵巢组与(1)比, 60, 81 和 150 天, 骨小梁的面积分别增加(217 %, 108 % 和 101 %), 并降低骨高转化率至对照组. 与 60, 81 天组比, 150 天组不能维持较高的骨量. (3) 用 Ris 60 天, 撤药观察, 21 天时与(Ris-on)组无差异, 90 天时骨小梁面积增高(+26 %), 并超过年龄对照组(+27 %). **结论**: Ris 使用/撤药的方案对去卵巢大鼠有预防骨质疏松的作用.

本刊主编丁光生教授于 1997 年 12 月荣获第五届中国韬奋出版奖, 特此表示祝贺! 并感谢丁光生教授将所获奖金 4000 元捐赠给《中国药理学报》基金会.

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