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蛇床子总香豆素对类固醇性骨质疏松的作用

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Effect of total coumarins from dried fruits of *Cnidium monnieri* on glucocorticoid-induced osteoporosis in rats

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ABSTRACT Twenty-four 3-month-old ♂ SD rats were divided into 3 groups: Group 1 — control (ig water). Group 2 — ig prednisone 45 $\mu g \cdot kg^{-1}$ twice a week. Group 3— treated with total coumarins of dried fruits of *Cnidium monnieri* (L) Cuss (TCCM) and prednisone. TCCM was given ig 5 $g \cdot kg^{-1}$, 6 days per week. After 90 d, rats were killed. The proximal tibiae of rats were processed undecalcified for histomorphometric analysis. In comparison with control rats, the bone resorption was enhanced and bone formation decreased. The trabecular bone areas were characterized by reduction of 40 % in rats which received prednisone. Trabecular bone areas of rats treated with TCCM increased 56 % compared with rats receiving prednisone. All indices of bone histomorphometry were near to those in the control. The results showed that TCCM prevented glucocorticoid-induced osteoporosis.

KEY WORDS coumarins; prednisone; osteoporosis; *Cnidium monnieri*

摘要 蛇床子总香豆素(TCCM) 5 $g \cdot kg^{-1}$ ig, 每周6次, 泼尼松45 $\mu g \cdot kg^{-1}$ ig, 每周2次, 持续90 d. 不脱钙骨制片测量. 结果: 泼尼松组大鼠胫骨骨小梁骨吸收增加、骨形成减少, 骨小梁面积减少40 %. TCCM加泼尼松组骨小梁面积增加56 %, 而各项骨计量学指标接近对照组. 提示TCCM能防治泼尼松引起的骨质疏松.

关键词 香豆素类; 泼尼松; 骨质疏松症; 蛇床

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需要长期用糖皮质激素(glucocorticoids, GC)治疗的病人中, 约有半数病人发生骨质疏

松，甚至股骨头无菌性坏死[1,2]。虽有使用钙、维生素 D、NaF 等治疗的报道[3,4]，但尚不满意。蛇床子的提取物—蛇床子总香豆素（total coumarins from dried fruits of *Cnidium monnieri* (L) Cuss，TCCM）有类似性激素作用，能使子宫及卵巢重量增加，睾丸增大[5]，本文探讨 TCCM 对泼尼松引起骨质疏松的防治作用，为临床应用提供理论依据。

MATERIALS AND METHODS

蛇床子素和总线性呋喃香豆素的提纯 蛇床子是伞形科蛇床属蛇床的果实，其主要有效成分是6种香豆素（Tab 1）。

Tab 1. Chemical constituents of *Cnidium monnieri*.

	R_f	mp (℃)	λ_{max} (nm)
Osthol ($C_{15}H_{16}O_3$)	0.639	82—84	322
Imperatorin ($C_{16}H_{14}O_4$)	0.50	99—101.5	249
Bergapten ($C_{12}H_8O_4$)	0.538	194—195	249
Isopimpinelline ($C_{13}H_{10}O_4$)	0.456	149—151	248
Xanthotoxin ($C_{12}H_8O_4$)	0.49	149—151	248
Xanthotoxol ($C_{11}H_6O_4$)	0.397	254—256	250

它们的成份分别与已知样品测混合 mp 均不下降。因这6种香豆素均从蛇床子中分离所得，故称为蛇床子总香豆素（TCCM）。本实验用的 TCCM 由湛江制药厂药物研究所提纯[6]和鉴定[5,7]。

动物模型 SD 大鼠♂，24只3月龄，体重306±s 15 g，（广东实验动物中心提供）随机分3组，每组8鼠。第1组：正常对照（每天用凉开水 ig），第2组：泼尼松（福建省湖州制药厂出品）每周星期三、六、用泼尼松 45 $\mu g \cdot kg^{-1}$ ig，其余时间用水 ig，第3组：泼尼松（用量同第2组）+TCCM，5 $g \cdot kg^{-1}$ ig，每周6次，（TCCM 含蛇床子素，占蛇床子果实的2 %，总线性呋喃香豆素占蛇床子果实的0.8 %）。3组大鼠在同一间明亮、清洁、通风良好、室温25℃的动物房中饲养3个月（饲料均由本院实验动物中心配制），每周称大鼠体重一次。分别在处死前的第10天和第2天给大鼠 tetracycline（上海新亚制药厂）25 $mg \cdot kg^{-1}$ sc，使骨表面形成荧光双标记，以动态观察两次注射期间内骨矿化情况。

骨片的制作 饲养3个月后处死大鼠，用台金骨锯（美国产）把胫骨横断为三段，然后胫骨近端再冠状锯开，置10 %磷酸缓冲液配制的福尔马林中固定，Villanneva 染色，EtOH 和 Me_2CO 脱水，甲基丙烯酸甲脂（$C_5H_8O_2$）包埋不脱钙骨，锯片厚300 μm，然后人工磨至20 μm，脱水，透明，封片[8]。

体视学测量的仪器及方法 用数字图象分析系统（日本产，由 Nikon 萤光显微镜、Mactablet 测量板、Macintosh 电子计算机组成），及配体视学电脑程序（KSS Computer Engineers，Magna UT，USA）。放大39.63 和313 倍，对胫骨近端从距骺浅1 mm 处至远端3 mm 范围内的骨小梁进行骨计量学测量。

骨计量学术语的涵义、缩写和参数计算见 Tab 2 和 Tab 3。

Tab 2. Measurement for trabecular bone histomophometry.

Parameters	Units
Total tissue area (T.A)	mm^2
Trabecular bone area (TbA)	mm^2
Trabecular bone perimeter (TbP)	μm
Single tetracycline label perimeter (STLP)	mm
Double tetracycline label perimeter (DTLP)	mm
Interlabel width (ILW)	mm
Eroded (Howship's lacuna) perimeter (EP)	mm
Osteoid perimeter (OP)	mm
Osteoid width (OW)	μm

统计学处理 百分率（%）用（$\bar{x}_c/\bar{x}_1$）100—100 公式计算。各参数值均数差异的显著性用 t 检验。

RESULTS

大鼠体重。处死时，对照组为375±37 g，泼尼松组为364±24 g，TCCM 治疗组为371±32 g，3组间的体重差异无显著性（$P>0.05$）。

与对照组比较，（1）泼尼松组大鼠胫骨近端骨吸收增加（吸收陷窝周长+353 %），骨形成减少（骨形成率−49 %，矿化沉积速率−28%，类骨质百分比−47 %），骨质丢失（骨小梁面积−40 %，骨小梁周长−33 %，骨小梁厚度−18 %，骨小梁密度−34 %，骨小梁间

Tab 3. Histomophometric parameters calculated.

Parameters	Formulae	Units
Percentage of trabecular area (TbA %)	(TbA/T.A)×100	%
Trabecular number (TbN)	(1.199/2)×(TbP/T.A)	条/mm
Trabecular thickness (TbT)	(2000/1.199)×(TbA/TbP)	μm
Trabecular separation (TbS)	(200/1.199)×(T.A−TbA)/TbP	μm
Percentage of labeled perimeter (LP %)	[(DTLP+STLP/2)/TbP]×100	%
Percentage of eroded perimeter (EP %)	(EP/TbP)×100	%
Osteoid area (OA)	OP·OW/1000	mm^2
Percentage of osteoid area (OA %)	(OA/T.A)×100	%
Mineral apposition rate (MAR)	ILW/Interval	μm/d
Bone formation rate (BFR)	(DTLP+STLP/2)×MAR/TbP×100	μm/d×100
Mineralization lag time (MLT)	OW/MAR	day

隙+54 %). 但二组的荧光(四环素)标记周长百分比及矿化延迟时间无显著差异. (2) TCCM 治疗组大鼠的骨吸收减少−77 %, 骨形成增加(类骨质面积百分比+67 %, 矿化沉积率+29 %, 骨形成率+71 %), 骨量增加(骨小梁面积+56 %, 周长+40 %, 厚度+17 %, 密度+40 %, 间隙−30 %). 荧光(四环素)标记周长百分比及矿化延迟时间的无显著差异 (Tab 4).

DISCUSSION

本文泼尼松组的大鼠胫骨近端骨小梁的骨形成减少, 表现为类骨质分泌减少, 矿化沉积速率下降, 骨吸收明显增加. 与文献[9]结果相符. 性激素是骨代谢的强大调节剂[10-12], 其减少在糖皮质激素引起骨质疏松的过程中起重要作用[13]. TCCM 对于雄性动物, 有类似雄激素作用, 可增加骨的形成, 减少骨吸收.

用 TCCM 治疗的大鼠骨小梁的骨吸收明显减少, 各项骨计量学指标接近对照组. 推测 TCCM 还直接作用于骨, 影响骨细胞, 以抵消泼尼松对骨骼的副作用, 抑制骨吸收, 使骨形成增加, 使骨量基本维持在原有水平.

本实验的泼尼松组和对照组的四环素标记周长百分比及矿化延迟时间无显著差异. 故认为 GC 可能不影响骨的矿化. 加 TCCM 治

Tab 4. Proximal tibital histomorphometric data after prednisone and TCCM treatment. $n=8$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control. $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ *vs* prednisone.

	Control	Prednisone		Prednisone + TCCM		
	$\bar{x}\pm s$	$\bar{x}\pm s$	%	$\bar{x}\pm s$	%	%−1
TbA %	12.6±1.8	7.5±1.0	−40[c]	11.8±2.1	−7[a]	+56[f]
TbP	55.8±15.6	37.3±4.8	−33[b]	52.3±8.9	−6[a]	+40[f]
TbT	53.5±7.9	44.0±3.1	−18[b]	51.5±3.4	−4[a]	+17[f]
TbN	2.4±0.4	1.6±0.2	−34[b]	2.2±0.3	−8[a]	+40[f]
TbS	380±93	587±56	+54[c]	407±61	+7[a]	−30[f]
LP %	6.1±1.1	4.3±2.4	−29[a]	5.7±1.0	−6[a]	+33[f]
EP %	0.4±0.1	1.8±0.6	+353[c]	0.4±0.1	+3[a]	−77[f]
OA %	0.7±0.1	0.4±0.2	−47[c]	0.7±0.2	−12[a]	+67[f]
MAR	1.6±0.1	1.2±0.1	−28[c]	1.5±0.1	−7[a]	+29[f]
BFR	10.0±2.3	5.1±2.9	−49[c]	8.7±1.3	−13[a]	+71
MLT	5.8±0.5	7.2±1.7	+23[a]	7.0±1.1	+20[a]	−3[d]

疗组与激素组比较，此二项指标的差异也无显著性，说明 TCCM 只增加类骨质，而没有影响矿化的副作用(某些二磷酸盐类药物—抗骨质疏松药有矿化缺陷的副作用)。

本文提示：在使用糖皮质激素的同时加服 TCCM，能很有效防治类固醇性骨质疏松，望在临床试用。

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