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Effects of total coumarins of *Cnidium monnieri* on bone density and biomechanics of glucocorticoids-induced osteoporosis in rats[1]

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KEY WORDS *Cnidium monnieri*; coumarins; prednisone; osteoporosis; bone density; femur; biomechanics

AIM: To evaluate the effects of total coumarins from dried fruits of *Cnidium monnieri* (TCCM) on glucocorticoids (GC)-induced osteoporosis (OP) in rats. **METHODS**: Single photon absorptiometric and biomechanical character measurements of femurs were used. **RESULTS**: The bone density (BD) indices in proximal, middle, and distal segments in GC group were decreased by 12 % ($P < 0.05$), 14 % ($P < 0.05$), and 12 % ($P < 0.05$), respectively *vs* control group. The BD on proximal, middle, and distal segments in GC-TCCM group were increased by 26 % ($P < 0.01$), 34 % ($P < 0.01$), and 31 % ($P < 0.01$), respectively *vs* GC group. The biomechanical competence in femoral middle segments in GC group tended to decrease *vs* control group. In GC-TCCM group, the torsional strength, energy, maximal torsional angle, and rigidity were increased by 15 % ($P < 0.05$), 32 % ($P < 0.05$), 14 % ($P > 0.05$), and 13 % ($P > 0.05$), respectively *vs* the GC group. **CONCLUSION**: TCCM not only prevented glucocorticoids-induced osteoporosis but also increased the torsional strength of femurs in rats.

We have previously reported that the glucocorticoids (GC)-induced skeletal histomorphometric changes in SD ♂ rats, including the amount of cancellous and cortical bone mass and their structural architecture[1,2], and that total coumarins from dried fruits of *Cnidium monnieri* (TCCM) prevented GC-induced bone mass loss by using bone histomorphometric technique[3]. However, there has been no report on the bone density (BD) and biomechanical effects of TCCM on GC-induced skeletal changes. To evaluate comprehensively the effect of TCCM in preventing GC-osteoporosis (OP), we would observe the BD and biomechanical characteristic effect of TCCM on prednisone-induced OP in rats by using single photon absorptiometry and SWD-10 material test machine, so as to provide reference data for exploitation of this new drug.

MATERIALS AND METHODS

Drugs Prednisone acetate, 5 mg/tablet (930907), produced by Pharmaceutical Factory of Affiliated Hospital of Guangdong Medical College. *Cnidium monnieri* (L) Cuss was bought from Zhanjiang Medicinal Crops Corporation, Guangdong. Consulted the relevant documents[4,5], TCCM was extracted from dried fruits of *Cnidium monnieri* (L) Cuss by using acetone extract method in Department of Chemistry, Guangdong Medical College, and its yield was 4.4 %. TCCM consisted mainly of 7 constituents: osthol, palmition acid, xanthotoxin, xanthotoxol, alloisoinperatorin, imperatorin, and bergapten[6]. In the present study, TCCM crystals was given ig 20 mg·kg^{-1}, which corresponded to crude drugs 5 g·kg^{-1}.

Instruments SD-1000 single photon absorptiometry with radio-active source ^{241}Am, which was made in Beijing Geological Research Institute of Nuclear Industry, for measurement of bone density. SWD-10 electronic universal material testing machine was made by Changchun Testing Machine Group Co, China.

Rats Sprague-Dawley (SD) rats, 24 ♂ of 3-month-old and weighing 340 ± 6 g (Guangdong Animal Experimental Center), were acclimated for 1 wk. They were randomly divided into 3 groups: (A) control group was given ig normal saline 2 mL·kg^{-1}, (B) GC-OP group was given ig prednisone acetate 4.5 mg·kg^{-1}, twice a wk, and (C) TCCM treated group was given prednisone as group B plus TCCM 20 mg·kg^{-1}·d^{-1}, *ig*, 6 times a wk. The rats were housed at 25 ℃ and allowed free access to water and food (Animal Center of Guangdong Medical College). The rats were weighed weekly and the volume of the drug was adjusted accordingly.

Measurements of BD and biomechanics After 90 d, the femurs were stripped of muscles and placed in 10 % phosphate-buffered formalin (pH 7.2) for 24 h, and transferred to 70 % ethanol. Then, 3 lines were drawn: A line was drawn

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at the distalis of the basis on lesser trochanter in proximal femur; B line was drawn at the proximalis on the patellar surface in distal femur; and C line was drawn between A and B lines. The same line was scanned transversely 3 times using single photon absorptiometry. Bone mineral content and bone width were obtained and BD was calculated by computer coupled with single photon absorptiometry. After that, 2 ends of the femurs were embedded (bulk: 1 cm × 1 cm × 1 cm) by liquid methyl methacrylate of self-coagulation (Shanghai Tooth Material Factory, 9299) and powdery methyl methacrylate of self-coagulation (The Oral Cavity Material Factory, the Second Medical University of Shanghai, 881124). The embedded bulks were placed in SWD-10 material testing machine coupled to a computer and carried out twisting until they fractured. The torsion-angle curve and the indices of mechanics were given by computer.

Biomechanics parameters Torsional strength (M_{max}) reflected the load amount exerted on the material when it was destroyed. Maximal torsional angle expressed the deformation angle produced before the material was destroyed. They indicated the ability of bone anti-deformation and tenacity. Rigidity (k) was expressed by slope of elastic range curve and determined bony hardness. Energy (W) reflected the energy stored before the structure was destroyed and the sum of the ability of material resisting deformation.

Statistical analyses Data were expressed as $\bar{x} \pm s$ and compared with Dunnett t-test. The % was calculated from $\bar{x}_2/\bar{x}_1 \times 100 - 100$.

RESULTS

Body weight At the end of experiment, the body weight in control, GC, and GC-TCCM group were 375 ± 37 g, 364 ± 24 g, and 371 ± 32 g, respectively. There was no significant difference between the 3 groups.

BD In GC group, the BD in proximal, middle, and distal segments of femurs were decreased by 12 % ($P < 0.05$), 14 % ($P < 0.05$), and 12 % ($P < 0.05$), respectively, in comparison to control group. The BD on proximal, middle, and distal segments in GC-TCCM group were increased by 26 % ($P < 0.01$), 34 % ($P < 0.01$), and 31 % ($P < 0.01$), respectively, compared with GC group (Tab 1).

Biomechanical characters In GC group, the biomechanical characters in femoral middle segments tended to decrease: the torsional strength, the maximal torsional angle, rigidity, and energy were decreased by 7 %, 8 %, 14 %, and 13 %, respectively, in comparison with control group. In GC-TCCM group, the torsional strength, energy, maximal torsional angle, and rigidity increased by 15 % ($P < 0.05$), 32 % ($P < 0.05$), 14 % ($P > 0.05$), and 13 % ($P > 0.05$), respectively, compared with the GC group (Tab 1).

DISCUSSION

Our findings showed that when GC was used for a long time, the BD in proximal, middle, and distal segments of femora were significantly decreased in

Tab 1. Bone density [BMC/BW (g/cm²)] and biomechanical parameters of rat femurs after GC and GC + TCCM treatment. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* control; $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* GC.

Parameters ($n = 8$ rats)	Group A Control	Group B GC	Group C GC + TCCM
Bone density			
Proximal segments	0.34 ± 0.03	0.30 ± 0.02	0.37 ± 0.01
		% − 12[b]	% 10[b] 26[f]
Middle segments	0.32 ± 0.05	0.28 ± 0.01	0.37 ± 0.01
		% − 14[b]	% 16[b] 34[f]
Distant segments	0.38 ± 0.05	0.34 ± 0.03	0.44 ± 0.04
		% − 12[b]	% 15[b] 31[f]
Biomechanical test			
Torsional strength (M_{max})/Nm	0.685 ± 0.057	0.638 ± 0.057	0.733 ± 0.066
		% − 7[a]	% 7[a] 15[e]
Maximum tosional angle/deg	11.891 ± 2.076	10.967 ± 2.663	12.542 ± 1.699
		% − 8[a]	% 5[a] 14[d]
Rigidity (K)/(Nm/deg)	0.048 ± 0.011	0.041 ± 0.009	0.046 ± 0.009
		% − 14[a]	% − 3[a] 13[d]
Energy (W)/J	0.0046 ± 0.0006	0.0040 ± 0.0013	0.0053 ± 0.0008
		% − 13[a]	% 14[e] 32[e]

comparison with control group. On the other hand, TCCM treated group can arrest GC-induced BD loss. Even though the BD in three segments of TCCM group increased over to control group level. These results is consistant with the previous bone histomorphmetric report[3] by our lab. However, it should be emphasized that the BD measurement can't divide cancellous and cortical bone, but its best advantage is a non-invasion method and very easy to do. We can take bone out of the body to do and also do in alive rats, help us to know the dynamic changes and give us a quick tendency[7, 8].

Our study also further confirmed that ability of bone antideformation and its tenacity also declined slightly in GC group. These results suggested that the architecture of bone changed due to its organic and inorganic constituents lost, and also showed a similar change to the previous report[9] by Ding *et al*. Thereby, it is understood that the patients receiving large doses of glucocorticoids would result in fractures easily. On the contrary, in the GC-TCCM group had a striking increase of the bone anti-deformation and a pronounced reinforcement of the bone tenacity, so the anti-tortional strength and energy significantly increased by 15 % and 32 %, respectively. Thus, TCCM which showed estrogen-like effect[10] could effectively strengthen the ability of bone antitortion because it can increase bone formation through reinforcing activity of osteoblasts and decrease the bone resorption through depressing the function of osteoclasts, and keep fracture from being happened accordingly.

However, the reason why no differences between GC and control group in the indices of biomechanics is not clear. It will be further studied in the future whether the drug dose was not used sufficiently in experiments or other reasons.

In summary, our results suggested that TCCM be a potentially useful agent which not only could reverse prednisone-induced bone mass loss, but also increase the anti-torsional strength of femurs in rats.

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蛇床子总香豆素对糖皮质激素致大鼠骨质疏松的骨密度和生物力学性能的影响[1]

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关键词 蛇床; 香豆素类; 泼尼松; 骨质疏松症; 骨密度; 股骨; 生物力学

目的: 评价蛇床子总香豆素(TCCM)对糖皮质激素(GC)致大鼠骨质疏松的骨密度(BD)和生物力学性能的影响. 方法: 24 只 3 月龄 ♀ SD 大鼠, 体重 340 ± 5 g, 随机分为对照(A)组、GC(B)组和预防(C)组, B 和 C 组均喂醋酸泼尼松 4.5 mg·kg^{-1}, 每周 2 次; C 组还喂 TCCM 20 mg·kg^{-1} ig, 每周 6 次. 持续 90 d. 结果: B 组(与 A 组比较)大鼠股骨近、中和远段 BD 分别减少了 12 %、14 % 和 12 %; C 组(与 B 组比较) BD 则分别增加了 26 %、34 % 和 31 %. C 组(与 B 组比较)股骨的扭转强度和能量分别增加了 15 % 和 32 %. 结论: TCCM 除能防止 GC 所致骨量丢失外, 还能增加其抗扭转强度.