Synergistic effect of total isoflavones of pueraria and vitamin D on prevention and treatment of osteoporosis*★

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Abstract

BACKGROUND: Total isoflavones of pueraria (TIP) possesses estradiol-like structures and has inhibition effect on bone loss or osteopenia in ovariectomized rats. However, studies have demonstrated that the prevention and treatment effect of TIP on osteoporosis in ovariectomized rats is poor, especially in single utilization.

OBJECTIVE: To investigate the combination effect of TIP and vitamin D on the treatment of osteoporosis in ovariectomized rats.

METHODS: Totally 81 female sprague-drawly (SD) rats of 3-month-old were randomly assigned into 9 groups. Exception those in the sham-surgery group, all rats were prepared for ovariectomized models. Vitamin D or low-, middle- and high-dose TIP or low-, middle- and high-dose TIP combined with vitamin D were intragastric administrated in the vitamin D, TIP or combination groups, respectively. There was no drug medication in the model and sham-surgery groups. The uterus coefficient was calculated at 3 months after medication. The serum alkaline phosphatase (ALP) activity, calcium, phosphonium, bone gla protein, estradiol levels, as well as bone mineral density of femur was determined.

RESULTS AND CONCLUSION: Compared with the model group, the uterus coefficient and estradiol level was obviously increased in the middle- and high-dose TIP groups and all combination groups ($P < 0.05$); the ALP and bone gla protein levels significant decreased ($P < 0.05$); and the bone mineral density of central and distal femur were notably increased ($P < 0.05$); in particular, the result was more manifest in the high-dose combination group. TIP and vitamin D presented with synergism in uterus coefficient, estradiol, ALP, bone gla protein levels and bone mineral density of distal femur, but the effect was not significant in the calcium and phosphonium levels in the TIP and/or vitamin D groups. The findings demonstrated that Tip combined with vitamin D can induce synergism on prevention and cure of osteporosis in ovariectomized rat.

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INTRODUCTION

Postmenopausal osteoporosis is a kind of primary osteoporosis. The main reasons are ovarian declination and estrogen descend[1-2], which result in bone absorption larger than bone formation, and presented with regression of bone mineral density (BMD) and bone strength[3-4]. Studies have demonstrated that total isoflavones of pueraria (TIP) is a phytoestrogen, which possesses estrogen-like structural features[5-6], and produces biologic activity when combined with isoflavonoids-estrogen receptor. TIP provides a new approach for clinical treatment of osteoporosis as a substitute of estrogen[7-9]. However, some researches showed that the single use of TIP can not receive good outcomes due to weak drug action[10]. Considering vitamin D also has auxiliary function on prevention of bone mineral loss[11], we assume that whether the combination of TIP and vitamin D play superior role than both of them? Accordingly, here, ovariectomized rats served as animals in the postmenopausal osteoporosis models, and to observe the effects of TIP combined with vitamin D on serum bone metabolic markers and femoral BMD.

MATERIALS AND METHODS

Design

A randomized, controlled, animal experiment.

Time and setting

The experiment was performed at the University of South China, from 2008 to 2009.

Experimental animals

A total of 81 3-month SD rats, with clean grade, weighing (270±31) g, were provided by Experimental Animal Center, University of South China, certificate No. SCXK(xiang)2004-0009. All rats were housed at 24 ℃ with 60%－70% humidity for 1 week before experimentation.

Reagents and instruments are listed as the following:

Reagent and instrument	Source
Total isoflavones of pueraria	Sitong Phyto Active Ingredients Inc.
Vitamin D	Tongxiang Kangyuan Bio-products Co., Ltd.
Biochemical analyzer	Beijing Zhongke Fubang Medical Equipment Co., Ltd.
SD-1000C bone mineral content measuring apparatus	Beijing Research Institute of Uranium Geology

Methods

Grouping and model preparation

Totally 81 rats were randomly divided into 9 groups based on body weights, with 9 rats in each group. Seventy-two rats were prepared for ovariectomized models except those in the sham-surgery group, which only removed fat surrounding ovary. According to 4×2 factorial design, ovarictomized rats were equally divided into 8 groups and prepared for osteoporotic models. Rats with failure model preparation were rejected accordingly serum estrogen levels measured by cytology and vaginal smear at 7－12 days. Vitamin D (0.2 μg/kg), or TIP with low-, middle- and high-dose (25, 50 and 100 mg/kg), or low-, middle- and high-dose TIP combined vitamin D (combination, 25, 50 and 100 mg/kg TIP+0.2 μg/kg vitamin D) were intragastric administrated in the vitamin D, TIP or combination groups, respectively. All medication was performed

once per day for 3 successive months. There was no drug medication in the model and sham-surgery groups.

Specimen preparation

After medication, rat blood was collected by unilateral eyeball enucleation, that is, rats were fixed with one eye upward, both lateral neck were pressed by left thumb and forefinger, made the eyeball evagination, and extirpated the eyeball with forceps and collected blood using anticoagulative tube. The blood was placed at room temperature for 0.5 hour, followed by 5 minutes centrifugation at speed of 1 000 r/min. The serum was preserved at 20 ℃ for further examination. Their bilateral femurs were removed. After all soft tissues around the bone were taken clearly, the femoral BMD was measured.

Calculation of uterus coefficient

Rat uterus was separated and weighted, the uterus coefficient was calculated according to: uterus coefficient=uterus wet weight (mg)/body weight (100 g) × 100%.

Determination of serum estradiol level

Rat blood was centrifugated and serum estradiol level was determined by an automatic biochemistry analyzer.

Measurement of bone metabolic markers

Serum alkaline phosphatase (ALP) activity, calcium, phosphonium, bone gla protein contents were measured with an automatic biochemistry analyzer.

Detection of femoral BMD

Femoral BMD was detected by SD-1000C BMD measuring apparatus.

Main outcome measures

The uterus coefficient, serum ALP activity, calcium, phosphonium, bone gla protein, estradiol levels, as well as femoral BMD were determined.

Statistical analysis

Using SPSS 13.0 statistical software for data processing and the data were expressed as Mean±SD. The interaction between TIP and vitamin D was determined by multiple factor variance analysis. The interaction types were judged by intuitive analysis, and the intergroup comparison was performed by LSD method. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Quantitative analysis of experimental animals

Totally 81 rats were collected in the study. During experimentation, 2 rats died of anesthetic accident, 5 rats died of bleeding combined with infection, and the other 75 rats were included in the final analysis.

Effects of TIP and vitamin D on uterus coefficient and serum estradiol levels

Compared with the model group, the uterus coefficient and estradiol level was obviously increased in the middle-, high-dose TIP groups and all combination groups ($P < 0.05$), in particular, the result was more manifest in the high-dose combination group. The uterus coefficient of the vitamin D group was increased than that of the model group ($P < 0.05$), but there was no significant difference in serum estradiol level between two groups ($P > 0.05$; Table 1). TIP and vitamin D presented with alliance protection on uterus coefficient and serum estradiol levels in ovariectomized rats. There was a synergistic effect between TIP and vitamin D (Table 2).

Table 1 Effects of TIP and vitamin D on uterus coefficient and serum estradiol level in ovariectomized rats ($\bar{x}\pm s$)

Group	*n*	Uterus coefficient (%)	Estradiol (pg/L)
Model	9	1.05±0.16	3.00±1.42
Low dose TIP	7	1.01±0.23	4.45±0.49
Moderate dose TIP	9	1.66±0.27[a]	15.21±2.56[a]
High dose TIP	8	2.01±0.35[a]	18.91±5.23[a]
Vitamin D	7	1.40±0.34[a]	4.86±0.85
Low-dose combination	9	2.22±0.30[a]	13.90±2.50[a]
Moderate-dose combination	8	2.20±0.28[a]	26.81±2.30[a]
High-dose combination	9	2.75±0.17[a]	29.72±2.66[a]

TIP: total isoflavones of pueraria; [a]$P < 0.05$, *vs.* model group

Table 2 Variance analysis of the effect of TIP and vitamin D on uteru coefficient and serum estradiol level in ovariectomized rats

Factor	Uterus coefficient (%)		Estradiol	
	F	*P*	*F*	*P*
TIP	54.62	0.000	206.60	0.000
Vitamin	114.72	0.000	158.12	0.000
DCombination	7.60	0.000	10.90	0.000

TIP: total isoflavones of pueraria

Effects of TIP and vitamin D on bone metabolic markers (Table 3)

Table 3 Effects of TIP and vitamin D on bone metabolic markers ($\bar{x}\pm s$)

Group	*n*	ALP(nkat/L)	Ca (mmol/L)
Model	9	101.00±2.90	2.54±0.14
Low-dose TIP	7	97.23±4.20	2.57±0.12
Moderate-dose TIP	9	92.44±2.60[a]	2.60±0.07
High-dose TIP	8	86.03±4.50[a]	2.62±0.13
Vitamin D	7	93.30±1.60[a]	2.58±0.09
Low-dose combination	9	87.17±1.60[a]	2.67±0.13[a]
Moderate-dose combination	8	79.01±2.80[a]	2.55±0.12
High-dose combination	9	72.82±4.50[a]	2.54±0.16
Group	***n***	**P (mmol/L)**	**BGP (μg/L)**
Model	9	1.73±0.10	4.80±0.12
Low-dose TIP	7	1.69±0.07	4.70±0.14[a]
Moderate-dose TIP	9	1.71±0.07	4.62±0.09[a]
High-dose TIP	8	1.71±0.10	4.50±0.24[a]
Vitamin D	7	1.73±0.14	4.67±0.15[a]
Low-dose combination	9	1.72±0.10	3.37±0.10[a]
Moderate-dose combination	8	1.78±0.12	3.06±0.10[a]
High-dose combination	9	1.75±0.14	2.91±0.07[a]

TIP: total isoflavones of pueraria; ALP: alkaline phosphatase;BGP: bone gla protein; [a]$P < 0.05$, *vs.* model group

Compared with the model group, the ALP and bone gla protein levels were obviously decreased in the middle- and high-dose TIP groups, vitamin D group and all combination groups ($P < 0.05$); in particular, the result was more manifest in the high-dose combination group. However, TIP and vitamin D had little effect on calcium and phosphonium levels. Only the calcium level was notably increased in the low-dose combination group ($P < 0.05$). There was synergistic effect between TIP and vitamin D ($P < 0.05$; Table 4).

Table 4 Variance analysis of the effect of TIP and vitamin D on bone metabolic markers ($\bar{x}\pm s$)

Factor	ALP		Ca	
	F	*P*	*F*	*P*
TIP	112.31	0.000	0.64	0.59
Vitamin D	124.14	0.000	0.01	0.93
Combination	5.50	0.002	1.82	0.15

Factor	P		BGP	
	F	*F*	*F*	*P*
TIP	0.41	189.87	189.87	0.000
Vitamin D	1.93	1235.28	1235.28	0.000
Combination	0.24	107.31	107.31	0.000

TIP: total isoflavones of pueraria; ALP: alkaline phosphatase; BGP: bone gla protein

Effects of TIP and vitamin D on rat femoral BMD

The BMD of central and distal femur were notably increased in the middle- and high-dose TIP groups, vitamin D group and all combination groups than that of the model group ($P < 0.05$), in particular, in the high-dose combination group. There was no significant difference between vitamin D and model groups in central and distal femoral BMD ($P > 0.05$; Table 5). TIP and vitamin D had interaction effect on central and distal femoral BMD, that is, a synergistic effect (Table 6).

Table 5 Effects of TIP and vitamin D on rat femoral BMD ($\bar{x}\pm s$, g/cm^3)

Group	*n*	Central BMD	Distal BMD
Model	9	0.181±0.021	0.206±0.025
Low-dose TIP	7	0.194±0.015	0.234±0.028
Moderate-dose TIP	9	0.214±0.023[a]	0.239±0.015[a]
High-dose TIP	8	0.246±0.010[a]	0.243±0.017[a]
Vitamin D	7	0.202±0.012	0.215±0.021
Low-dose combination	9	0.255±0.020[a]	0.288±0.027[a]
Moderate-dose combination	8	0.279±0.019[a]	0.305±0.033[a]
High-dose combination	9	0.286±0.036[a]	0.308±0.027[a]

TIP: total isoflavones of pueraria; BMD: bone mineral density; [a]$P < 0.05$, *vs.* model group

Table 6 Variance analysis of the effect of TIP and vitamin D on rat femoral BMD ($\bar{x}\pm s$)

Factor	Distal BMD		Central BMD	
	F	*P*	*F*	*P*
TIP	13.16	0.000	21.60	0.000
Vitamin D	45.89	0.000	68.85	0.000
Combination	4.02	0.012	1.51	0.220

TIP: total isoflavones of pueraria; BMD: bone mineral density

DISCUSSION

Studies have demonstrated that TIP has estrogen-like effect[12-13], which can improve bone metabolism and has anti-osteoporosis action. In the experiment, healthy, adult, female, SD rats were housed in the same conditions and performed ovariectomy, they can correctly simulate bone loss and reactions to intervention factors as postmenopausal osteoporosis[14-15].

The experimental results showed that TIP could increase uterus weight and elevate serum estradiol level in ovariectomized rats, suggesting TIP with moderate- or high-dose, similar to endogenous estrogen, can promote uterus weight and vaginal epithelium cornification, the result is consistent with previous studies[16]. The findings demonstrated that vitamin D combined with some a TIP not only can block bone loss and bone structure degeneration in ovariectomized rats, but also has obviously amelioration and recovery effect on experimental osteoporosis. However, low-dose TIP has poor ability to reverse BMD, suggesting there is dose-effect relationship. The ovariectomy and medication have variant effects on each part of bone tissues, namely, the bone loss is most obviously in the cancellous bone, but the central femur is not sensitive, this may be because the synergism of TIP and vitamin D affect distal femur rather than central femur. In order to supplement bone loss resulted from ovariectomy, TIP with moderate- or high-dose can suppress osteocytes destruction caused by ovariectomy, increase new bone formation, and maintain bone transformation in a balance. The results is consistent with studies that phytoestrogen has a dose-depended manner in improving bone metabolism[17]. The experiment found that blood calcium fluctuated in a small range, which only has significance utilizing low-dose TIP combined with vitamin D. This may a comprehensive performance of multiple channels regulate calcium levels, but the detail needs to be explored.

TIP has an estrogen-like effect, that is, it can accelerate osteoblast proliferation, suppress osteoclast differentiation, elevate calcium absorption and utilization, increase 1，25-dihydroxyvitamin D_3, 1, 25-$(OH)_2O_3$ synthesis. Vitamin D can induce osteoblast proliferation, and enhance TIP effect, which complement each other substantially[18-20]. The results of experimentation showed that though TIP combined with vitamin D not has synergism on each side of bone metabolism, they indeed obtain satisfactory therapeutic efficacy in treating osteoporotic rats, and the protection effect superior to single medication. Moderate- or high-dose of TIP combined with vitamin D yielded the best effect, which hope to receive good result on prevention and cure of postmenopausal osteoporosis.

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葛根异黄酮联合维生素 D 防治骨质疏松的协同效应*★

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摘要

背景：葛根异黄酮具有类雌二醇结构特征，对去卵巢大鼠的骨质丢失和骨量的减少有一定的抑制作用。但也有报道其对去卵巢大鼠骨质疏松的防治效果较弱且单独使用效果不明显。

目的：观察葛根异黄酮与维生素 D 联合对去卵巢大鼠骨质疏松的作用。

方法：将 81 只 3 月龄雌性 SD 大鼠随机分为 9 组：除假手术组外其他各组均行双侧卵巢摘除造模。低、中、高剂量 TIP 组，VitD 组，低、中、高剂量联合组在去卵巢基础上分别灌胃给予相应剂量的葛根异黄酮和(或)维生素 D；模型组和假手术组不给予药物治疗。给药 3 个月，计算大鼠子宫系数；测定大鼠血清碱性磷酸酶、血钙、血磷、骨钙素及血清雌二醇水平；检测大鼠股骨骨密度。

结果与结论：与模型组比较，中、高剂量 TIP 组，低、中、高剂量联合组大鼠子宫系数、雌二醇水平明显增高($P < 0.05$)，血清碱性磷酸酶、骨钙素水平明显降低($P < 0.05$)，股骨中心及远心骨密度均明显增加($P < 0.05$)，均以高剂量联合组作用效果最明显。且在子宫系数，雌二醇、血清碱性磷酸酶、骨钙素水平及股骨远心端骨密度方面，葛根异黄酮和维生素 D 均表现为协同作用。在血钙、血磷方面，葛根异黄酮和(或)维生素 D 的作用不明显。说明葛根异黄酮和维生素 D 联合使用对去卵巢大鼠骨质疏松的防治有协同效应。

关键词：葛根异黄酮；维生素 D；联合作用；去卵巢；骨质疏松

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来自本文课题的更多信息--

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本文创新性：

提供证据：在 CNKI 数据库中检索，检索词为“葛根异黄酮、维生素 D、去卵巢”，共检索到 9 篇相关文献。未见与文章密切相关的研究

创新点说明：葛根为药食两用植物，成本低，患者容易接受，若开发出产品，有非常好的市场需求和光明的发展前景。实验探索了植物雌激素葛根异黄酮联合维生素 D 防治绝经后骨质疏松的新方案，从医学营养学角度拓展葛根防治疾病的新方向。