

Effects of *Radix Puerariae* flavones on liver lipid metabolism in ovariectomized rats

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Abstract

AIM: To study the effects of *Radix Puerariae* flavones (RPF) on liver lipid metabolism in ovariectomized (OVX) rats.

METHODS: Forty adult female Wistar rats were randomly divided into four groups: OVX group; sham-OVX group; OVX+estrogen group and OVX+RPF group. One week after operation rats of the first two groups were treated with physiological saline, rats of OVX+estrogen group with estrogen (1 mg/kg·b.w.) and rats of OVX+RPF group with RPF (100 mg/kg·b.w.), respectively for 5 weeks. After the rats were killed, their body weight, the weight of the abdominal fat and uterus were measured, and the levels of total cholesterol (TC) and triglyceride (TG) in liver homogenate were determined.

RESULTS: Compared with the sham-OVX group, the body mass of the rats in OVX group was found increased significantly; more abdominal fat in store; TC and TG in liver increased and uterine became further atrophy. As a result, the RPF was found to have an inhibitive action on those changes of various degrees.

CONCLUSION: RPF has estrogen-like effect on lipid metabolism in liver and adipose tissue.

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INTRODUCTION

Estrogen plays an important role not only in maintaining the reproductive function and the secondary sex characters of the female animals, but also in so-classical target tissues, such as the brain, bone, liver, kidney and the cardiovascular system^[1,2]. With the decreases of estrogen in postmenopausal women, the incidences of fracture in osteoporosis, cardiovascular and neurodegenerative diseases often increase^[3-5]. Estrogens replacement therapy is able to attenuate symptoms of menopausal syndrome and to reduce the incidence of above-mentioned diseases^[6-9].

Phytoestrogens are naturally occurring plant chemicals that

can produce an estrogen-like effect in the body, used as a natural alternative to hormone replacement therapy and to reduce menopausal symptoms. They are not chemically the same as the estrogens made in the body, but when digested and absorbed they can act somewhat as estrogen in the body^[10]. *Radix Puerariae* is the dried root of *Pueraria lobata* Ohwior *Pueraria thomsonii* Benth. Sweet in taste and neutral in nature, it can strengthen the spleen and stomach, invigorate the spleen and replenish *Qi*. Its main component is flavone. Recent research has found leguminous plants rich in flavone have a certain kind of estrogen-like effect^[11-13]. In this study extraction of *Radix Puerariae* was given to ovariectomized (OVX) rats and its estrogen-like effects were observed.

Previous studies in our laboratory have illustrated that when the female rats were OVX, the estrogen level in their body decreased, which might lead to the body weight gains, abdominal fat stores, increase of liver fat and atrophy of uterine^[12]. Therefore, the OVX rats can be used as models to reflect the pathologic changes in perimenopausal or postmenopausal women.

MATERIALS AND METHODS

Animals

All of the 40 female Wistar rats, 220±10 g in body mass, were purchased from the Experimental Animal Center of Chinese Academy of Medical Sciences. They were raised in an air-conditioned animal house at 25±1 °C with a light-dark cycle of 12 h, and fed with free forge and running water. The rats were randomly divided into four groups: OVX group; sham-OVX group; OVX+estrogen group and OVX+RPF group. After anesthetized with 15 g/L sodium pentobarbital intraperitoneally, the rats of OVX, OVX + estrogen and OVX+RPF groups were OVX. Rats from sham-OVX received only a squeeze in the connecting point between the ovary and the fallopian tube without having their ovary removed. Starting from the second week of the operation, the rats of OVX group and sham-OVX group were treated with saline, the rats of OVX+estrogen group were treated with estrogen (1 mg/kg·b.w.) and rats from OVX+RPF group were treated with RPF (100 mg/kg·b.w.) respectively for 5 wk. The average feed consumption and mass of the rats from various groups were measured once a week.

Drugs and reagents

The RPF, provided by Biochemistry Department of Beijing University of Chinese Medicine, was made into suspension of required concentration (10 g/L). The positive control drug was nilestriol (batch number: 20000403) provided by Beijing Four Rings Pharmaceutical Factory. TG and TC kit were provided by Beijing Zhong-Sheng High-Technology Bioengineering Company.

Instruments

The Semi-automatic Biochemistry Analyzer MD 100 was made in USA. The electronic analytical balance was produced by Shimadzu Co. in Japan and the high speed centrifuge TLLC

was manufactured by Beijing Sihuan Scientific Instrumental Company.

Collection and measurement methods of specimens

Five weeks after the operation, the animals were anesthetized with 15 g/L sodium pentobarbital and the liver, abdominal fat and uterus were taken weighed and frozen. Some liver specimens were collected after operation, fixed in 40 g/L formaldehyde, embedded in paraffin, sectioned and stained with hematoxylin and eosin for light microscopy. Some liver specimens were frozen stored at -20 °C. Liver triglyceride and total cholesterol concentration were measured with the TC kit and the TG kit on a MD100 Semi-automatic biochemistry analyzer respectively after lipid extraction.

Statistical analysis

Data were presented as mean±SD, and Student's *t* test was used to determine changes between different groups. *P*<0.05 was considered significant.

RESULTS

Effect of RPF on body weight of OVX rats

The mean body mass of the OVX group increased significantly after the operation, compared to the OVX group, RPF was found to have a long term effect on body weight gains (*P*<0.05). The mean body weight of the OVX group started to exceed that of the sham-OVX group significantly ever from the first week (*P*<0.05). The mean body mass of OVX+RPF group had a statistical difference from the mean body weight of OVX group after 3 wk (*P*<0.05). From the fifth week, the body mass of all rats began to drop, but the final data showed that RPF could control the weight gain caused by ovariectomy. During feeding, rats from the OVX+estrogen group were found with colporrhagia, magersucht, and anorexia, but no such symptoms found in the OVX+RPF group (Table 1).

Table 1 Effect of RPF on body mass of OVX rats

Group	<i>n</i>	Body mass after operation (g)	Body weight during recovery period (g)	Wk 1 (g)	Wk 2 (g)	Wk 3 (g)	Wk 4 (g)	Wk 5 (g)	Change rate
OVX	8	215±15	233±17	250±16	266±16 ^c	276±15 ^c	284±16 ^c	268±20 ^c	22.1%
Sham OVX	9	235±4.7	230±4	232±7	235±6	241±9	246±11	237±14	7.1%
OVX+estrogen	10	236±17	242.4±21	249±18	248±16 ^a	239±14 ^a	248±18 ^a	239±19 ^a	1.1%
OVX+RPF	10	216±15	233.3±16	243±17	251±20	249±16 ^a	246±19 ^a	245±17 ^a	5.6%

Results expressed as mean±SD. ^a*P*<0.05 vs OVX group, ^c*P*<0.05 vs sham-OVX.

Table 2 Effect of RPF on abdominal fat and fat factor of OVX rats

Group	<i>n</i>	Abdominal fat (g)	Compared with OVX (%)	Fat factor (%)	Compared with OVX (%)
OVX	8	13.2±2.1 ^c	—	5.1±0.4 ^c	—
Sham OVX	9	10.6±3.2	80.4	4.6±1.2	91.2
OVX+estrogen	10	6.8±3.6 ^a	52.1	3.1±1.6 ^a	60.1
OVX+RPF	10	8.5±2.5 ^a	64.4	3.7±1.2 ^a	72.1

Results expressed as mean±SD. ^a*P*<0.05 vs OVX group, ^c*P*<0.05 vs sham-OVX.

Table 3 Effect of RPF on liver lipid metabolism of OVX rats

Group	<i>n</i>	TC (mg/dL)	Compared with OVX (%)	TG (mg/dL)	Compared with OVX (%)
OVX	8	16.4±5.0 ^c	—	119±26 ^c	—
Sham-OVX	9	11.4±4.4	81±27	65±18	54±15
OVX+estrogen	10	10.2±1.7 ^a	62±10	54±31 ^a	45±26
OVX+RPF	10	10.2±2.0 ^a	76±15	46±24 ^a	39±20

Results expressed as mean±SD. ^a*P*<0.05 vs OVX group, ^c*P*<0.05 vs sham-OVX, OVX+estrogen and OVX+RPF group.

Effect of RPF on abdominal fat and fat factor of OVX rats

The mean weight of abdominal fat of the OVX group was obviously higher than that of the sham-OVX group (*P*<0.05). Both estrogen and RPF could inhibit the storing of abdominal fat caused by ovariectomy, the mean data was only 52.1% and 64.35% compared with OVX group, respectively (Table 2).

Effect of RPF on liver lipid metabolism of OVX rats

TC and TG in liver of the OVX group were 16.4±5.0 mg/dL and 119±26 mg/dL, respectively, which were significantly higher than other groups (*P*<0.05). In the sham-OVX group they were only 11.4±4.4 mg/dL and 65±18 mg/dL. The RPF was found to have a marked effect on controlling liver TC level, with TC 10.2±2.0 mg/dL and TG 46±24 mg/dL which were even lower than sham-OVX group and OVX+estrogen group (Table 3).

Effect of RPF on wet uterus weight of OVX rats

Six weeks after the operation, the uteri of the rats from the OVX group were found with severe atrophy with a quarter of the uterus weight of the sham OVX group. The uterus weight of the estrogen group was higher than the sham-OVX group (*P*<0.05). RPF has again been proved to have some effects on maintaining the uterus weight (*P*<0.05) in OVX group (Table 4).

Table 4 Effect of RPF on wet uterus weight of OVX rats

Group	<i>n</i>	Uterus (g)	Uterus factor (%)
OVX	8	0.11±0.02 ^c	0.40±0.10 ^c
Sham-OVX	9	0.39±0.14	1.70±0.60
OVX+estrogen	10	0.46±0.34 ^a	2.10±1.50 ^a
OVX+RPF	10	0.15±0.03 ^a	0.70±0.10 ^a

Results expressed as mean±SD. ^a*P*<0.05 vs OVX group, ^c*P*<0.05 vs sham-OVX.

DISCUSSION

Unlike the animal models that are often prepared by giving high fat diets for hyperlipemia study, the OVX rats were used in the present study. Results of the experiments showed that the animals with ovariectomy tended to have body weight gain, accumulated abdominal fat, increased TC and TG levels in liver and atrophied uterus, which are very similar to menopausal symptoms. Experience from our past studies has proved that animal models thus prepared are stable, which can be used in the study of lipid metabolic disturbance.

Current studies on phytoestrogen both at home and abroad are mainly focused on soy bean and its extracts, and there are very rare reports of *Radix Puerariae*, a plant from the leguminous family. In fact, the phytoestrogen content in *Radix Puerariae* is much higher than that in soy bean. The aim of the present research was to study the effects of extracts from *Radix Puerariae* on lipid metabolism in OVX rats, which mimicked the postmenopausal situation of the disorders of lipid metabolism. And we obtained a series of satisfactory results.

We found in the study that there was an obvious lipid metabolic disturbance in OVX rats. Five weeks after the operation, the TC concentration in liver in OVX group was found obviously higher than those in other groups. RPF could lower the TC concentration to the normal value. However, the mechanism for this change is not quite clear yet. The increased insulin concentration in OVX rats may accelerate the dephosphorylation effect of the hydroxymethylglutaryl CoA reductase (HMGCoA reductase), which is a rate-limiting enzyme of the composition of cholesterol. As a result, it accelerates the activity of the enzyme^[14-16]. Insulin could also induce the composition of the HMGCoA reductase directly to increase the composition of cholesterol^[17-19]. For the cholesterol metabolism *in vivo*, there are two transport pathways-the endogenous cholesterol transport and the reverse cholesterol transport. In the former, the cholesterol by exogenous absorption and synthesis in the liver is utilized when it, in the form of LDL-C, is combined with the receptors of the extra hepatic tissue, and the ligand combined with LDL receptors are mainly the apolipoprotein of apo B100 and apo E^[20-23]. In the latter, the extrahepatic cholesterol, in the form of HDL-C, is conveyed back to the liver and expelled out of the body after further metabolism^[24]. Some experiments have shown that estrogen can enhance the activity of the LDL receptors and promote the endogenous transport so as to lower the TC levels^[25]. In conclusion, the high insulin level in OVX rats may lead to a high level of cholesterol concentration, while, the low level of estrogen concentration may result in the low level of LDL-R concentration and activity, both of which can cause an accumulation of LDL. The increased production and decreased consumption together make a high level of cholesterol concentration in the body, followed by the hypercholeste and adiposis hepatica.

A high level of TG concentration was also found in the OVX rats. Extrinsic estrogen can be supplemented to reduce the TG accumulation in liver. It was found in our study that RPF had a similar effect. The histological sections revealed a great number of grease spots in the OVX group, while not so many in the estrogen treated group and RPF treated group. The mechanism may be as follows: First, glucose was promoted to change into TG by a high level of insulin concentration in OVX rats^[26]. Second, the high level of TC directly damaged the structure of hepatic sinusoid and chylomicrons that contained a high level of TG coming into the Disse's spaces without been treated. Therefore, the liver cell ingested a large amount of TG from the blood. Profound studies are still needed to further confirm its mechanisms.

We also noticed the side effects and risks of estrogen in this study. In clinic, the commonly encountered side effects of HRT are edema (fluid retention), nausea, breast tenderness,

and headache. Other side effects include rash, increased growth of facial hair, dizziness, and hypophrodisia. More serious potential health risks to consider of HRT are breast cancer, uterine cancer, blood clotting, and liver and gallbladder diseases^[27-29]. Today, selective estrogen receptor modulators (SERMs) have been used as an alternative approach to activate estrogen signaling pathways in a tissue-specific manner^[30,31]. Phytoestrogens have a similar chemical structure to estrogen, and could bind to the estrogen receptor (ER)-beta. Once they bind each other, the ER is occupied, and the real estrogen can not get in. Phytoestrogens then dispatch estrogen-like messages to the cells. Although it is a weak message, it is strong enough to produce some of the positive effects of estrogen but it is still too weak to stimulate the growth of cancer cells^[32,33]. When the estrogen levels are too high, this competition appears to reduce the effects of estrogen by replacing estrogen with the weaker phytoestrogen. When the estrogen levels are too low, it appears that phytoestrogen simulates the effects of estrogen and partially makes up for the deficiency^[34].

In conclusion, our data suggest that as a natural estrogen replacement, the RPF could regulate the lipid metabolic disturbance in liver in the OVX rats. Furthermore, there may be a possible protective action against cardiovascular diseases and osteoporosis caused by deficiency of estrogen in menopausal women. However, our data was limited. More work is still needed to confirm its profound pharmacological effects.

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