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Brief communication

Effects of genistein supplementation on gingival blood flow disturbances after menopause: an *in vivo* study using ovariectomized rats

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Background: Estrogen synthesis decreases in the postmenopausal period inducing gingival periodontal disturbance. Estrogen replacement therapy has been used to reduce such gingival complications, although it has been limited by the adverse effect of estrogen.

Objective: To investigate the effects of supplementation of genistein, a phytoestrogen derived from the soybean, on blood flow disturbance in the gingiva after menopause, using an ovariectomized rat model.

Materials and methods: Female Wistar rats (220 - 280 g) were subjected to a bilateral ovariectomy (OVX rats). The rats were classified into four groups: sham-operated animals treated with vehicle (DMSO 100 μl/day,s.c) (Sham (veh); n = 6), OVX rats treated with vehicle (DMSO 100 μl/day,s.c) (OVX (veh); n = 6), OVX rats treated with 17β-estradiol (20 μg/kg/day,s.c) (OVX (E_2); n = 6) and OVX treated with genistein (0.25 mg/kg.BW/day,s.c) (OVX (gen); n = 6). Plasma estradiol, body weight, and gingival blood flow (GBF) were measured 3 weeks after OVX. For the GBF measurement, laser Doppler perfusion flowmetry was used.

Results: Three weeks after OVX, plasma estradiol levels in three groups of OVX rats were significantly lower than the sham-operated control ($p < 0.05$). Body weight increased in OVX (veh) and OVX (gen) groups ($p < 0.001$). The GBF increased significantly in OVX (veh) compared to the Sham (veh) group ($p < 0.05$), indicating that GBF disturbance due to the lack of estrogen was improved by oral supplemention of 17β-estradiol or genistein.

Conclusion: Genistein supplementation improves disturbed gingival blood flow induced in ovariectomized-rats. Genistein might have oral health benefits after menopause.

Keywords: Genistein; gingival blood flow, laser Doppler flowmetry, ovariectomy, rats.

Menopause, due to a decrease in the synthesis of hormones (mainly of estrogens), induces various disorders in the cardiovascular and bone systems. Postmenopausal estrogen replacement therapy (ERT) has been widely used for women's well-being after the menopause [1, 2]. In dentistry, ERT may reduce gingival periodontal disturbances that are intimately connected with tooth loss, thus resulting in better tooth retention in the menopause [3, 4]. On the other hand, it has been reported that ERT may raise the risk of various diseases such as cardiac coronary disease and breast cancer [5, 6]. For this reason, the therapeutic potential of ERT for oral health benefits after menopause has been limited.

Compounds that are estrogen agonist to the bone and cardiovascular systems that appear to have no side effects have been studied [7, 8]. Among theses compounds, phytoestrogens have been well verified. Phytoestrogens represent a family of plant compounds with both estrogenic and antiestrogenic properties. Especially, soybean and its isoflavone derivatives (mainly genistein) have been studied intensively [9]. The present study is aimed at exploring the therapeutic potential of genistein for the prevention of dental diseases in postmenopausal women.

It has been reported that gingival vessels have become dilated, convoluted and looped during the development of an irregular pattern in the vascular

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plexus in periodontitis. Such vascular morphological changes might be a reflection of blood flow disturbances in the gingiva [10, 11]. In the present study, we investigated flow changes in the gingiva after menopause by using an ovariectomized-rat model without and with genistein supplementation. Focus was put on the effect of genestein on the gingival blood flow measured using a laser Doppler tissue perfusion flowmeter.

Materials and methods

Twenty-four female Wistar rats aged 10 weeks (220-280 g) were used in this study. The animals were obtained from the National Laboratory Animal Center of Salaya Campus, Mahidol University, Bangkok, and were fed on normal food and tap water *ad libitum*. The rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (45 mg/kg BW), and a bilateral ovariectomy (OVX) was made (OVX rats) [12].

We examined four groups of rats: sham-operated rats treated with vehicle (DMSO 100 µ1/day, s.c.) (Sham (veh); n=6), OVX rats treated with vehicle (DMSO 100 µ1/day, s.c.) (OVX (veh); n=6), OVX rats treated with 17β-estradiol (20 µg/kg/day, s.c.) (OVX (E_2); n=6), and OVX rats treated with genistein (0.25 mg/kg/day, s.c.) (OVX (gen); n=6). Treatment with DMSO, 17β-estradiol or genistein was given immediately after the OVX surgery; it was continued for three weeks during which time the endothelial function was restored by genistein supplementation in the ovariectomized rats [1, 2].

Gingival blood flow measurement

Gingival blood flow was measured 3 weeks after the OVX surgery. The rats were anesthetized with sodium pentobarbital (45 mg/kg BW *i.p.*). A laser Doppler flowmeter (Advance, ALF 21, Tokyo, Japan) was used. A 1.0 mm diameter fiber-optic probe was put vertically on a gingival site behind the incisors. Any pressure exerted on the tissue was made as little as possible [13]. The gingival blood flow was measured at 3 points on the tissue, 3 times for each point, and their average value was used for each rat.

Plasma estradiol levels were measured after the GBF measurement. The abdomen was opened, and blood samples (3 ml) were collected from the abdominal vein in a polypropylene tubing containing heparin (50,000 IU). After centrifugation at 3500 x g at 4 °C for 10 minutes, each sample was stored at -70 °C until analysis. Plasma estradiol levels were determined by the electrochemiluminescence immunoassay (ECLIT) with a commercially available kit. The functional sensitivity was 44 pmol/1 (12 pg/ml).

Data analysis

All data were presented as Means SEM. For comparison among groups of animals, one way analysis of variance (one-way ANOVA) was used and differences in pairs of means among groups were made by least significant difference (LSD) test. A *p*-value less than 0.05 was considered to be significantly different.

Results and discussion

Table 1 shows plasma-estradiol values and body weight changes measured three weeks after OVX surgery in Sham (veh), OVX (veh), OVX (E_2), and OVX (gen) groups. The plasma-estradiol levels in OVX (veh) were significantly reduced, compared to the sham-operated control rats ($p<0.05$). Estrogen replacement therapy and genistein supplementation increased plasma 17β-estradiol to levels that are not significantly different from those of sham-operated rats. The increase in body weight was seen in OVX (veh) and OVX (gen) groups ($p<0.001$). The increase might be mediated directly by the effect of 17β-estradiol on lipoprotein metabolism.

Table 1. Plasma 17β-estradiol and body weight changes three weeks after OVX in Sham (veh), OVX (veh), OVX (E_2), and OVX (gen) groups.

Status	Plasma 17 -estradiol (pg/ml)	Body weight change (%)
Sham (veh)	23.20 ± 3.84	5.17 ± 0.46
OVX (veh)	16.43 ± 1.06*	33.67 ± 1.47**
OVX (E_2)	19.10 ± 1.84	3.89 ± 1.24
OVX (gen)	17.05 ± 1.20	20.00 ± 2.85**

*$p<0.05$ significantly different compared to Sham (veh); **$p<0.001$ significantly different compared to Sham (veh).

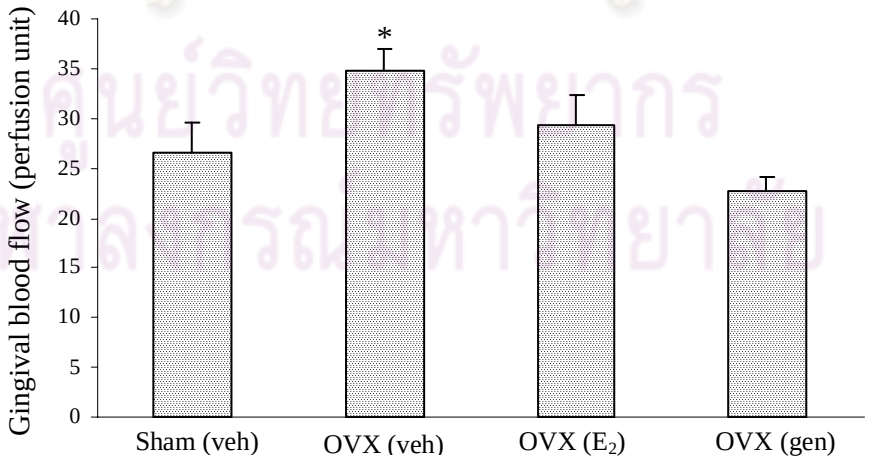
Figure 1 shows gingival blood flows (GBF) measured three weeks after the OVX operation in Sham (veh), OVX (veh), OVX (E_2), and OVX (gen) groups. The GBF was significantly increased in OVX (veh), compared to Sham (veh) ($p<0.05$). Interestingly, GBF could be restored to the Sham (veh) control level in three weeks after supplementation with estrogen or genistein.

We have evaluated the effect of phytoestrogen (genistein) on changes in the GBF by using laser Doppler perfusion flowmetry in bilateral OVX rats without and with genistein supplementation. In general, laser Doppler perfusion flowmetry is a non-invasive method that is capable of instantaneously and continuously assessing local (perfusion) blood flow in various tissues and organs. The measuring system has been accepted for measurement of gingival blood flow under normal and inflammatory conditions [14]. In gingival inflammation, its first manifestation may be capillary dilatation and an increase in blood flow. According to a recent study by Kerdvongbundit et al, the GBF in inflamed gingiva increased, and the blood flow was returned to a normal level after the inflammation subsided [15]. Accordingly, a clinical sign of inflammation may be changes in gingival blood flow. In the present experiment, estrogen deficiency caused a significant increase in GBF. Estrogen and genistein supplementation could restore the increment in GBF. Consequently, the present results imply that deprivation of estrogen may influence periodontal disease by decreasing the tonus of blood vessels in the gingiva.

The existence of estrogen receptors in the gingiva was not proved in our study, though they may play an important role in maintaining or improving the GBF. However, it has been proposed that the gingiva contains receptors that bind specifically to 17 β-estradiol [16]. These receptors are found in the basal layer of the gingival epithelium, in fibroblast and in the lamina propria of the endothelium of small vessels. Therefore, it is reasonable to consider gingival tissue as a target organ for estrogens.

Genistein, the principal isoflavone found in soybeans, is structurally similar to the potent estrogen, 17β-estradiol. This confers ability to bind to estrogen receptors, and thus genistein mimics the biological activity of 17β-estradiol. The estrogenic potency of genistein was shown through its interaction with estrogen subtype β rather than subtype α [17]. Consequently, the response to genistein on GBF is probably favored by the existence of estrogen receptors localized in the gingiva.

In conclusion, genistein supplementation effectively reduces the gingival blood flow disturbance in OVX-rat model. Genistein may be useful as a therapeutic agent giving oral health benefits in postmenopausal women.



*$p<0.05$ significantly different compared to Sham (veh).

Fig. 1 Gingival blood flows measured by laser Doppler perfusion flowmetry in Sham (veh), OVX (veh), OVX (E_2), and OVX (gen) groups three weeks after OVX.

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