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Structural and histomorphometric evaluations of ferutinin effects on the uterus of ovariectomized rats during osteoporosis treatment

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ABSTRACT

Aims: The effects of chronic administration of Ferutinin (phytoestrogen found in the plants of genus *Ferula*), compared with those elicited by estradiol benzoate, were evaluated, following ovariectomy, on the uterus of ovariectomized rats as regard weight, size, structure and histomorphometry.
Main methods: The experimental study included 40 female Sprague–Dawley rats, assigned to two different protocols, i.e. *preventive* and *recovering*. In the preventive protocol, ferutinin (2 mg/kg/day) was orally administered for 30 days, starting from the day after ovariectomy; in the recovering protocol, ferutinin was administered, at the same dosage, for 30 days starting from the 60th day after ovariectomy, when osteoporosis was clearly established. Its effects were compared with those of estradiol benzoate (1.5 μg per rat twice a week, subcutaneously injected) vs. vehicle-treated ovariectomized controls and vehicle-treated sham-operated controls. Uteri were removed, weighed and analysed under both the structural and histomorphometrical points of view.
Key findings: Our data show that ferutinin acts, similarly to estradiol benzoate, on the uterus stimulating endometrial and myometrial hypertrophy; this notwithstanding, the phytoestrogen ferutinin, in contrast to estrogen treatment, appears to increase apoptosis in uterine luminal and glandular epithelia.
Significance: Ferutinin, used in osteoporosis treatment primarily for bone mass recovering, seems in line with an eventual protective function against uterine carcinoma, unlike estrogens so far employed in hormone replacement therapy (HRT).

Introduction

Ferutinin (jaeschkeanadiol *p*-hydroxybenzoate) is a phytoestrogenic compound found in plant roots of genus *Ferula* (Abourashed et al., 2001). Its estrogenic property was firstly demonstrated *in vitro* (Appendino et al., 2002). Specifically, ferutinin displayed the ability to bind estrogen receptors α (ERα) with a higher affinity (IC50 = 33.1 nM) in comparison with estrogen receptor β (ERβ) (IC50 = 180.5 nM) (Ikeda et al., 2002). Ferutinin was also found to act as agonist for ERα and as agonist/antagonist for ERβ: therefore it could be considered a selective estrogen receptor modulator (SERM) (Appendino et al., 2002; Ikeda et al., 2002). Recently, we evaluated the ferutinin effects on sexual behavior of hormone-primed and non-hormone-primed female rats; in hormone-primed females, the acute administration of ferutinin significantly inhibited female receptivity (Zavatti et al., 2006), whereas in non hormone-primed rats the chronic administration of the compound was able to restore a normal sexual function, previously suppressed by ovariectomy (Zavatti et al., 2009). Further experiments performed administering ferutinin, alone or with estradiol benzoate (EB), in ovariectomized progesterone primed rats

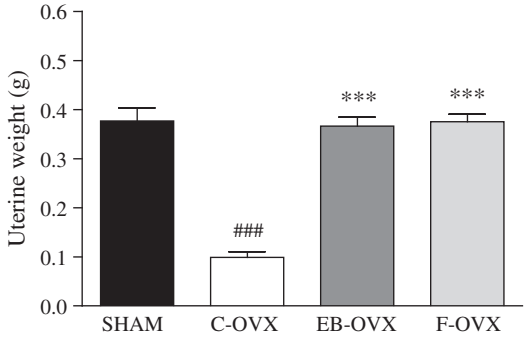

Fig. 1. Mean values of uterine weight in the preventive study. Values are expressed as mean ± SEM. ***$P<0.001$ versus C-OVX; ###$P<0.001$ versus SHAM (ANOVA followed by Newman–Keuls test). SHAM sham-operated controls receiving vehicle; C-OVX ovariectomized controls receiving vehicle; F-OVX ovariectomized treated with ferutinin; EB-OVX ovariectomized treated with estradiol benzoate.

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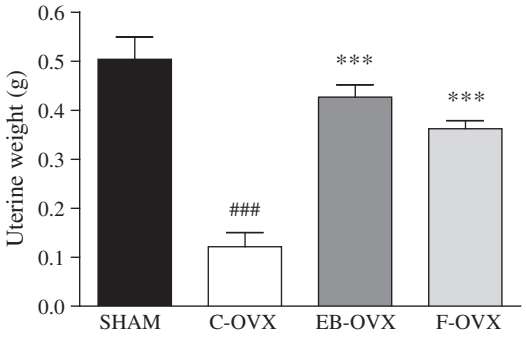

Fig. 2. Mean values of uterine weight in the recovering study. Values are expressed as mean ± SEM. $^{***}P<0.001$ versus C-OVX; $^{\#\#\#}P<0.001$ versus SHAM (ANOVA followed by Newman–Keuls test). SHAM sham-operated controls receiving vehicle; C-OVX ovariectomized controls receiving vehicle; F-OVX ovariectomized treated with ferutinin; EB-OVX ovariectomized treated with estradiol benzoate.

demonstrated ferutinin property in increasing ERα expression in the hypothalamus when administered alone, like estradiol, but in decreasing the response to estradiol when administered in combination (Zanoli et al., 2009). Thus ferutinin seems to display estrogenic or antiestrogenic activity, through hypothalamic ERα, depending on absence/presence of estrogen priming. From a clinical viewpoint our findings suggest potential therapeutic effects of ferutinin in estrogen-deficiency like menopause. This hypothesis was confirmed by our recent results from osteoporotic ovariectomized rats; ferutinin was found to counteract the reduction in bone density due to estrogen deficiency, following chronic treatment starting the day after ovariectomy (Palumbo et al., 2009) or 2 months later, when osteoporosis was clearly evident (Ferretti et al., 2010). In both studies, ferutinin displayed the same effects on bone mass observed with estradiol benzoate, thus suggesting that it could prevent osteoporosis and enhance bone loss recovery in osteoporotic ovariectomized rats.

Overall, our *in vivo* findings confirm ferutinin estrogenic property, suggesting its potential benefit in reducing symptoms and degenerative processes associated to menopause. Now it is crucial to evaluate ferutinin side effects, specifically on the organs which are reputed to be the target of estrogen effects, like uterus, vagina, mammary glands. It is well known that estrogens stimulate endometrial proliferation and their administration in HRT was associated to an increased risk of cancer. Phytoestrogens are claimed to have beneficial effects with a minor incidence of undesired side effects in comparison with estrogen therapy. In general, phytoestrogens showed a higher binding affinity for ERβ than ERα (Kuiper et al., 1998) and displayed both agonist and antagonist effects (Patisaul et al., 2005). Proliferative activity in estrogen-responsive cells can be either enhanced or suppressed by phytoestrogens depending on their concentration and relative potency (Whitten and Patisaul, 2001). Clinical reports about phytoestrogen effect on endometrial cancer are limited to case-controlled observational studies (Johnson et al., 2001).

The present study was designed to compare the effects of the chronic ferutinin treatment with those induced by estradiol benzoate on the uterus of ovariectomized rats.

Materials and methods

Animals and treatments

All animal handling and experimental conditions were carried out according to the Italian law (D.L. n. 116/1992) and European legislation (EEC n. 86/609). The experimental design and procedures were conducted under protocols approved by the Bioethical Committee of the Italian National Institute of Health.

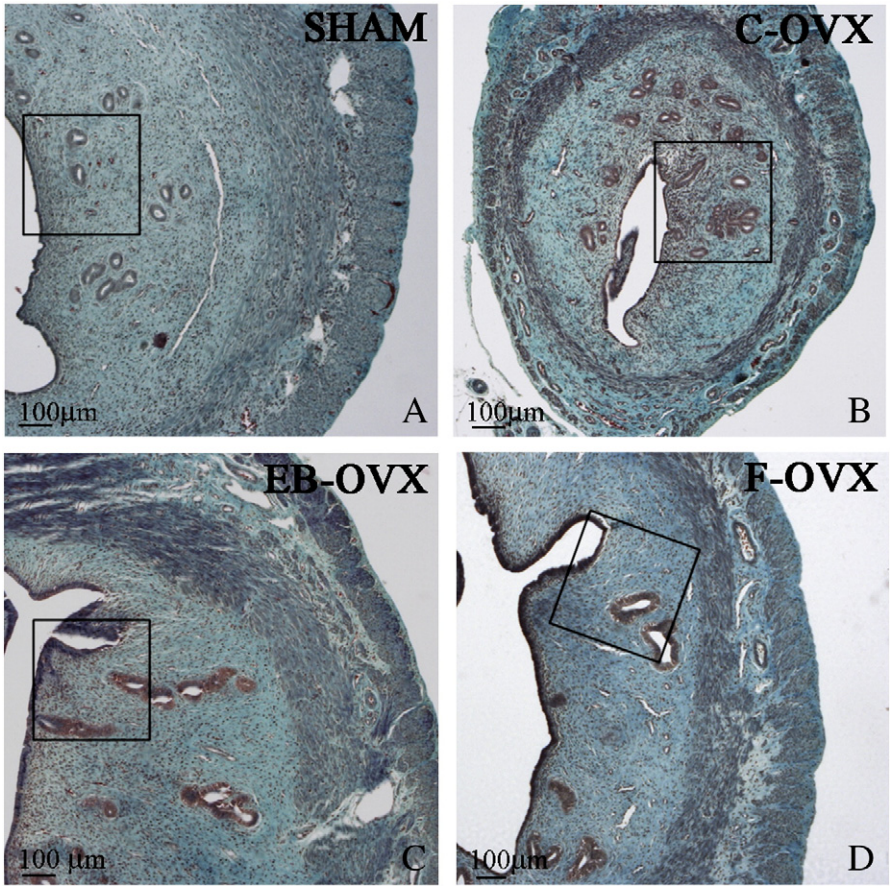

Fig. 3. LM micrographs of Gomori trichrome–stained cross sections showing the uterine structure in the preventive study. SHAM sham-operated controls receiving vehicle; C-OVX ovariectomized controls receiving vehicle; F-OVX ovariectomized treated with ferutinin; EB-OVX ovariectomized treated with estradiol benzoate.