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Tissue specificity of 8-prenylnaringenin: Protection from ovariectomy induced bone loss with minimal trophic effects on the uterus

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Abstract

Plant secondary metabolites with estrogenic activity (phyto-estrogens) have been studied in the past as a potential alternative to classical hormone-replacement therapy (HRT) in menopausal women. No final verdict on the efficacy of soy or red clover based pharmaceutical preparations has been reached despite numerous clinical studies. We have studied the novel and most potent phyto-estrogen 8-prenylnaringenin (8-PN) in adult ovariectomized rats, an established animal model to mimic hormone dependent osteoporosis in menopausal women. Our results demonstrate that 8-PN can completely protect from ovariectomy induced bone-loss while exhibiting minimal, (dose independent) trophic effects on uterus and endometrium. It is estimated that at equivalent bone protective doses of 17β-estradiol and 8-PN, the phyto-estrogen has a 10-fold lower stimulatory effect on uterus and endometrium. The bone tissue specific effect of 8-PN was confirmed in a transgenic reporter mouse model (ERE-Luc mice). Here we also found pronounced estrogenic activity in prostate. Present results add important aspects to the pharmacological profile of 8-PN and position this compound as an interesting alternative new candidate for treatment of peri- and postmenopausal symptoms.

Keywords: 8-Prenylnaringenin; Phyto-estrogen; Tissue specific estrogenicity; HRT; Osteoporosis

1. Introduction

A recently published and widely recognized study including peri- and postmenopausal women has questioned the health benefit of classic long-term hormone replacement therapy (HRT) [1]. This has rekindled an interest in alternatives to classical HRT such as dietary estrogenic compounds (phyto-estrogens) and the potential benefits or risks they may cause. Plant-derived estrogenic compounds were first recognized in the 1940s by their negative impact on fertility of sheep herds grazing on pastures containing red clover. Immature females showed signs of estrus, ewes became insusceptible and pregnant animals frequently miscarried [2]. Red clover contains high amounts of the isoflavones formononetin and biochanin A, later recognized as estrogenic [3]. Four plant derived families of phenolic compounds are conventionally classified as phyto-estrogens: the isoflavonoids, stilbenes, lignans and coumestans. Because of their prominent role in the diet, the soy derived isoflavanoids genistein and daidzein have been studied in most detail. Recently, however, a prenylated flavanon, 8-prenylnaringenin (8-PN), has been characterised as a novel phyto-estrogen, unique with respect to receptor specificity and potency, which is far higher than the one of any other phyto-estrogen so far investigated [4,5].

The dominant form of estrogen in the body is 17β-estradiol. Estrogens bind to intracellular receptors (ERs) which then dimerize and translocate into the nucleus and bind to DNA motifs known as estrogen responsive elements

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(EREs), whose structures are believed to influence recruitment of co-activators to the ER at target gene promoters [6], and thus, may serve as the key regulator for biological activity. Any compound that induces receptor dimerization and subsequent binding to the ERE, can be considered an estrogen. There are two known estrogen receptors (ERα and ERβ) which may be localized within the same cell, vary in tissue concentrations and distributions and may exhibit different biological effects dependent on the presence of various co-factors [7]. Both receptor subtypes function in normal ovarian and follicular development, vascular endothelial cells, myocardial cells, smooth muscle and breast tissue [7]. ERα is involved in bone maturation and maintenance of bone mineral density in both genders. ERβ is more important in maintaining follicular stimulation and luteinizing hormone concentrations in blood. Physiological estrogens, produced in the ovaries and testis, exhibit a multitude of biological effects in addition to their well-known role in the reproductive system of males and females. Various classes of phyto-estrogens and diverse compounds within each class interfere with the estrogen-mediated biological pathways in different ways.

Some synthetic drugs act as mixed agonists/antagonists and are referred to as selective estrogen receptor modulators (SERMs). For example, the "anti-estrogen" Tamoxifen acts as an estrogen antagonist in breast tissue but exhibits agonistic effects in the uterus, bone and vascular system [8]. The SERM Raloxifen (Evista®) has proven benefit on maintenance of bone mineral density in menopausal women but worsens menopausal symptoms such as hot flashes. Several recent reviews highlight the interaction of estrogen receptors and SERMs [7,9–11]. The existance of these mixed agonistic/antagonistic effects is used as an explanation for the different profile phyto-estrogens show compared to estradiol.

At least 105 clinical studies related to the effects of phyto-estrogens on bone density, cardiovascular health, cancer prevention, cognitive ability and menopausal symptoms have been reported and recently reviewed [12–15]. However, no final verdict on their efficacy in the treatment of estrogen deficiency syndromes has been reached.

One reason for the disappointing clinical results mainly obtained with the soy phyto-estrogens may be related to the fact that they predominantly interact with the ERβ while there is firm evidence that the bone protective activity of estrogens is mediated by ERα. But the low potency and the missing general drug development know-how may also have contributed to the non-satisfactory situation.

We have recently shown that racemic 8-prenylnaringenin (8-PN), present in hop (*Humulus lupulus*) and beer [16], is the first and so far only phyto-estrogen that exhibits a preference for ERα [5] and is a potent anti-angiogen [17]. This has encouraged us to test this compound and its 2S(−) enantiomer (2S-8-PN) for tissue selective effects in two different animal models. The well-established bone mineral density (BMD) model in ovariectomized rats mimics postmenopausal osteoporosis, while the transgenic ERE-luc mouse model [18] provides information about short-term estrogenic effects in various tissues. Results clearly demonstrate that 8-PN is a compound that shows tissue specific estrogenic activity different from 17β-estradiol. 8-PN compensated ovariectomy-induced loss of bone mineral density in adult rats with minimal effects on uterus weight and endometrium. In the other model, the bone tissue specificity of 8-PN was not only confirmed but also indications for a prostate tissue selectivity were obtained.

2. Methods

2.1. Materials and equipment

8-PN was synthesized according to a published method [19] modified for larger amounts, higher yield and lower costs (data on file). Purity of racemic substance was >98.5%. Separation of 2S(−) and 2R(+) enantiomers was achieved by chiral HPLC (Chiralpack AD 20 μm, 250 mm × 50.8 mm, 60 ml/min, isocratic elution with hexane/ethanol 9:1, v/v) resulting in purities of 99.8% (2R(+)) and 93.4% (2S(−)), respectively.

Bone mineral density measurement was carried out by pQCT (XCT-960A, Stratec, Germany).

2.2. BMD rat studies

Female 12–14-week-old Sprague–Dawley CD® BR rats (breeder Charles River, Germany) were used. Animals were randomly assigned to study groups of $N = 6$–8. Mean initial body weights per group ranged from 262 to 274 g. The experimental procedures were approved by the local Provincial State Office of Western Finland. Two studies were performed. In the first study, the dose dependent effects of 2S-8-PN was evaluated in relation to SHAM and control animals while in the second study the effects of one fixed dose of 2S-8-PN or rac. 8-PN, respectively, were compared to SHAM, control and 17β-estradiol treated animal groups. Both studies followed identical general procedures.

2.2.1. Rat bone mineral density model

Animals of group 1 were sham-operated (SHAM) while animals of other groups underwent bilateral ovariectomy (OVX) under anesthesia using an s.c. injection of a mixture of one part fentanylcitrate (0.315 mg/ml), two parts fluanisone (10 mg/ml, Hypnorm®, Janssen Pharmaceutica, Belgium) and one part midazolam (5 mg/ml, Dormicum, Roche Oy, Finland). Injection volume was 2.5 ml/kg. Buprenorphine (0.1 mg/0.33 ml/kg s.c., Temgesic®, Reckitt & Colman, Hull, UK) was given at the day of surgery and 1 day thereafter. Animals were kept individually in Macrolon III cages with ad libitum access to tap water and RM1 E SQC pellets (Witham, UK). Housing temperature was 21 ± 1.5 °C and relative humidity 30–65%. Artificial light was provided for 12 h/day. Body weights were recorded before ovariectomy (initial BW) and one day before necropsy (final BW). BMD