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Subtype-specific activation of estrogen receptors by a special extract of *Rheum rhaponticum* (ERr 731®), its aglycones and structurally related compounds in U2OS human osteosarcoma cells

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Abstract

The special extract ERr 731® from the roots of *Rheum rhaponticum* is the major constituent of Phytoestrol® N which is used for the alleviation of menopausal symptoms. Recently, we demonstrated that ERr 731® and its aglycones *trans*-rhapontigenin and desoxyrhapontigenin as single test substances do not activate the estrogen receptor-α (ERα) in human endometrial adenoarcinoma cells. However, these substances together with the structurally related hydroxystilbenes *cis*-rhapontigenin, resveratrol and piceatannol activated the ERβ-dependent reporter gene activity. To investigate if these substances are tissue selective ER activators, ERr 731® and the single test substances were examined in bone-derived U2OS cells stably expressing ERα or transiently expressing ERβ. In the ERα expressing U2OS cells, a weak, but statistically significant ERα-coupled luciferase activity was detected with ERr 731® and desoxyrhapontigenin which was 10-times lower than with 10^{-8} M 17β-estradiol. In the ERβ test system, all test substances significantly induced the luciferase activity in a magnitude comparable to 17β-estradiol. All effects were abolished with the pure ER antagonist ICI 182 780, indicating an ER-specific effect. Intracellular actions were also examined with the glycosylated ERr 731® constituents rhaponticin and desoxyrhaponticin. Treatment of U2OS cells with defined mixtures of both glycosides resulted in a reporter gene activity comparable to that of ERr 731®, thereby providing evidence for the existence of cellular uptake mechanisms for glycosylated hydroxystilbenes. This report confirms the strong ERβ-dependent activity of ERr 731® and provides evidence for a tissue selective ER agonistic activity by ERr 731® and its aglycones, as demonstrated by the activation of ERα in bone cells but not in endometrial cells.

Keywords: Estrogen receptor; Estrogen responsive element; Hydroxystilbenes; Phytoestrol N; Rhapontigenin; Desoxyrhapontigenin; Resveratrol; Piceatannol; Osteosarcoma cells; Menopause; Climacteric

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Introduction

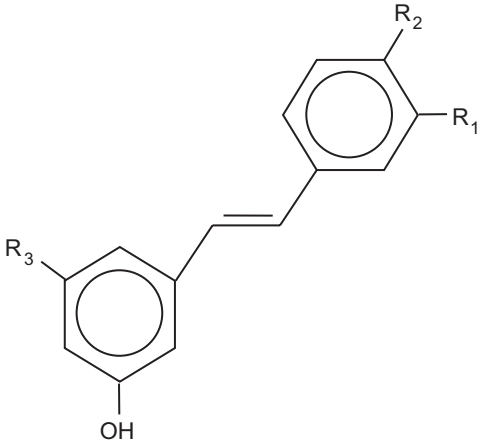
Hormone therapy (HT) is very effective in relieving climacteric complaints during perimenopause and postmenopause as well as preventing the long-term consequences of estrogen deficiency such as osteoporosis (Lindsay and Tohme, 1990; Skafar et al., 1997). HT works by replacing one or both of the two female sex steroids estrogen and progestogen. Due to the association of HT with an increased risk of breast and endometrial cancer and venous thromboembolism (Rosendaal et al., 2002; Ross et al., 2000), many women refuse to take HT. These patients however still seek relief from their climateric symptoms and thus, the demand for alternative treatments, in particular herbal remedies, which are claimed to not display the potential risks associated with HT, are high.

Additionally, untreated postmenopausal cessation of ovarian estrogen production leads to the loss of bone mass and density, a condition commonly referred to as osteoporosis. During the reproductive period of females, the mass and density of the bone is maintained by a subtle balance of the activity of the bone forming osteoblastic and bone degrading osteoclastic cells, which are regulated by estradiol. Two basic mechanisms can explain the bone sparing activity of estradiol, (a) the stimulation of osteoblasts or (b) the inhibition of osteoclasts. The main bone protective activity of estradiol appears to result from the suppression of osteoclasts rather than from the stimulation of osteoblasts. However, there are also indications that estradiol physiologically stimulates osteoblasts, since it has been shown to induce gene expression in these cells e.g. osteocalcin or collagen type-1. These results make it desirable for any HT replacement therapy to have, in addition to the relief of menopausal symptoms, osteoporosis protection properties.

The special extract from the roots of *Rheum rhaponticum* (L.), referred to as ERr 731® (trade name Phytoestrol® N), has been regularly prescribed for climacteric complaints since 1993, without the occurrence of any safety related side effects such as endometrial hyperplasia, spotting or breakthrough bleeding. Recently, a 12 week double-blind, placebo-controlled clinical trial in 109 perimenopausal women has been completed demonstrating the clinical efficacy (proof of principle of the biological activity) of ERr 731® (Heger et al., 2006; Kaszkin-Bettag et al., 2007). However, in contrast to the clinical effectiveness of ERr 731®, little to date has been known about the molecular mechanism of either the extract or its potential metabolites, that can explain the clinical observations.

The plant *Rheum rhaponticum*, commonly known as Sibiric Rhubarb, originates from Central Asia. It was introduced into Europe in the 17th century, and has been cultivated since then in Western Europe, East Asia and the United States. The standardised extract ERr 731® (drug:extract ratio 16–26:1, extraction solvent calciumoxide:water 1:38 (mass/mass)), consists mainly of rhaponticin and desoxyrhaponticin and small amounts of the aglycones *trans*-rhapontigenin and desoxyrhapontigenin (both together about 5%). When ingested, however, it is proposed that large amounts of the aglycones may appear due to the deglycosylation of rhaponticin and desoxyrhaponticin by intestinal bacteria (Park et al., 2002; Kobashi and Akao, 1997). So far, it is not clear which molecule(s) are implicated as the primary mediators of the clinical effects of ERr 731®. The structures of *trans*-rhapontigenin and desoxyrhapontigenin are shown in Fig. 1. Both molecules have a hydroxystilbene backbone and are structurally related to resveratrol (Aggarwal et al., 2004).

From the clinical observations with ERr 731®, the question arises whether ERr 731® exerts its biological effects via binding to, and activation of, estrogen receptors. In a first *in vitro* study we investigated ERr 731®, the aglycones of its constituents, *trans*-rhapontigenin and desoxyrhapontigenin, as well as the structurally related compounds *cis*-rhapontigenin, piceatannol and resveratrol for potential activity on the estrogen receptors-α (ERα) and -β (ERβ) (Wober et al., 2007). ERα-mediated activities were investigated using two independent assays, (1) the yeast ERα reporter assay, and (2) stimulation of alkaline phosphatase in the human endometrial adenocarcinoma Ishikawa cell line expressing ERα. ERα and ERβ dependent activities were investigated in the human endometrial HEC-1B adenocarcinoma cells transiently transfected with the human ERα or ERβ. In this study we failed to detect any ERα



	R1	R2	R3
Rhaponticin	OH	OCH_3	O-Glc
Desoxyrhaponticin	H	OCH_3	O-Glc
Rhapontigenin	OH	OCH_3	OH
Desoxyrhapontigenin	H	OCH_3	OH
Resveratrol	H	OH	OH
Piceatannol	OH	OH	OH

Fig. 1. Structure of hydroxystilbene derivatives.