Toxicology Letters 192 (2010) 271–277

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Chemopreventive actions by enterolactone and 13 VIOXX®-related lactone derivatives in H295R human adrenocortical carcinoma cells

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ARTICLE INFO

Article history:
Received 29 July 2009
Received in revised form 29 October 2009
Accepted 1 November 2009
Available online 11 November 2009

Keywords:
CYP17
Chemoprevention
Lactone derivatives
Sex steroidogenesis
H295R

ABSTRACT

Cytochrome P450c17 (CYP17) has been linked to various hormone-related diseases, including breast cancer, thus being a potential target for cancer chemoprevention. We studied the naturally occurring phytochemical enterolactone (ENL) and 13 VIOXX®-related lactone derivatives (CRI-1 to CRI-13) for their effects on CYP17 activity and expression and on cell cycle status in the human H295R adrenocorticocarcinoma cell line. Of the tested compounds, only CRI-3, -7, -10 and -12 showed to be inhibitors of CYP17 activity in H295R cells. This inhibition was not due to decreased mRNA expression, but was apparently caused by post-translational modification of the CYP17 enzyme. The MAPK kinase (MEK) inhibitor PD98059 induced CYP17 activity by 24%, while co-incubation of the CRI-s with PD98059, reduced CYP17 activity even further than the reduction caused by the CRI-s alone. In addition, CRI-3, -7, -10 and -12 arrested the cell cycle in the G(2)/M phase. The structure–activity similarities of the CRI-s with known micro-tubule binding agents strongly suggest that cell cycle arrest is a result of interaction with tubulin. We conclude that the proposed cancer chemopreventive actions of ENL are not mediated through interaction with CYP17 or cell cycle status. Of the VIOXX®-related lactone derivatives, CRI-7 could prove useful in the prevention of hormone-dependent cancers, such as breast cancer, since *in vitro* it shows low cytotoxicity, it is a potent inhibitor of CYP17 activity and strong inducer of cell cycle arrest.

1. Introduction

During the past years, a shift in cancer research interest is seen from the treatment of cancer toward cancer chemoprevention, which is the use of natural, synthetic or biologic chemical agents that reverse, suppress or prevent carcinogenic progression to invasive cancer (Tsao et al., 2004). It is generally accepted that diet plays an important role in cancer incidence and prevention. The American Institute for Cancer Research/World Cancer Research Fund has calculated that as much as 30–40% of all cancers can be prevented by appropriate diets, physical activity and maintenance of appropriate body weight (Glade, 1999). Besides nutritional compounds, the daily diet also comprises a large group of phytochemicals. Phytochemicals are a diverse group of chemicals which do not possess a nutritional value and that can be found in fruits, vegetables, grains and other plant foods. Phytochemicals have been suggested to exert a variety of health effects including the protection against hormone-dependent cancers, such as breast cancer (Murillo and Mehta, 2001; Adlercreutz, 2002; Limer and Speirs, 2004). This hypothesis is mainly based on the fact that the incidence and mortality of breast cancer are much lower in Asian countries than in Western countries (Magee and Rowland, 2004). This difference is often attributed to the traditional Asian diet, which is low in fat and high in fiber and phytochemicals (Tham et al., 1998). Uptil now, over 5000 phytochemicals have been identified and classified (Liu, 2004). The term phytoestrogens is also often used to specify phytochemicals that are structurally similar to estrogens or are converted in the gut to compounds that exert estrogen-like actions (usually estrogen receptor, ER-mediated). Phytoestrogens are generally classified as isoflavones, coumestans and lignans (Bingham et al., 1998). Most extensively studied are the isoflavones, with genistein and daidzein being the most important, which are present in large amounts in soybeans and soy products. Coumestans, like coumestrol, can mainly be found in alfalfa and clover sprouts, thus not comprising a substantial part of the human daily diet. Lignans, on the other hand, are widespread in the Western diet and can be found in high fiber foods and whole grain products, but also in berries and garlic (Mazur and Adlercreutz, 1998). The main dietary, plant-derived lignans, matairesinol and seco-isolariciresinol, are converted by intestinal bacteria to the active compounds enterodiol (END) and enterolactone (ENL). END can be further converted into ENL in the mammalian gut (Borriello et al., 1985).

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doi:10.1016/j.toxlet.2009.11.001

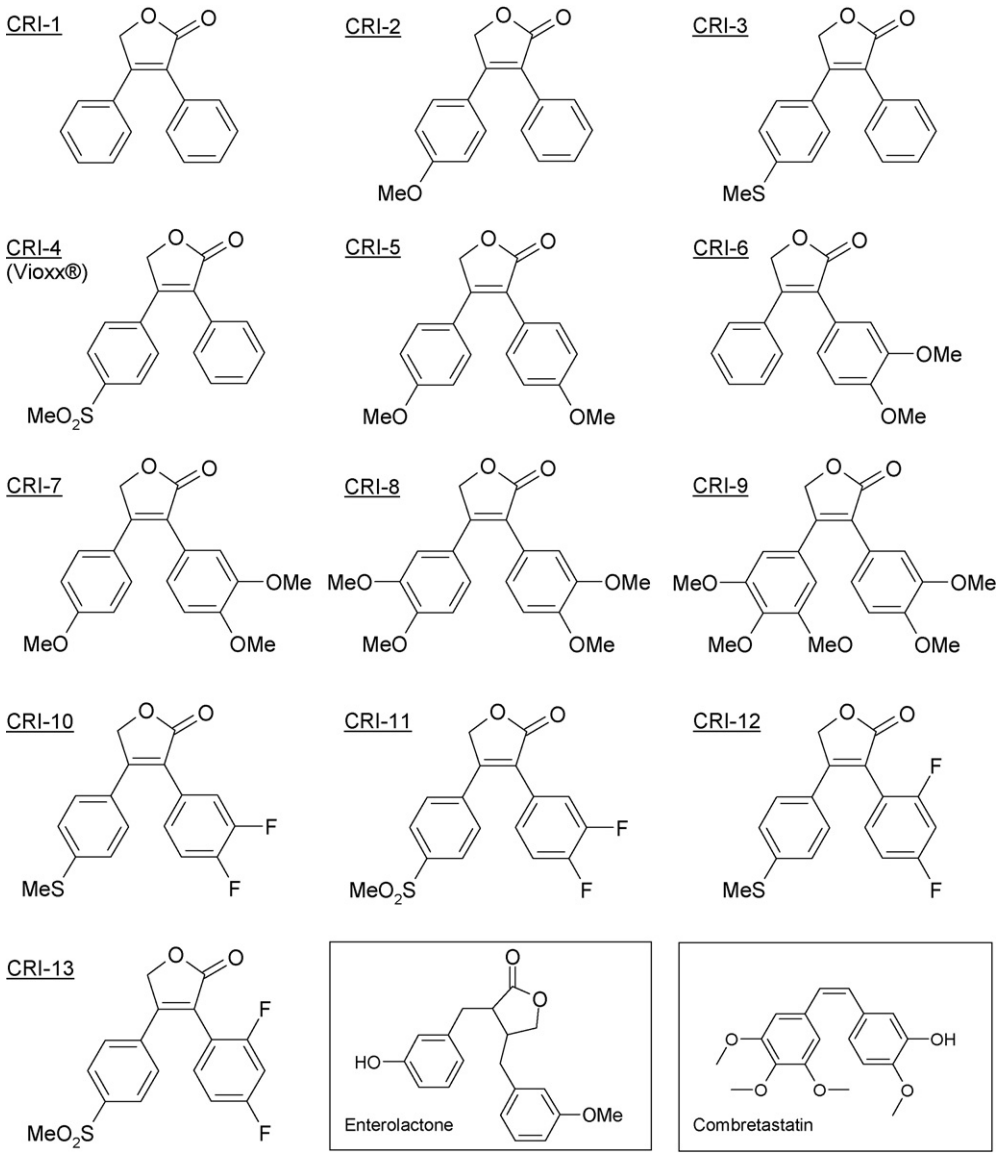

Fig. 1. Chemical structures of the 13 VIOXX®-related lactone derivatives used in this study, enterolactone (ENL) and combretastatin.

Many epidemiological studies have been performed to investigate the effects of phytochemicals on hormone-dependent cancers, such as breast cancer. However, the experimental designs (cohort, case–control, prospective studies), research methods (such as food questionnaire, phytochemical urinary or plasma levels) and results (decreased, increased, no effect on cancer risk) are very diverse (Adlercreutz, 2002; Peeters et al., 2003; Dixon, 2004; Magee and Rowland, 2004; van Gils et al., 2005). Still, while only a few studies have investigated the effects of lignans, mainly ENL, on hormone-dependent cancers, most studies describe a modest protective trend between lignan intake and breast cancer risk (Keinan-Boker et al., 2004; Linseisen et al., 2004; Olsen et al., 2004).

The suggested protection against hormone-dependent cancer by phytochemicals is often attributed to their ability to bind to the ER (Kuiper et al., 1997; Pearce and Jordan, 2004). However, mounting evidence suggests that phytochemicals can alter endogenous sex steroidogenesis and metabolism, thus altering the natural hormonal homeostasis of a cell, which can influence the initiation, promotion or proliferation of hormone-dependent tumors (Kirk et al., 2001; Sanderson and van den Berg, 2003; Castagnetta et al., 2004). Lignans also appear to have an effect on hormone balance. *In vitro*, ENL appears to be a moderate inhibitor of aromatase (Makela et al., 2000) and 5α-reductase (Evans et al., 1995). *In vivo*, they are positively correlated with plasma sex hormone binding globulin (SHBG) levels and negatively correlated with free plasma estradiol and testosterone (Adlercreutz et al., 1986).

De novo synthesis of sex steroid hormones involves the multiple enzymatic conversions of cholesterol and includes several rate-limiting steps, which makes fine-regulation of sex steroid levels possible. The final step in estrogen synthesis, for example, is the aromatization of androgens to estrogens by cytochrome P450 19 (CYP19 or aromatase) (Adams and Li, 1975). CYP19 has been shown to play an important role in breast tumor growth and suppression of local estrogen synthesis by aromatase inhibitors has been proven to be an effective treatment in post-menopausal breast cancer. Another key enzyme in sex steroidogenesis is cytochrome P450c17 (CYP17). The CYP17 enzyme is linked to various hormone-related diseases, such as polycystic ovarian syndrome (PCOS), congenital adrenal hyperplasia, prostate cancer and breast cancer, underlining the importance of CYP17 in human sex steroid homeostasis. CYP17 displays both 17α-hydroxylase and 17,20-lyase activity. This dual function of CYP17 allows the adrenal glands and gonads to