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Estrogenic and genotoxic potential of equol and two hydroxylated metabolites of Daidzein in cultured human Ishikawa cells

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Abstract

The soy isoflavone daidzein (DAI) is known to undergo metabolism to equol (EQO) and to 3′-hydroxy-DAI (3′-HO-DAI) and 6-hydroxy-DAI (6-HO-DAI) in humans. In order to better understand the implications of soy diets for human health, the hormonal and genotoxic activities of these DAI metabolites were studied in cultured human endometrial carcinoma cells. When the estrogenicity was tested by cell-free binding to recombinant human estrogen receptor (ER) α and β as well as by the induction of enzyme activity and gene expression of alkaline phosphatase (ALP) in Ishikawa cells, the ranking order was EQO > DAI > 3′-HO-DAI > 6-HO-DAI. All compounds had a higher affinity to ERβ than to ERα. No significant anti-estrogenic effects of the DAI metabolites were observed in the cells at non-cytotoxic concentrations. The in vitro genotoxicity was assessed by analyzing effects on cell cycle distribution and cell morphology as well as the induction of micronuclei (MN). EQO caused a slight increase in G1 and decrease in S phase of the cell cycle, and slightly but significantly induced kinetochore-positive as well as kinetochore-negative MN and an elevated proportion of abnormal mitotic spindles. 3′-HO-DAI, but not 6-HO-DAI, induced kinetochore-negative MN. The observation that major human metabolites of DAI exhibit estrogenic and genotoxic potential may be of relevance for the safety evaluation of diets containing soy isoflavones.

Keywords: Daidzein; Equol; Isoflavones; Micronuclei; Estrogen; Ishikawa cells

Abbreviations: ALP, alkaline phosphatase; BSA, bovine serum albumin; CREST, calcinosis Reynaud's phenomenon esophageal mobility abnormalities sclerodactyly and telangiectasia; DAI, daidzein 7-hydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one; DAPI, 4,4′-diamidino-2-phenylindole; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; E2, 17β-estradiol; EDTA, ethylenediaminetetraacetic acid; ER, estrogen receptor; EQO, equol 3,4-dihydro-3-(4-hydroxyphenyl)-2H-1-benzopyran-7-ol; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; GEN, genistein 5,7-dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one; HEPES, N-(2-hydroxyethyl)piperazine-N'-ethanesulfonic acid; HO, hydroxy; HPRT, hypoxanthine-guanine phoshoribosyltransferase; MN, micronuclei; MuLV, murine lymphoma virus; PBS-CMF, phosphate-buffered saline free of calcium and magnesium; PCR, polymerase chain reaction; PMSF, phenylmethanesulfonylfluorid; RT, reverse transcription; S.D, standard deviation; TAE, TRIS acetate–EDTA buffer

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1. Introduction

There is substantial evidence from human epidemiological studies and from animal experiments that the isoflavones present in soy may have a beneficial effect on hormone-related neoplasia, e.g. cancer of the breast and prostate (Adlercreutz, 2002). On the other hand, some soy isoflavones have been reported to exhibit genotoxic potential in cultured cells (Munro et al., 2003). The major isoflavones present in soy are daidzein (DAI, Fig. 1) and genistein (GEN, Fig. 1), differing by just one hydroxyl group. GEN but not DAI acts as a clastogen in mammalian cells in vitro, indicating that small differences of the chemical structure can profoundly affect the biological activity of isoflavones. It has recently been shown that DAI is metabolized by rat liver microsomes to a variety of catechol metabolites (Kulling et al., 2000). The major in vitro metabolites of DAI were identified as 3′-HO-DAI, 6-HO-DAI and 8-HO-DAI (Fig. 1). These hydroxylated metabolites of DAI have also been demonstrated in incubations with human hepatic microsomes and in the urine of humans after ingestion of soy food (Kulling et al., 2002). In addition to hydroxylation, DAI is known to undergo biotransformation to equol (EQO, Fig. 1). This reductive metabolism is mediated by colonic bacteria and occurs in about one third to one half of all human individuals. EQO has been identified in human breast (Maubach et al., 2003) and prostate (Hong et al., 2002) tissue.

It was the aim of the present study to clarify the estrogenic as well as the genotoxic properties of the major DAI metabolites 3′-HO-DAI, 6-HO-DAI and EQO. The endogeneous estrogen 17β-estradiol (E2) and its 4-hydroxylated metabolite 4-HO-E2, which is believed to be involved in the mechanism of E2-mediated carcinogenesis (Liehr, 2000) were included in our study. The estrogenic and anti-estrogenic potential was determined at two in vitro endpoints, i.e. (1) the binding affinity to the human estrogen receptor-α (ERα) and β (ERβ) under cell-free conditions, and (2) the expression of the alkaline phosphatase (ALP) gene which was determined by mRNA quantification and measurement

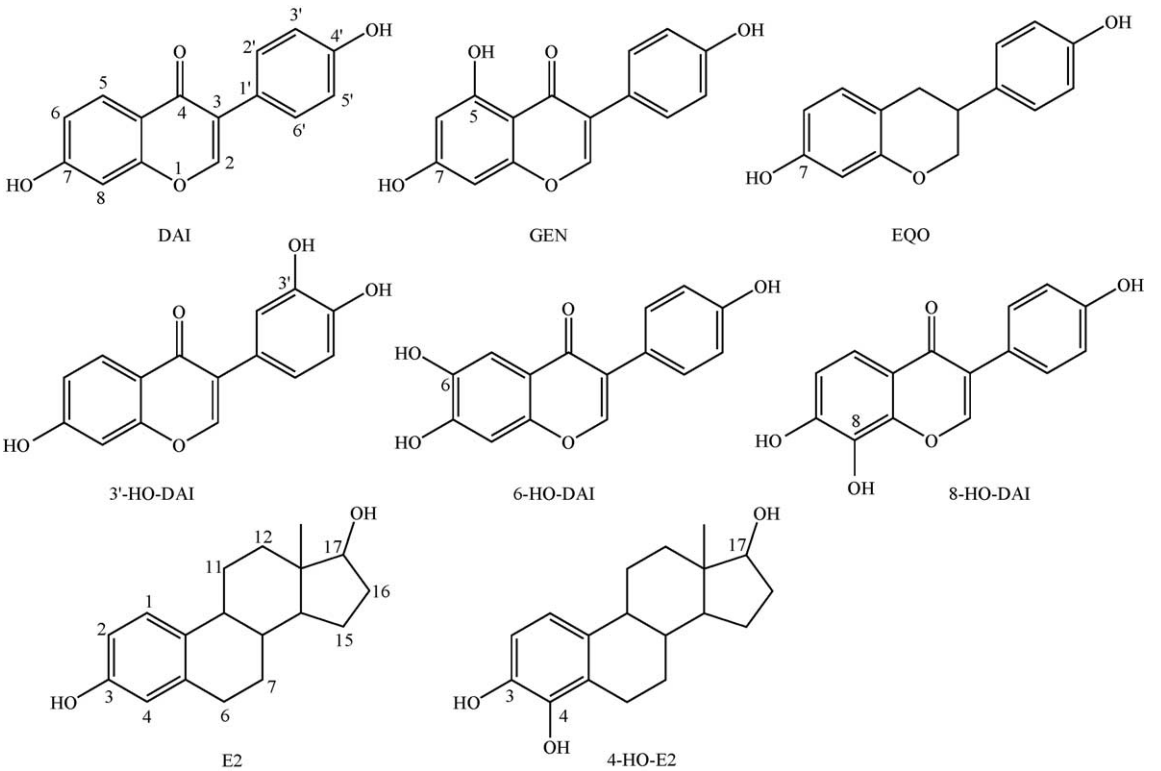

Fig. 1. Chemical structures of isoflavonoids, E2 and 4-HO-E2.