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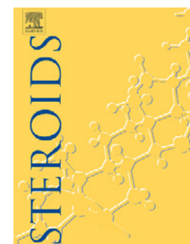

Effects of endocrine disruptors on genes associated with 17β-estradiol metabolism and excretion

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abstract

In order to provide a global analysis of the effects of endocrine disruptors on the hormone cellular bioavailability, we combined 17β-estradiol (E2) cellular flow studies with real-time PCR and Western blot expression measurements of genes involved in the hormone metabolism and excretion. Three endocrine disruptors commonly found in food were chosen for this study, which was conducted in the estrogen receptor (ER) negative hepatoblastoma HepG2 cell line: bisphenol A (BPA), genistein (GEN) and resveratrol (RES). We showed that 24 h after a single dose treatment with genistein, resveratrol or bisphenol A, the expression of ATP-binding cassette transporters (the multidrug resistance or MDR, and the multidrug resistance associated proteins or MRP) uridine diphosphate-glucuronosyltransferases (UGT) and/or sulfotransferases (ST) involved in 17β-estradiol elimination process were significantly modulated and that 17β-estradiol cellular flow was modified. Resveratrol induced MDR1 and MRP3 expressions, bisphenol A induced MRP2 and MRP3 expressions, and both enhanced 17β-estradiol efflux. Genistein, on the other hand, inhibited ST1E1 and UGT1A1 expressions, and led to 17β-estradiol cellular retention.

Thus, we demonstrate that bisphenol A, genistein and resveratrol modulate 17β-estradiol cellular bioavailability in HepG2 and that these modulations most probably involve regulations of 17β-estradiol phase II and III metabolism proteins. Up to now, the estrogenicity of environmental estrogenic pollutants has been based on the property of these compounds to bind to ERs. Our results obtained with ER negative cells provide strong evidence for the existence of ER-independent pathways leading to endocrine disruption.

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1. Introduction

Endocrine disrupting compounds (EDCs) are chemicals with the potential to elicit negative effects on endocrine systems of humans and other animals. They interfere with the functioning of the endocrine system in at least three possible manners: by mimicking the action of a naturally produced hormone, binding to their hormone receptors; by blocking the receptors in target cells for these hormones and therefore preventing the action of natural hormones; or by altering the synthesis and function of hormone receptors and modifying the synthesis, transport, metabolism and excretion of hormones. The most studied of all EDCs are environmental estrogens which we will designate as e-EDCs (estrogenic EDCs), that mimic the gonadal hormone 17β-estradiol (E2). Various natural and synthetic chemical compounds have been identified that induce estrogen-like responses including pharmaceuticals, pesticides, industrial chemicals, heavy metals and phytoestrogens [1]. Up to now, the estrogenicity of e-EDCs has been based on the property of these compounds to bind to estrogen receptors (ERs), which subsequently exert transcriptional effects when binding to the estrogen response element (ERE) on the promoter of target genes. However, several nuclear receptors other than the ERs could be involved in E2 target genes regulation by e-EDCs.

Hormonal estrogens are eliminated from the body principally by the liver, where the major pathway of E2 elimination comprises its conjugation by phase II detoxifying enzymes and subsequent excretion of the conjugate by phase III transporter proteins into the bile.

There are evidences that e-EDCs might influence the biological effects of E2 by modulating its metabolism and disrupting the balanced generation of metabolites [2–5]. However, while most of these studies investigated the modulation of E2 phase II enzymes activities, fewer studies have actually measured genes expression (always in ER positive models), and none has investigated the effects of e-EDCs on phase III E2 excretion, neither on transport activities nor on transporters expression.

This paper focuses on the effects of three e-EDCs commonly found in human diet on the regulation of E2 cellular elimination in the human hepatoblastoma HepG2. This cell line was used as a model because it is well differentiated, it has retained many hepatocyte-specific functions and it expresses most of the proteins involved in E2 phase II and III metabolism of the human liver. Conversely, HepG2 cells expresses ER receptor at very low levels which are unsufficient for ligand to induce transactivation of an ERE-containing synthetic target gene in transient transfection [6,7], thus enabling the investigation of an ER-independent effect of these e-EDCs.

The e-EDCs chosen for this study mimic or induce estrogen-like response with varying degrees of potency. The chemical bisphenol A (BPA) has been shown to be released from polycarbonate flasks during sterilization, inner coating of food cans and dental sealants [8]. The isoflavone genistein (GEN) and the stilbene resveratrol (RES) were chosen as representative estrogenic polyphenols to which we are exposed, as they are natural compounds of food and wine [9,10].

In human hepatocytes, E2 glucuronides are formed by UDP (uridine diphosphate)-glucuronosyltransferases (UGTs) 1A1, 2B7 and with a minor contribution by UGT1A3, 1A4 and 2B15 [11,12]. The estrogen sulfotransferase (SULT1E1), and to a lesser extent the thermostable phenolsulfotransferase (SULT1A1) catalyze the formation of E2 sulfoconjugates [13,14]. Major transporters responsible for E2 excretion by hepatocytes are ATP-binding cassette proteins with varying affinities for E2 conjugates. These include, for the most important, P-glycoprotein, encoded by MDR1 [15] (multidrug resistance) gene and the multidrug resistance proteins MRP1 [16], MRP2 [17], MRP3 [18] and MRP4 [19].

E2, BPA, RES and GEN effects on E2 cellular metabolism in HepG2 were first investigated by measurements of the expressions of genes involved in E2 phase II and III metabolism, and then by E2 cellular flow studies. E2 flow studies are a combined measurement of E2 influx and efflux from the cells. Due to E2 hydrophobicity, E2 influx can be considered as passive. On the other hand, E2 efflux results from active excretion by transporters of conjugates issued from E2 phase II metabolism. Thus the efflux represents the transport activity of E2 across the cell, such that the E2 elimination process can be analyzed as a whole by this method.

The present experiments were designed to test the hypothesis that endocrine disrupting chemicals can modulate E2 bioavailability through a modification of E2 metabolism and elimination. The tests examined ER-independent pathways leading to endocrine disruption.

2. Experimental

2.1. Chemicals

17β-estradiol (E2), bisphenol A, *trans*-resveratrol, dimethylsulfoxide (DMSO) and 30% acrylamide/bis-acrylamide were purchased from Sigma–Aldrich (St. Quentin Fallavier, France). Genistein (GEN) was obtained from AApin Chemicals (Abingdon, UK). [^{3}H]-17β-estradiol ([^{3}H]-E2; specific activity: 1.84 TBq/mmol; labeled in 6 and 7) was purchased from PerkinElmer Life and Analytical Sciences (Boston, US). Chemicals used for Western blotting and cellular incorporation kinetic, unless specified, were purchased from Sigma–Aldrich.

2.2. Cell and culture media

Cell culture media, L-glutamine and non-essential L-aminoacids were purchased from Sigma–Aldrich. Heat-inactivated fetal bovine serum (FBS) was obtained from Invitrogen (Cergy-Pontoise, France). The HepG2 human hepatoblastoma cell line was obtained from the ECACC (European Collection of Cell Culture, Salisbury, UK). Routine monitoring has shown the HepG2 cells to be mycoplasma free (Mycoalert Kit from Cambrex, Verviers, Belgium). The cells were grown in monolayer culture and maintained in phenol-red Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 2 mM L-glutamine, 1% non-essential L-aminoacids, and 10% FBS (v/v) in an humidified atmosphere of 5% CO_2 and at 37 °C. All the experiments were carried out

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