Human Adrenocarcinoma (H295R) Cells as Potential Predictors of Bisphenols Ability to Interfere with Steroid Hormone Production: A Mini Review

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Abstract

The focus on the refinement, reduction and replacement of animal use in toxicity testing requires the development of cell-based systems that mimic the effects of xenobiotics including bisphenols in human tissues. The human H295R adreno-carcinoma cell line provides a good in vitro system for the analysis of the human adrenal steroidogenic pathway at the level of hormone production and gene expression, because it expresses genes that encode for all the key enzymes for steroidogenesis. In this review we provide an introduction to H295R cell line and its use for research of toxicological effects of bisphenols, which represents an important contaminant.

Keywords: bisphenols; endocrine disruptors; H295R; steroidogenesis

1. Introduction

Over the past three decades, there has been increasing concern about the possible impacts of exposure to chemicals in the environment on endocrine and reproductive systems in humans and wildlife [1]. Endocrine disruption is a toxicity of both physiological and regulatory importance. Steroid hormones regulate reproduction, development, and other biological processes. Thus, chemicals that can disrupt the production of steroids may be directly linked to adverse outcomes for these processes. It is a priority to identify chemicals that may interact with production of these hormones [2]. H295R cells are a transformed human adrenal cell line which secretes all the steroid intermediates of the steroidogenesis pathway, and has been found useful for studying steroidogenesis [3]. Bisphenols (BPs) are a group of chemical compounds that consist of two phenolic rings joined together through a bridging carbon or other chemical structures, BPs are commonly used to produce polycarbonates and epoxy [4, 5]. H295R cells have been used to evaluate effects of xenobiotics on hormone production, as well as steroidogenic enzyme activity and expression [3, 6; 7].

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2. Steroidogenesis in Human H295R Cells

The H295R assay is an *in vitro* method for detecting chemical disruption of the catalytic events of steroidogenesis and has been used predominantly to predict chemical perturbation of 17β-estradiol (E2) and testosterone (T) synthesis [8]. The H295R cell line demonstrates the biological characteristics of zonally undifferentiated human fetal adrenal cells, but produces the steroid hormones found in the adult adrenal cortex and the gonads, allowing testing for effects on both corticosteroid synthesis and the production of sex steroid hormones such as androgens and estrogens simultaneously (Figure 1) [9, 10]. This cell line has been used widely as a cell model for evaluating the chemical disruption of the steroidogenesis pathway because of the

ability to measure the alteration in gene transcription, enzyme activity and hormone production at the same time [10-13]. The US EPA endocrine disruptor screening program (EDSP), ToxCast, European REACH, as well as the global Organization for Economic Cooperation and Development (OECD) have all employed hormone measurement in the H295R cell model for identification of endocrine disrupting chemicals [3].

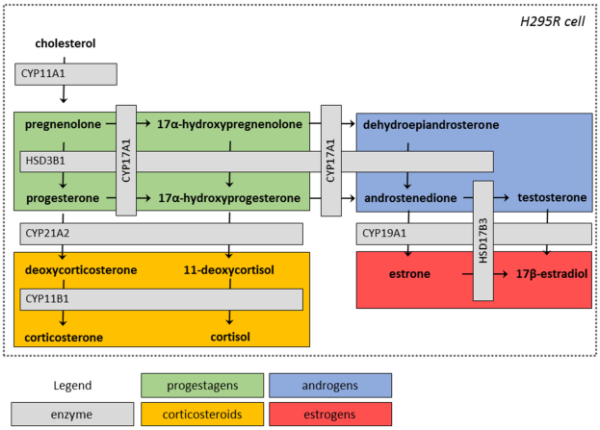

Figure 1. Representation of the steroid biosynthesis pathway expressed in H295R cells [2].

In steroidogenesis, cholesterol is first shuttled to the inner mitochondrial membrane in a rate-limiting step by steroidogenic acute regulatory protein (StAR). Cholesterol is then converted to pregnenolone by side chain cleavage enzyme (CYP11A1). Progesterone is produced by 3 beta-hydroxysteroid dehydrogenase (3βHSD2) action on pregnenolone. CYP17A1 catalyzes the 17-hydroxylation of pregnenolone and progesterone to 17α-hydroxy intermediates and the 17,20 lyase reactions leading to DHEA and along with 17beta-hydroxysteroid dehydrogenase (17βHSD) activity, to testosterone. Cortisol is synthesized from the 17α-hydroxy intermediates by the enzymes 3βHSD, 21-hydrolase (CYP21A2), and 11-beta hydroxylase (CYP11B1). Estradiol is converted from testosterone by the enzyme aromatase (CYP19A1). Estradiol can alternatively be converted by17βHSD from estrone, a hormone produced by aromatase activity on androstenedione [3].

3. Bisphenols as endocrine disruptors

The bisphenols are a group of chemical compounds with two hydroxyphenyl functionalities. Most of them are based on diphenylmethane. The exceptions are bisphenol S, P, and M. Among BPs, bisphenol A (BPA) is the most widely used and investigated chemical [5]. A large number of studies has shown that BPA has adverse effects on human health, including interrupted steroid hormone synthesis [13, 14], reproductive toxicity [15, 16], immunotoxic potential [17], disrupted development of mammary gland [18], changes in obesity and diabetes associated parameters [19, 20]. The theory of endocrine disruption proposes that low exposure to chemicals that interact with hormone receptors, hormone metabolism or other processes involved in hormone homeostasis can interfere with reproduction, development, and hormonally mediated processes in general [21, 22]. BPA also is associated with heart disease, abnormal liver function, etc [23-27]. According to Feng et al., 2016, hormone level results demonstrated that BPA analogues, such as BPF, BPS and BPAF are capable of altering steroidogenesis in H295R cells at non-cytotoxic concentrations. BPA and BPS exhibited inhibition of hormone production; BPF predominantly led to increased progesterone and 17β-estradiol levels; and BPAF showed induction of progesterone and reduction of testosterone [13]. Due to its ubiquitous nature and potential health hazard for human beings, the use of Bisphenol BPA has been regulated in many countries. As a result of the restriction, structural anologues such as bisphenol AF (BPAF), bisphenol B (BPB), bisphenol S (BPS) and bisphenol F (BPF) have already been used for industrial applications as alternatives to BPA. For example, BPA was banned in baby bottles in Canada, France, and the European Union in 2008, 2010, and 2011, respectively [13]. Structural anologues of BPA such as BPAF, BPB, BPF and BPS have already been used as alternatives for BPA. BPS is used for a variety of industrial applications such as a wash fastening agent, an electroplating solvent, and thermal receipt papers [28]. BPB is used in the manifucture of phenolic and polycarbonate resins. It can be found in products such as food packaging, paper plates, cutlery and small appliances such as roasters [29]. BPF can be used in the production of epoxy resins and polycarbonates, especially for lining of solid/high built systems. Its use is important in the production of tank and pipe linings, industrial floors, road and bridge deck toppings, structural

adhesives, grouts, coatings and electrical varnishes [30, 31]. BPAF is mainly used as a crosslinker in the synthesis of specialty fluoroelastomers. BPAF is also used as precipitation agent for polymer-preparation emulsions; monomer for polyimides, polyamides, polyesters, polycarbonates, and other specialty polymers [32]. Due to the widespread consumer and commercial use of BPA alternatives, these chemicals have been detected in many products worldwide. These products include food, personal care products, food cans, dental fillings, and paper products [33-35]. BPS and BPF were also detected in environmental samples such as surface water, sediment, and sewage in China [36-39]. In indoor dust, BPS, BPF, BPA, and BPAF have been detected at the following concentrations: BPS, 0.34 μg/g; BPF, 0.054 μg/g; BPA, 1.33 μg/g; BPAF, 0.00069 μg/g [35]. In 267 foodstuffs from the United States, the mean concentrations of BPS were 0.130, BPF - 0.929, BPA - 3.00, and BPAF were 0.012 ng/g [33, 34]. The Estimated Daily Dietary Intakes (EDI) of BPS, BPF, BPA, and BPAF for adults were 1.31, 7.46, 44.6, and 0.275 ng/kg bw/day, respectively [33, 34]. In 100 urine samples from non-occupational Americans, BPS was detected in 78% of samples with 0.13 ng/mL median concentration, BPF was detected in 55% of samples with 0.08 ng/mL median concentration, and BPA was detected in 95% of samples with 0.72 ng/mL median concentration [35]. It seems that these chemicals may become worldwide food contaminants and environmental pollutants in the future [13].

4. Conclusions

The endocrine disrupting effects of bisphenols, their interactions with each other or with other substances, are still the subject of current studies to better understand the mechanism of action. Therefore, it is necessary to continue the studies by using H295R cell line and gradually reduce the risk of endangering animal or human health.

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