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Serum free BG-1 cell proliferation assay: A sensitive method for determining organochlorine pesticide estrogen receptor activation at the nanomolar range

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Abstract

Most xenobiotic estrogeniety assay methods rely on direct agonist action on the estrogen receptor (ER) to approximate activation potential. Such methods do have drawbacks since some ER activating pesticides are weak or non-agonistic in ligand-binding assays. This study discusses a method that detects pesticide estrogenic actions regardless of ER ligand binding ability. Using a serum-free BG-1 ovarian cell culture model, we investigated the ability of several organochlorine (OC) pesticides to stimulate known estrogenic actions. We observed concentration dependent ER mediated cell proliferation in BG-1 cells using heptachlor epoxide (HE), β-hexachlorohexane (β-HCH), and endosulfan (Endo). In addition, we observed upregulation of the ERE-dependent proteins progesterone receptor and PS2. Gel-shift/EMSA studies for ERE binding further supported these OC's ERE activating abilities. All of these effects were abolished using ICI 164,384(ICI). Using the same culture conditions, we tested the blocking action of growth factor antibodies for erbB2(9G6) and insulin-like growth factor (IGF-Ab) receptors and discovered they inhibited BG-1 proliferation (9G6: HE and β-HCH/ IGF-Ab: Endo.) This experiment confirms the existence of a possible cross-talk between ER and growth factor receptors in OC ligand-dependent activation and also validates this sensitive method for determining both ligand-dependent and independent estrogenic activity of selected pesticides.
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1. Introduction

The cellular consequences of estrogen receptor (ER) activation have been well characterized in many cell models. ERs have been implicated in a variety of cellular functions including regulation of protein synthesis and cellular proliferation. This is especially true in areas that define female secondary sexual characteristics such as the breast, uterus and ovarian tissue systems. ER activation has been shown to lead to induction of progesterone receptors in MCF-7 cells (Petz and Nardulli, 2000; Petz et al., 2002) and increases in uterine mass (Menditto and Turrio-Baldassarri, 1999), both important processes in the female reproductive system. Animal knockout models deleting the estrogen receptor are often characterized by infertility (Britt and Findlay, 2002). Despite this clarity in the ER's cellular function, there has been increasing evidence that its activation can involve a multitude of complex mechanisms.

Abbreviations: ER, estrogen receptor; E_2, 17-β-Estradiol; LIA, ligand-independent activation; OC, organochlorine; ERE, estrogen response element; PKA, cAMP-dependant protein kinase; HCH, hexachlorocyclohexane; HE, heptachlor epoxide; ENDO, endosulfan I; PR progesterone receptor; FBS, fetal bovine serum; 4-OH-Tam, 4-OH-Tamoxifen; HRG, heregulin; EGF, epidermal growth factor; IGF, insulin-like growth factor; ICI, ICI 164,384.

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Direct binding of the ER by endocrinic steroids, 17-β-Estradiol (E_2), and other estrogens remains the most widely recognized form of ER activation and is commonly referred to as ligand-dependant ER activation (Menditto and Turrio-Baldassarri, 1999). Recent evidence in the literature suggests, however, that the ER may be activated by other mechanisms that do not require direct binding of ligands to the receptor. In several estrogen responsive cells lines, peptide growth factors such as EGF and IGF have been shown to act in this manner as surrogates for estrogen (Ignar-Trowbridge et al., 1993; Jazaeri et al., 2001). In these cases, the ER is activated without direct ligand binding. Therefore, this process is referred to as ligand-independent activation (LIA) of the ER.

Recent studies, including those done in our laboratory, have demonstrated that pesticidal organochlorine compounds (OC) may be exerting their effects by way of LIA of the ER (Hatakeyama et al., 2002; Hatakeyama et al., 2003). It is clear that, OCs do disrupt the endocrine systems through "estrogen-like" properties particularly when tested in vivo (e.g. increases in uterine weight) (Hatakeyama et al., 2002; Tessier and Matsumura, 2001). Furthermore, OCs have been shown to activate the genes that are regulated by an ERE (Hatakeyama et al., 2002). The ERE is normally activated by ligand bound estrogen receptors (ER). However, OCs cannot be considered as direct estrogen mimics, since several studies have shown that these compounds show only weak, marginal or no agonistic actions on the ER (Soto et al., 1995). This is an extremely important point, since many of established xenoestrogen screening methods used today are based on direct receptor activation through their agonistic actions: an assay principle which would not work well on OCs. Therefore, estrogeneity of most OCs may have to be assessed by different approaches based of the knowledge on their molecular action mechanism. One such possible mechanism is based on the experimental observations that certain pesticides directly activate tyrosine kinase associated with growth factor receptors such as cNeu (=erbB2) receptors (Tessier and Matsumura, 2001). It has already been shown that growth factors such as EGF and TGF-α can "cross-talk" and activate the ER system using these same receptors through the LIA of ER in BG-1 cells (Gehm et al., 2000; Ignar-Trowbridge et al., 1996; Ignar-Trowbridge et al., 1993; Jazaeri et al., 2001). Preliminary experiments which demonstrate this "cross-talk" ability of pesticides have already been completed in a standard human breast cancer cell line, MCF-7, and a prostate cancer cell line (LNCAP) in our laboratory (Hatakeyama et al., 2002; Tessier and Matsumura, 2001). Other possible mechanisms for LIA include signal transduction components downstream of tyrosine receptor activation and release of endogenous tyrosine receptor ligands such as TGF-α and Insulin-like growth factor (IGF). For example, it has been shown that activation of the cAMP-dependant protein kinase (PKA) or protein kinase C (Riby et al., 2000; Rowan et al., 2000) can lead to similar cellular effects seen in LIA by growth factors and exogenously added compounds.

Previous work in our laboratory has established the ability of pesticides to activate cNeu (=erbB2) associated kinases in breast carcinoma (Hatakeyama et al., 2002) and prostate cancer cells (Tessier and Matsumura, 2001). "Cross-talk" parameters such as increased foci formation (Hatakeyama et al., 2002), cell proliferation, and ERE binding and activation (Hatakeyama et al., 2003) were demonstrated in breast cancer (MCF-7) cells lines thereby confirming the estrogenic ability (estrogeneity) of certain pesticides.

Because the actions of hormones are often tissue specific, this paper examines how broadly that our OC LIA principle is applicable in hormone responsive ovarian cell line. While many systems have similar mechanisms of ERE activation by estrogen and have exhibited "cross-talk" abilities by a variety of stimuli, there are particular tissue specific differences in the types and expressions of tyrosine kinase receptors and their subsequent actions. For example, the BG-1 ovarian cancer cells (as opposed to MCF-7 breast cancer cells) tend to be more receptive to IGF receptor stimuli (Ignar-Trowbridge et al., 1993) than cNeu (=erbB2) activation for "cross-talk" initiated cell proliferation. In addition, BG-1 cells express twice a much ER as MCF-7 cells and it must be emphasized that these pesticides have been previously found to be estrogenic in the "uterotropic" tests in vivo, thus implicating the importance and susceptibility of this model. These cellular differences, in turn, may lead to differences in pesticide induced estrogenic effects.

Therefore, the main objective of this study is to determine first, whether OC pesticides can act estrogenic in our BG-1 ovarian cell line system, second, to determine the optimal experimental conditions to investigate the OC estogeniety in BG-1 cells, and third, to establish possible mechanisms of how OCs can activate ER without acting as direct ER ligands.

2. Materials and methods

2.1. Materials

17-β-Estradiol (E_2) and 4-OH-Tamoxifen (4-OH-Tam) were purchased from Sigma Biochemicals (St. Louis, MO). β and α-isomers of hexachlorocyclohexane (β-HCH and α-HCH) were purified in this laboratory from a chemical standard stock supplied by the US. EPA. Arochlor 1248 was obtained from Polyscience Corp (Niles, IL). 2,2′,3,5′,6-Pentachlorobiphenyl (PCB 95) and endosulfan I were purchased from Accustandard Inc. (New Haven CT). *cis*-Permethrin and Chlorthalonil were ordered from Chem Service (West Chester, PA). Heptachlor Epoxide (HE) was obtained from Dow chemical Corp. (Midland, MI). ICI 164,384 was obtained from Zeneca Corporation. E_2 and pesticides were kept as high concentration stock solutions in ethanol. Human recombinant EGF, IGF, and choleratoxin were purchased from Gibco BRL (Gaithersburg, MD). H-89 was obtained from Calbiochem

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