REGULATION OF SYNTHESIS AND SECRETION OF SEX HORMONE-BINDING GLOBULIN IN THE MCF-7 CELL LINE BY 17β ESTRADIOL, PCB3 AND ITS HYDROXYLATED METABOLITES.

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Introduction

The relationship between plasma sex hormone binding globulin (SHBG)and breast cancer has been widely investigated over the last twenty years. The estrogen-dependence of some breast tumors and their singular behavior, as well as the ability of SHBG to bind circulating estradiol at high affinity, induced many investigators to search for possible SHBG involvement in the pathogenesis of neoplasms.

It has been reported that human SHBG binds phytoestrogens [1,2], fatty acids [3] and several pharmaceutical agents [4], although little is known about its interactions with synthetic non-steroidal compounds [1,5]. A chemical that has low affinity for extracellular binding proteins may potentially be more potent *in vivo* than compounds that are extensively bound by extracellular proteins.

In the present study, we investigated the effects that exposure of MCF-7 cells to E2, PCB3 and their hydroxylated metabolites (2-0H-E2 and 4-0H-E2, and 4-OH-PCB3 and 3,4-diOH-PCB3) had on intracellular and extracellular SHBG levels. We found that SHBG modulates cell proliferation.

Material and methods

Cell proliferation

The Alamar Blue assay (BioSource Int, USA) contains a redox-reactive compound, and is designed to quantify living cells per well based on their metabolic activity. Thus, this assay is used to indirectly measure the inhibition of proliferation and/or the cytotoxicity of test compounds. The Alamar Blue assay is based on the quantitative metabolic conversion of the blue, non-fluorescent resazurin to pink, fluorescent resorufin by living cells. Alamar Blue was aseptically added to culture wells in an amount equal to 10% of the incubation volume at 24 and 72 hrs. After incubating for 4 hrs, the absorbance of the medium was measured at 530 nm excitation and 590 nm emission wavelengths using a microELISA plate reader.

Measurement of SHBG

The SHBG concentrations from homogenized MCF-7 cells and medium samples were determinate using the enzyme immunoassay SHBG ELISA (DRG Instruments GmbH, Germany). The sensitivity of the assay was 0.2 nmol/l, the intra-assay variation was 3.0%-8.6%, the inter-assay variation was 7.2%-11.6%, and the linear measuring range was 0-260 nmol/l.

Statistical analysis

Each treatment was repeated three times (n=3) in quadruplicate. The average of the quadruplet values was used for statistical calculations. Statistical analysis was performed using Stastistica 6.0. Data were analyzed using a 1-way analysis of variance (ANOVA) followed by a Tukey honestly significant difference (HSD) multiple range test. Results were considered statistically significant at the $P<0.05$ level.

Results

Natural 17 β-estradiol and its hydroxylated metabolites (2-OH-E2 and 4-OH-E2).

A positive SHBG response to estradiol was observed in MCF-7 cells. 17 β-estradiol and 4-OH-E2 had no effect on extracellular SHBG levels but increased intracellular SHBG levels. 2-OH-E2 had no effect on either extra- or intracellular SHBG levels (Fig. 1).

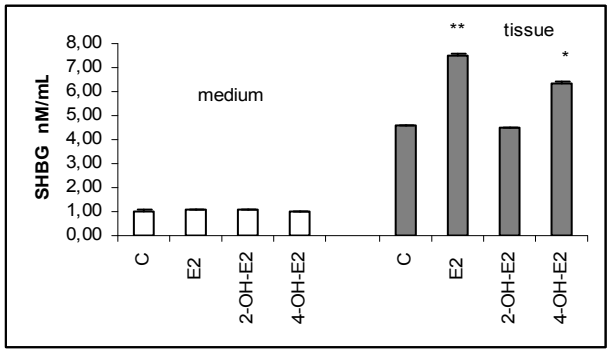

Fig. 1 The effect of 17 β-estradiol and its hydroxylated metabolites (2-0H-E2 and 4-OH-E2) on extracellular (open bars) and intracellular (grey bars) sex hormone-binding globulin (SHBG) concentration. MCF-7 were cultured for 24 hours in the absence (control C) and presence of 10nM testes compounds. * $p < 0.05$, ** $p<0.01$.

Both E2 and its hydroxylated metabolites (2-0H-E2 and 4-OH-E2) increased the number of proliferating cells, while SHBP itself did not produce any significant cell proliferation. Pre-incubation with SHBG did not modify cell proliferation. Simultaneous treatment with estradiol or its metabolites and SHBG revert stimulatory action on cell proliferation (Fig. 2).

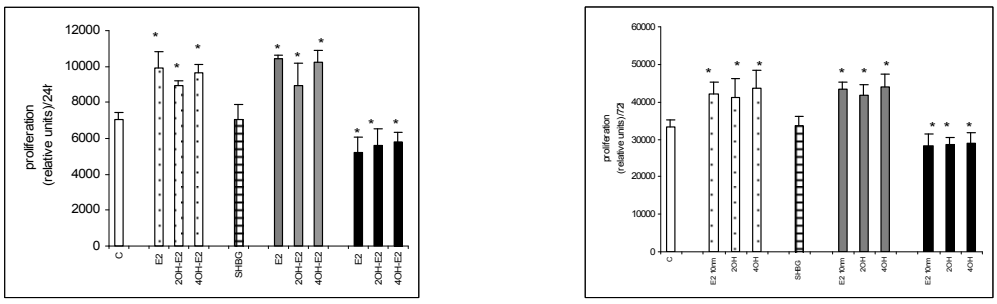

Fig. 2 The effect of 17 β-estradiol and its hydroxylated metabolites (2-OH-E2 and 4-OH-E2) on MCF-7 cell proliferation after a) 24h and b) 72h of exposure to 10 nM concentrations of these compounds. Dots bars- estradiol and metabolites alone, grey bars- SHBG+E2 or metabolites (preincubation with SHBG), black bars- SHBG+E2 (co-incubation with SHBG). (*) $p < 0.05$.

PCB3 and its hydroxylated metabolites (4-OH-PCB-3 and 3,4-diOH-PCB3).

PCB3 and 4-0H-PCB3 had no effect on either the extra- or intracellular SHBG levels. A tendency toward stimulatory action on intracellular SHBG levels and no effect on extracellular levels was noted in cells exposed to 3,4-di-OH-PCB3 (Fig. 3).

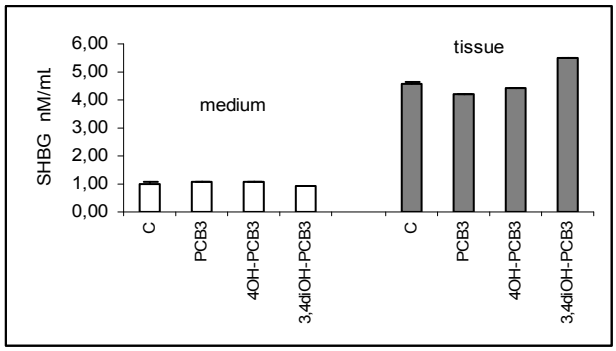

Fig. 3 The effect of PCB3 and its hydroxylated metabolites (4-0H-PCB3 and 3,4-diOH-PCB3) on extracellular (open bars) and intracellular (grey bars) sex hormone-binding globulin (SHBG) concentration in MCF-7 cell cultures. MCF-7 were cultured for 24 hours in the absence (control C) and presence of 60ng/ml testes compounds. (*) $p < 0.05$.

Both 24- and 72-hr exposures to PCB 3 and its hydroxylated metabolites did not affect cellular proliferation. Neither culture condition (pre-incubation and simultaneous SHBG treatment) affected cell proliferation (Fig. 4).

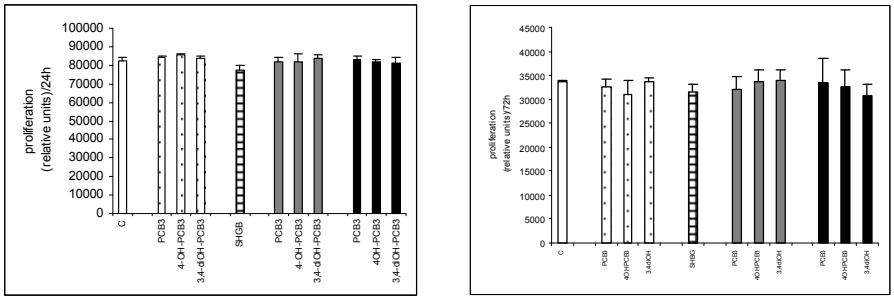

Fig. 4 Effect of PCB3 and its hydroxylated metabolites (4-OH-PCB3 and 3,4diOH-PCB3) on MCF-7 cell proliferation after a) 24h and b) 72h of exposure to 10 nM concentrations of these compounds. dots bar- PCB3 and metabolites alone, grey bars- SHBG+PCB3 or metabolites (preincubation with SHBG), black bars- SHBG+PCB3 (co-incubation with SHBG). (*) $p < 0.05$.

Discussion

An increase of SHBG under the influence of steroid and growth hormones has previously been described in HepG2 cells [6-9]. This data first showed the effect of E2 and its metabolites on SHBG levels in extra hepatic tissue. Here, we showed that simultaneous treatment with SHBG caused inhibition of cellular proliferation. Fortunati et al.[10] and Porto et al.[11] have detected a specific binding site for SHBG on MCF-7 breast cancer cells. In addition, the presence of a SHBG binding site in breast cancer has been related to a reduced proliferation rate [12]. The interaction of SHBG with its membrane-binding site induces the second messenger cAMP [13,14] and causes a complete inhibition of estradiol-induced MCF-7 cell proliferation[15]. In another report, Catalano et al.[16] suggested that the interaction of SHBG with MCF-7 cell membranes causes inhibition of the anti-apoptotic effect of estradiol, which might account for SHBG's inhibitory effect on breast cancer cell growth. To our knowledge, there is no data concerning the affinity of hydroxylated E2 metabolites for SHBG. Hryb et al.[17] showed that 2-methoxyestradiol, a biologically inert metabolite of estradiol, binds more tightly to SHBG than does either testosterone or estradiol. As shown by Rosner et al.,[18] 2-methoxyestradiol is also a stronger inhibitor of SHBG binding to RSHBG than either of these two steroids.

Although environmental contaminants (xenoestrogens) that interfere with estrogen signaling are of increasing concern, there is only limited information regarding their ability to interact with estrogen-binding proteins (SHBG) or receptors (ER). Any binding of xoestrogens to the high-affinity sites on SBP would be expected to displace biologically active steroid and may also specifically deliver the exogenous compound to the target sites within the organism. PCB3 and both of the investigated congeners had no effect on extracellular SHBG levels while 3,4-diOH-PCB3 slightly increased the intracellular SHBG level. However, co-treatment of all investigated compounds with SHBG did not alter cell proliferation. Danzo [19] reported that very high concentrations of nonylphenol (10,000 times greater than DHT) reduced the binding of [3H]DHT to SHBG by 70%, while other xenobiotic competitors (*o,p'*-DDT and pentachlorophenol) reduced binding by 20–30%, and methoxychlor, *p,p'*DDT, *p,p'*DDE, dieldrin, and atrazine showed no inhibition. Jury et al., [20] using a panel of polychlorinated biphenyls (PCBs) and hydroxy-polychlorinated biphenyls (HO-PCBs) examined in the screening assay, showed that although the PCBs have little or no ability to interact with SHBG, at least three HO-PCBs (HO-PCB2, HO-PCB3 and HO-PCB4) were identified as potential ligands for SHBG. These authors suggested that less substituted HO-PCBs may be of greater concern in terms of health risks, due to their increased activity at the receptor level. However, we conclude that further experiments be in a more physiological context, compared to previous methods. In recently published data, Gale et al [21] determined the ability of various xenoestrogens to bind to the recombinant channel catfish ERa and b and to a putative plasma SHBG from the adult female channel catfish (ccfSHBG), suggesting that the binding of xenoestrogens to SHBG must also be taken into account for proper assessment of endocrine disruption caused by environmental contaminants.

Conclusion Results from our investigations agree with other previous works that cast a skeptical light on the possibility that PCB3 and their metabolites interact with SHBG. Moreover, our results suggest other mechanism

of possible carcinogenic action of PCBs and its metabolites. Any binding of exoestrogens to the high-affinity sites on SBP could produce indirect effects by displacing endogenous (and highly active) steroids from the reservoir on plasma binding sites.

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