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Effects of Tris(4-chlorophenyl)methanol on Proliferation of Human Breast Cancer Cells

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Abstract

Tris(4-chlorophenyl)methanol (t-CP-OH), a bioaccumulating ubiquitous environmental contaminant, was tested for possible direct (anti)estrogenic activity in estrogen receptor positive human MCF-7 breast cancer cells and for (anti)androgenic potential using the androgen receptor positive human breast cancer cell line MFM-223. t-CP-OH up to 5 µmol/L did neither induce any cell proliferation in MCF-7 cells nor inhibit estrogen induced cell proliferation. 10 µmol/L of t-CP-OH were highly cytotoxic to MCF-7 cells. Proliferation of MFM-223 cells was induced by t-CP-OH in a dose-dependent manner with a maximum increase of 49 ± 7 % above the hormone free control at 5 µmol/L. t-CP-OH was able to compensate the antiproliferative effect of the androgens 5α-dihydrotestosterone and Mibolerone in AR positive MFM-223 cells thus representing an antiandrogenic effect.

Introduction

In recent years tris(4-chlorophenyl)methanol (t-CP-OH) has been found in marine mammals, birds, and fish, from various regions all over the world including Arctic and Antarctic [1, 2, 3, 4]. The levels detected in liver and adipose tissue on lipid weight basis were up to several mg/kg (ppm). Thus, t-CP-OH is a persistent organic pollutant (POP) with global distribution and strong accumulation in food chains.
In contrast to its ubiquitous environmental presence there are open questions regarding anthropogenic sources of t-CP-OH. Possible sources include optically active polymers, agrochemicals, and compounds used in the production of synthetic dyes. t-CP-OH is probably a metabolite of tris(4-chlorophenyl)methane, a side-product in technical-grade DDT [5]. Very little is known about the toxicology of t-CP-OH. Short-term oral exposure of rats led to induction of hepatic phase I and II enzymes, increase in liver and spleen weight, and systemic changes including the haematopoetic system[5].

Although no systematic testing of chemicals for their possible estrogenic properties has been carried out up to now, in recent years an increasing number of non-steroidal anthropogenic chemicals and naturally occurring compounds have been identified to act like endogenous estrogens [6,7,8]. Since a common chemical substructure responsible for the estrogenic action has not yet been identified, it is to be expected that more xenobiotics with estrogenic properties are present in the environment and biosphere. Recently we could demonstrate this for two widely used p-chlorocresols and the flame retardant Tetrabromo-Bisphenol-A [9].

There is wide concern about adverse effects of persistent chemicals with endocrine action on reproduction of humans and wildlife species. Various reproductive disruptions in wildlife populations of birds, amphibia, and fish in the presence of significant levels of estrogenic chemicals are well documented [6]. Therefore, we were interested whether the ubiquitous environmental contaminant tris(4-chlorophenyl)methanol has a potential to mimick or antagonize the effects of natural estrogens and androgens *in vitro*. The human estrogen receptor (ER) positive breast cancer cell line MCF-7 and the androgen receptor (AR) positive breast cancer cell line MFM-223 were used in the respective screening systems.

Experimental Methods

Chemicals: Tris(4-chlorophenyl)methanol (t-CP-OH) with 98 % purity was obtained from Lancaster (Nr. 6965), 4-t-Octylphenol with >90 % purity from Fluka. 0.01 M stock solutions were prepared in DMSO. 17ß-estradiol, 5α-dihydrotestosterone (both from Sigma), and Mibolerone (from NEN) were dissolved in ethanol at 1 mM. The stock solutions were diluted with experimental medium (see below) to the desired test concentrations so that the final concentration of the solvent in the medium did not exceed 0.1 % (v/v).

Cell culture and proliferation experiments:
Cultivation of MCF-7 cells and performance of the proliferation experiment was carried out following the principle method described by Soto et al.[7] with substantial modifications. Briefly, cells were cultivated in DME medium with 5 % fetal calf serum (FCS) in a humidified atmosphere with 5 % CO_2. Proliferation experiment was started by seeding cells in 24 well plates with a density of 10,000 cells per well. After 24 hours the medium was changed to the experimental medium, phenolred-free DME medium containing 5 % CD-FCS (FCS treated with charcoal-dextrane to remove all steroids following the protocol of Stanley et al. [10]). The negative controls consisted of four wells per assay without the addition of hormones. 17ß-estradiol (E2) in concentrations between 10^{-12} M and 10^{-9} M was used in the positive controls. t-CP-OH was tested in concentrations from 10^{-7} M to 10^{-5} M. Each concentration was tested in quadruplicate. Five days later the cells in each well were counted during the exponential phase of proliferation by measurement of total protein content using the sulforhodamine B (SRB) assay[11] and by determination of the mitochondrial metabolic activity using the MTT assay[12]. Within a certain range both endpoints are proportional to the cell number. The basic endpoint is the cell number relative to the hormone free control.
Testing for antiestrogenic activity was carried out by coincubation of MCF-7 cells with various concentrations of t-CP-OH together with 10^{-10} M E2 or $5*10^{-6}$ M 4-t-Octylphenol (4-t-OP), respectively. Cell numbers were compared to those achieved by E2 or 4-t-OP alone.

MFM-223 cells were cultivated at passages 40 to 54 in DME medium with 10 % FCS and 40 IU/L insulin [13]. Proliferation experiment was started by seeding cells in 24 well plates with a density of 20,000 cells per well. After 24 hours the medium was changed to the experimental medium, phenolred-free DME medium containing 10 % CD-FCS, insulin, and the test compound. 5α-dihydrotestosterone (DHT) and the synthetic androgen Mibolerone in concentrations between 10^{-12} M and 10^{-8} M were used in the positive controls. Medium and test compounds were renewed after four days. After seven days cells were counted using the SRB and MTT assay.

Results

1. Testing for (anti)estrogenic activity
t-CP-OH up to 5 µmol/L did not induce any cell proliferation in ER positive human MCF-7 breast cancer cells (Fig. 1). 10 µmol/L of t-CP-OH were highly cytotoxic to the cells.
Testing for antiestrogenicity was carried out by coincubation of various concentrations of t-CP-OH with 0.1 nmol/L of E2 and with 5 µmol/L of the xenoestrogen 4-t-Octylphenol (4-t-OP), respectively.

5 µmol/L of t-CP-OH revealed a moderate decrease of E2 and 4-t-OP induced cell proliferation (Fig. 2). The proliferative effect of E2 was reduced by 42 % (from 3.46 to 2.43) and the effect of 4-t-OP by 40 % (from 3.62 to 2.58), respectively. However, 5 µmol/L of t-CP-OH also reduced the cell number to 72 ± 5 % of that of the hormone free control. The strong cytotoxic concentration of 10 µmol/L of t-CP-OH also caused a similar decrease of cell number in the E2-treated group down to 44 ± 8 % of the hormone free control. Thus, the observed decrease of estrogen induced proliferation of MCF-7 cells by 5 µmol/L of t-CP-OH is regarded as the result of a cytotoxic effect of t-CP-OH and not of an antiestrogenic effect.

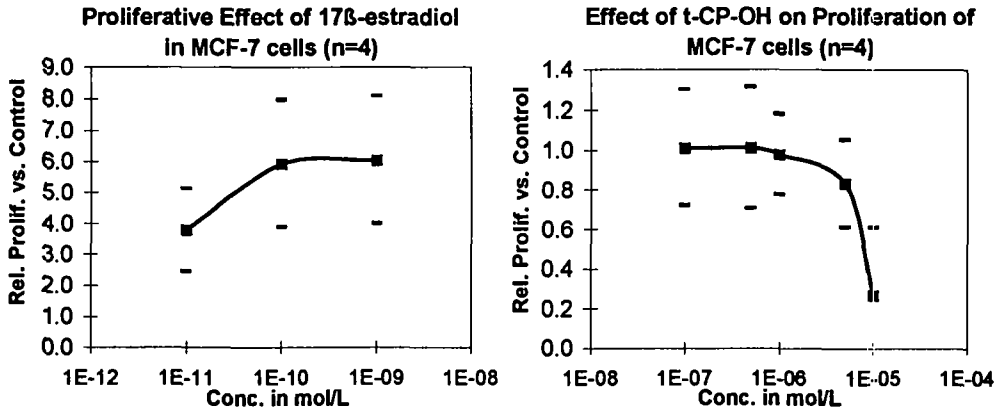


Fig. 1: Number of MCF-7 cells relative to the hormone free control after five days incubation with various concentrations of E2 and t-CP-OH. The diagrams represent mean values and standard deviations of four independent experiments each carried out in quadruplicate.

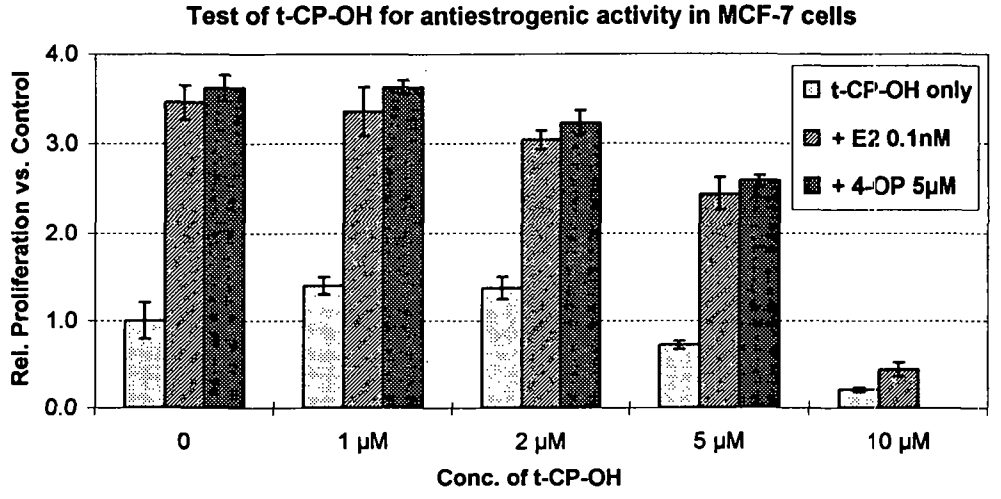
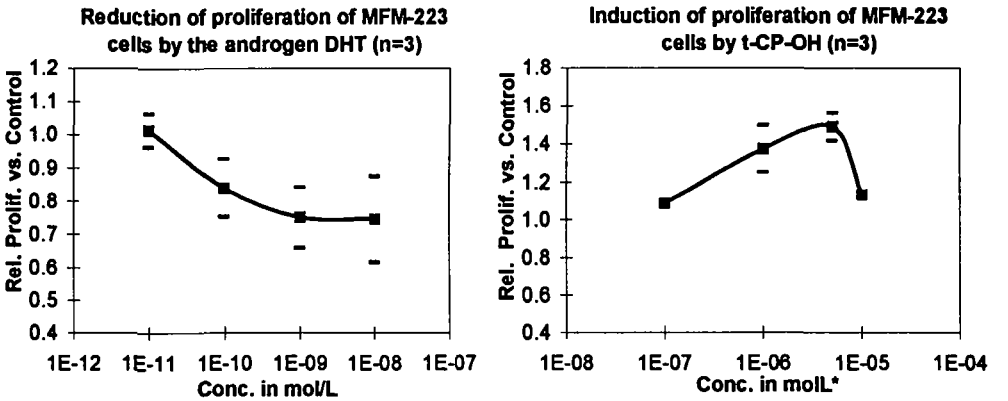


Fig. 2: Number of MCF-7 cells relative to the hormone free control after five days coincubation with various concentrations of t-CP-OH together with 0.1 nmol/L E2 and 5 µmol/L 4-t-OP, respectively. The diagram represents means and standard deviations of one experiment carried out in quadruplicate.

2. Testing for (anti)androgenic activity

Natural and synthetic androgens like 5α-dihydrotestosterone (DHT), Mibolerone, and Methyltrienolone (R1881) reduce cell proliferation in AR positive (and ER negative) MFM-223 breast cancer cells compared to a hormone free negative control [13]. This is illustrated in Fig. 3: 1 nMol/L of DHT reduce cell number to 75 ± 9 % of the hormone free control. t-CP-OH between 1 and 10 µmol/L did not inhibit cell proliferation thus not showing androgenic activity. In contrast, t-CP-OH induced a significant cell proliferation in human MFM-223 cells (Fig. 3). Compared to the hormone free negative control cell number was elevated by 49 ± 7 %. In contrast to MCF-7 cells 10 µmol/L of t-CP-OH were not yet cytotoxic to MFM-223 cells.



* mean of two experiments for 1E-07 and 1E-05 mol/L t-CP-OH

Fig. 3: Number of MFM-223 cells relative to the hormone free control after seven days incubation with various concentrations of DHT and t-CP-OH. The diagrams represent mean values and standard deviations of three independent experiments each carried out in quadruplicate.

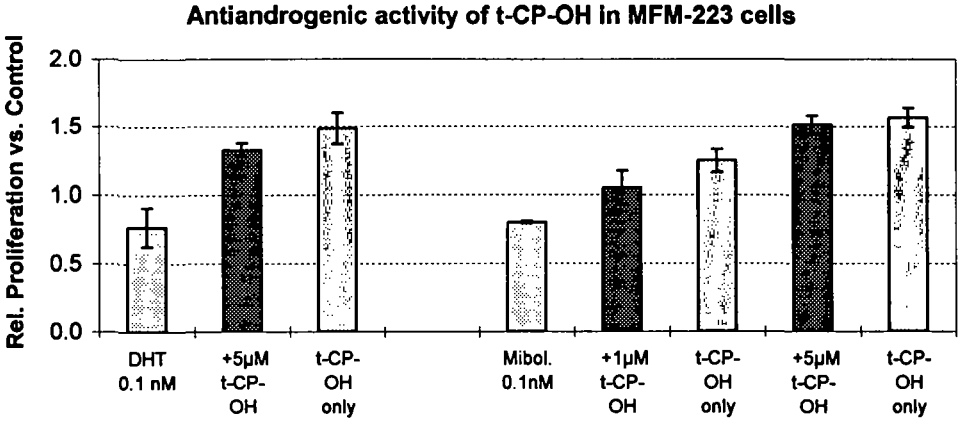


Fig. 4: Number of MFM-223 cells relative to the hormone free control after seven days coincubation with t-CP-OH together with 0.1 nM of the androgens DHT and Mibolerone, respectively. The diagram represents mean values and standard deviations of one experiment carried out in quadruplicate.

Testing for antiandrogenic activity was carried out by coincubation of MFM-223 cells with t-CP-OH together with 0.1 nM of DHT and Mibolerone, respectively. The antiproliferative effect of both androgens was completely inhibited by 5 µmol/L of t-CP-OH giving the same cell yield as t-CP-OH alone (Fig. 4).

Discussion

Breast cancer is by far the most frequent cancer in women in western countries. The continuously rising incidence all over the western world in the last decades can only partly be explained by known risk factors. Significant exposure to estrogenic chemicals is suspected to be an important cause [14].
As the majority of primary human breast cancers express the androgen receptor [15], persistent and bioaccumulating chemicals with a potential to interfere with the human androgen receptor might also play a significant role in the regulation of promotion and progression of breast cancer. However, AR mediated regulation of cell proliferation in breast cancer is still poorly investigated and understood. Thus, development and application of screening systems for detection of (anti)androgenic activities of chemicals remains an important task. The proliferation of human breast cancer cells is a biologically relevant *in vitro* model for the tumour promotion of mammary carcinoma. Using different carcinoma cell lines expressing either the estrogen or the androgen receptor (anti)estrogenic as well as (anti)androgenic effects of chemicals can be studied.
According to our knowledge this study provides the first data on the effect of t-CP-OH on human cells. Although it has been demonstrated that t-CP-OH is an ubiquitous contaminant in the marine environment there are at present neither data on occurrence in terrestrial ecosystems nor on human exposure.

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