Effects of Environmental Organochlorine Pesticides on Human Breast Cancer: Putative Involvement on Invasive Cell Ability

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ABSTRACT: Human exposure to persistent organic pollutants (POPs) is a certainty, even to long banned pesticides like *o,p′*-dichlorodiphenyltrichloroethane (*o,p′*-DDT), and its metabolites *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE), and *p,p′*-dichlorodiphenyldichloroethane (*p,p′*-DDD). POPs are known to be particularly toxic and have been associated with endocrine-disrupting effects in several mammals, including humans even at very low doses. As environmental estrogens, they could play a critical role in carcinogenesis, such as in breast cancer. With the purpose of evaluating their effect on breast cancer biology, *o,p′*-DDT, *p,p′*-DDE, and *p,p′*-DDD (50–1000 nM) were tested on two human breast adenocarcinoma cell lines: MCF-7 expressing estrogen receptor (ER) α and MDA-MB-231 negative for ERα, regarding cell proliferation and viability in addition to their invasive potential. Cell proliferation and viability were not equally affected by these compounds. In MCF-7 cells, the compounds were able to decrease cell proliferation and viability. On the other hand, no evident response was observed in treated MDA-MB-231 cells. Concerning the invasive potential, the less invasive cell line, MCF-7, had its invasion potential significantly induced, while the more invasive cell line MDA-MB-231, had its invasion potential dramatically reduced in the presence of the tested compounds. Altogether, the results showed that these compounds were able to modulate several cancer-related processes, namely in breast cancer cell lines, and underline the relevance of POP exposure to the risk of cancer development and progression, unraveling distinct pathways of action of these compounds on tumor cell biology. Environ Toxicol 30: 168–176, 2015.
Keywords: breast cancer; dichlorodiphenyltrichloroethane; endocrine disruptors; invasion; persistent organic pollutants, organochlorine pesticides

INTRODUCTION

A large variety of synthetic organic chemicals of different chemical classes have been released into the environment over the last few decades, and human exposure to these chemicals is a certainty, even to long banned organochlorine pesticides like dichlorodiphenyltrichloroethane (DDT), and its metabolites dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) (Ozonoff et al., 1994; Li et al., 2006; Porta, 2006). These man-made chemicals are environmentally persistent due to an intrinsic resistance to natural degradation processes and are lipophilic, bioaccumulate in the food chain, and may be found in human adipose tissue, blood, and breast milk (Li et al., 2006; Shakeel et al., 2010). For example, the half-life of DDE in the soil may be more than 20 years (Xu et al., 2010). In this regard, these compounds are termed persistent organic pollutants (POPs).

POPs remain one of the most important groups of pollutants to which humans are exposed to, primarily via dietary intake of dairy products, meat, and fish (Toppari et al., 1996; Li et al., 2006; Lee et al., 2007). It has been demonstrated that the entire population of the world presents detectable residues of more than one POP on their biological tissues (Snedeker, 2001; Zumbado et al., 2005; Valeron et al., 2009). Because of their persistence and toxicity, they are listed in the Stockholm Convention on Persistent Organic Pollutants, an international treaty designed to limit and ultimately eliminate their production, use, storage, and release.

In recent years, attention has been focused on the potential of some chemicals to act as endocrine disruptors, even at very low doses, being proposed for a number of adverse human health effects, including infertility, abnormal prenatal and childhood development, and cancers (Ozonoff et al., 1994; Lai et al., 2001). Evidence from experimental assays suggests that a number of POPs interfere with the function of the endocrine system by mimicking a hormone, blocking the effects of normal endogenous hormones, or by altering or modifying the synthesis, metabolism, or transport of hormones (Soto et al., 1995; Casals-Casas et al., 2008; Hanet et al., 2008; Swedenborg et al., 2009; Casals-Casas and Desvergne, 2011). Specifically, *o,p′*-DDT, the best known and most extensively studied xenoestrogen, acts through the classic estrogen receptor (ER) pathways (Mason and Schulte, 1981; Robison et al., 1985a; Steinmetz et al., 1996).

In 1993, Wolff et al. (1993) first reported the presumed positive association between *p,p′*-DDE—the main metabolite of DDT—and breast cancer. Breast cancer is one of the most commonly diagnosed cancers and the second leading cause of cancer deaths in women worldwide today (Wolff and Weston, 1997; Lacey et al., 2002). Several studies have established that estrogens are predominantly involved in the initiation and proliferation of breast cancer. On the other hand, the progression of a tumor from being *in situ* to invasive is a major prerequisite for cancer metastasis (Stetler-Stevenson et al., 1993; Chaffer and Weinberg, 2011). This well-known association between breast cancer and prolonged exposure to estrogens suggests that environmental estrogens, such as DDT, could play a critical role in the cellular and molecular changes that occur during breast carcinogenesis (Gellert et al., 1972; Aronson et al., 2000; Snedeker, 2001; Calle et al., 2002; Lopez-Cervantes et al., 2004; Valeron et al., 2009). Furthermore, because these chemicals are highly liposoluble and stored in the adipose tissue, there is also a suspected association between the incidence of breast cancer in women and levels of *o,p′*-DDT in breast adipose tissue (Falck et al., 1992). However, epidemiologic evidence has provided provocative but limited and inconsistent results, despite the knowledge that they promote tumorigenesis in rodents, as reported by the World Health Organization (Scribner and Mottet, 1981; Robison et al., 1985b; Lopez-Cervantes et al., 2004). These compounds are known to bind to the ER, induce tumor-cell proliferation and promote mammary tumor formation in rodents (Scribner and Mottet, 1981; Robison et al., 1985b; Korach et al., 1988). Some are considered carcinogenic, while others, like DDT, are tumor promoters (George and Shukla, 2011).

Taking into account the confirmed presence of POPs in human samples and their recognized endocrine disruption ability, this work aimed to evaluate the effect of DDT and its metabolites on several properties related to cancer, in particular breast cancer. For this purpose, *o,p′*-DDT, *p,p′*-DDE, and *p,p′*-DDD were tested on two human breast adenocarcinoma cell lines (MCF-7 and MDA-MB-231) and their effects on proliferation, viability, and invasion potential were evaluated. The major differences between these cell lines are the presence of the ER and their invasive and metastatic potential: MDA-MB-231 is a hormone-independent cell line (ERα(-)) and have higher invasive and metastatic potential than MCF-7, a hormone-dependent cell line (ERα(+)) (Brooks et al., 1973; Cailleau et al., 1978; Aube et al., 2011).

MATERIALS AND METHODS

Cells and Culture Conditions

MCF-7 cells were grown in Minimum Essential Medium (Sigma, St. Louis, MO), supplemented with 10% fetal bovine serum (FBS), 100 units mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, and 0.25 μg mL^{-1} amphotericin B (all from Sigma). MDA-MB 231 cells were grown in RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 2 mM glutamine, 15% fetal bovine serum, 100 units mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, and 0.25 μg mL^{-1} amphotericin B (all from Sigma). Both the cell lines were maintained in a humidified atmosphere of 5% CO_2–95% air. Culture medium was changed every 2–3 days, and the culture was split every 7 days, corresponding to 90–95% of confluence with the used cell culture conditions. For

subculturing, the cells were removed enzymatically (0.25% trypsin-EDTA, 5 min, 37°C), split 1:4, and subcultured in plastic culture dishes (21 cm^2; ø 60 mm; Orange Scientific, Belgium).

Cell Proliferation Determined by Methyl-[^{3}H]-thymidine Incorporation into DNA

MCF-7 and MDA-MB-231 cells were seeded into 24-well (1.65 cm^2; ø 14.5 mm; Orange Scientific, Belgium) plastic cell culture clusters in a final volume of 0.5 mL culture medium containing 10% and 15% FBS respectively. After 24 h in culture, cells were incubated with different concentrations (50–1000 nM) of *o,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD (Sigma-Aldrich, Madrid, Spain) in culture medium. A control treatment was made with 0.1% of dimethyl sulfoxide (DMSO), the vehicle of the tested compounds. After 24 h, the treatment medium was removed and the cells were incubated with 0.2 mL of methyl-[^{3}H]-thymidine (0.5 μCi/well; Amersham; Arlington Heights, IL) for 4 h. The medium was removed and the cells were fixed by incubation in 10% trichloroacetic acid (TCA, Merck, Darmstadt, Germany) for 1 h at 4°C. The cells were then washed twice with 10% TCA to remove unbound radioactivity. The plates were air-dried and the cells were lysed with 1 M NaOH (0.28 mL/well). A 0.25-mL aliquot of the lysate was neutralized with HCl and mixed with scintillation fluid. The radioactivity of the samples was quantified by a liquid scintillation counter. The counts (counts per min, cpm) of each treatment were averaged and expressed as percentage of controls (0.1% DMSO) and proliferation was evaluated using a method of relative quantification (adapted from Miranda et al., 1999).

Cellular Viability Determined by the LDH Assay

For the viability experiments, MCF-7 and MDA-MB-231 cells were seeded into 24-well (1.65 cm^2; ø 14.5 mm; Orange Scientific, Belgium) plastic cell culture clusters in a final volume of 0.5 mL culture medium containing 10% and 15% FBS, respectively. Cells were treated for 24 h, following the same treatment protocol as in proliferation evaluation, and cell viability was assessed by the lactate dehydrogenase (LDH) assay as described in the literature (Bergmeyer and Bernt, 1974). Briefly, culture medium was removed and cells were preincubated with the compounds in culture medium, at 37°C for 24 h. After treatment, cellular leakage of the cytosolic enzyme LDH into the extracellular (culture) medium was measured spectrophotometrically, by quantification of the decrease in absorbance of NADH during the reduction in pyruvate to lactate, and normalized to the intracellular LDH activity, measured after recovery of adherent cells by lysis with 0.1% Triton-X-100 in 5 mM Tris-HCl. Results were expressed in percentage of control.

Effect on Cell Invasion Potential

The effect of the compounds (*o,p′*-DDT and *p,p′*-DDD—50, 100, and 1000 nM; *p,p′*-DDE—50, 100, and 250 nM) on cell invasive potential was tested using Millipore QCM 24-well assay kit (Merck Millipore, Madrid, Spain), according to the manufacturer's instructions. Cells (7×10^5 cells/mL) were transferred to rehydrated kit inserts and treated with the compounds. Control inserts (without compounds) were also used. After incubation for 24 h at 37°C, invading cells were detached, lysed, and subsequently detected by CyQuant GR® dye. Detection was carried out at 480/520 nm using a Perkin-Elmer LS 45 fluorometer.

Statistical Analysis

Values are expressed as the arithmetic mean ± standard error of the mean (SEM) of control treatment (0.1% DMSO) percentage. All the assays were performed in triplicate at least three different times. The statistical significance of the differences between groups was determined via one-way analysis of variance (ANOVA), followed by Bonferroni's multiple comparison test. The differences were considered statistically significant when $P < 0.05$.

RESULTS

With the purpose of evaluating their effect on breast cancer biology, *o,p′*-DDT and its metabolites *p,p′*-DDE and *p,p′*-DDD were tested on two human breast adenocarcinoma cell lines: MCF-7 and MDA-MB-231. The treatments were performed using a range of concentration between 50 nM and 1 μM, as these compounds are known to be toxic and with endocrine-disrupting effects, even at very low doses. The structure of these compounds is illustrated in Figure 1.

Proliferation

The results obtained in the methyl-[^{3}H]-thymidine incorporation assay demonstrated that cell proliferation was not equally affected by the three compounds in the two cell lines (Fig. 2). In MCF-7, a significant decrease in cell proliferation was observed in the presence of *o,p′*-DDT (100–500 nM), when comparing with the control cells (35 193 ± 2899 cpm). In the presence of DDT metabolites, *p,p′*-DDD and *p,p′*-DDE, proliferation was not significantly affected by any of the tested concentrations (50–1000 nM), except for a significant reduction in 50 nM *p,p′*-DDD-treated MCF-7 cells. Additionally, the concentration response pattern differed between compounds, namely, when comparing the *o,p′*-DDT "U" shaped response with the concentration-dependent increase tendency observed in the *p,p′*-DDD treatment, where the smallest concentration had a significant reduction in proliferation. As for *p,p′*-DDE, no effects were observed.

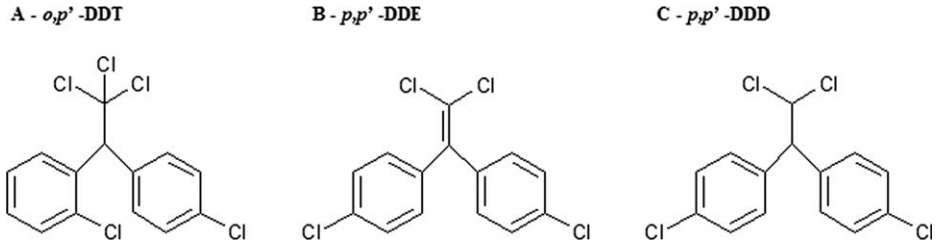

Fig. 1. Molecular structures of the tested organochlorine pesticides in MCF-7 and MDA-MB-231 cells: (A) *o, p′*-DDT, (B) *p, p′*-DDE, and (C) *p, p′*-DDD.

On the other hand, MDA-MB-231 response to the treatments was quite different and less marked than with MCF-7. Comparing with the control cells (63 122 ± 2330 cpm), proliferation was not significantly modified by the treatments (50–1000 nM), except for a significant reduction in proliferation with 250 nM of *p,p′*-DDD. Furthermore, no visible concentration response pattern was observed.

Viability

The effect on cell viability was evaluated through the LDH assay after 24 h of cell incubation with the compounds. As in cell proliferation, viability was not equally affected by these compounds (Fig. 3), either comparing between metabolites or cell types.

Regarding MCF-7 cells, the "U"-shaped concentration-response pattern observed in *o,p′*-DDT was maintained, comparing with the control viability (6.04 ± 0.86 Int/Ext LDH ratio). Thus, 100 and 250 nM, but not 500 nM of *o,p′*-DDT showed cytotoxicity. Regarding *p,p′*-DDD, the effect was less pronounced than with DDT, but the 50 and 500 nM of the compound also negatively affected cell viability, and this was not accompanied by a concentration-dependent increase tendency observed in the proliferation assay. Additionally, unlike upon proliferation, the two higher concentrations of *p,p′*-DDE (500 nM and 1 μM) negatively affected cell viability, imposing a reduction in approximately 50%. Once more, MDA-MB-231 response to the treatments was quite different as no effect was observed on cell viability.

Invasion Potential

Matrigel® inserts were used to evaluate invasion potential in the presence of the tested compounds. The choice of concentrations to be tested on either cell types was based upon the absence of a cytotoxic effect and the requirement to test the wider range of possible concentrations (Fig. 4). Thus, 50, 100, and 1000 nM were the tested concentrations for *o,p′*-DDT and *p,p′*-DDD, whereas for *p,p′*-DDE the highest concentration used was 250 nM.

The less invasive and metastatic cell line MCF-7 had its invasion potential significantly induced by almost all tested concentrations, in most cases increasing the potential in the order of 150% in relation to control cell potential (4.50 ± 1.06 Abs 480/520 nm). No significant increase effect was observed when 100 nM of *o,p′*-DDT and 50 nM of *p,p′*-DDD were tested. As for *p,p′*-DDE, it significantly increased the invasion potential in the smaller tested concentrations (50 and 100 nM), despite appearing to have less effects in the previous evaluated parameters. Curiously, the constitutively more invasive and, at the same time, hormone-independent cell line MDA-MB-231 had its invasion potential dramatically reduced to approximately 50% after most of the treatments, when compared with control (36.00 ± 4.04 Abs 480/520 nm). Only the higher concentration of *p,p′*-DDE (250 nM) had no effect, as also observed in the MCF-7 cell line.

DISCUSSION

Molecular data showed that compounds like POPs, a group of environmental pollutants with endocrine disrupting properties, may act as tumor promoters through hormone-mediated effects and can be associated with an increased risk for hormone-related cancers including breast and prostate cancers. Several POPs and some of their metabolites have been associated with estrogen-like effects in humans, even at very low doses (Gellert et al., 1972; Aronson et al., 2000; Snedeker, 2001; Calle et al., 2002; Lopez-Cervantes et al., 2004; Li et al., 2006; Valeron et al., 2009). The discovery that low concentrations of these compounds, detected in different human samples, may act on multiple biological mechanisms is leading to a reassessment of POPs' impact on human beings. However, human epidemiological studies on this subject have not been consistent nor conclusive (Snedeker, 2001; Lopez-Cervantes et al., 2004; Xu et al., 2010).

Taking into account (i) the confirmed presence of POPs in human samples in several studies, including in our recent project with Portuguese obese patients (Pestana et al., 2012), (ii) their possible distribution in the organism, (iii) and their previously described endocrine disruption ability, an evaluation of the effects on tumor cell biology, is crucial. In this regard, DDT and its metabolites (*p,p′*-DDE and *p,p′*-DDD) were chosen to be the focus of our investigation. Indeed, although DDT was banned in most of the world in the 1970s

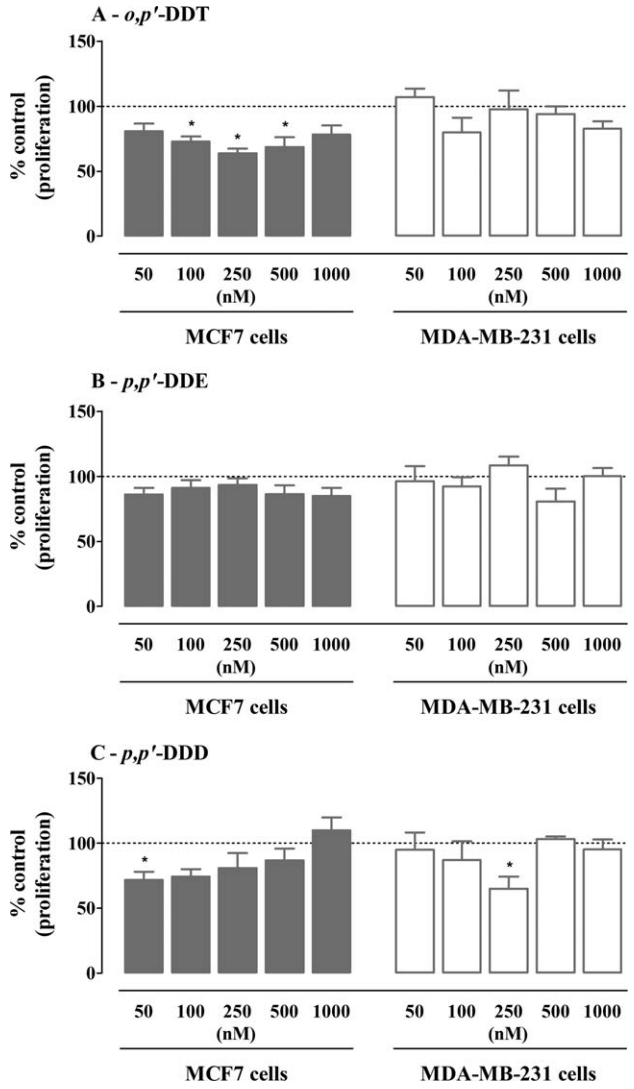

Fig. 2. Effects of (A) *o, p′*-DDT, (B) *p, p′*-DDE, and (C) *p, p′*-DDD on cell proliferation of two breast cancer cell lines: MCF-7 (estrogen receptor α positive) and MDA-MB-231 (estrogen receptor α negative). Cells were treated with the compounds, or vehicle, for 24 h. Cellular growth was measured by methyl-[^{3}H]-thymidine incorporation into DNA. Values are represented as mean of the percentage of control group ± SEM ($n \geq 6$). The absolute control values (cpm) were 35193 ± 2899 and 63122 ± 2330 for MCF-7 and MDA-MB-231 cells, respectively. Statistical analysis with one-way ANOVA, followed by Bonferroni's multiple comparison test: *significantly different from control (vehicle), $P < 0.05$.

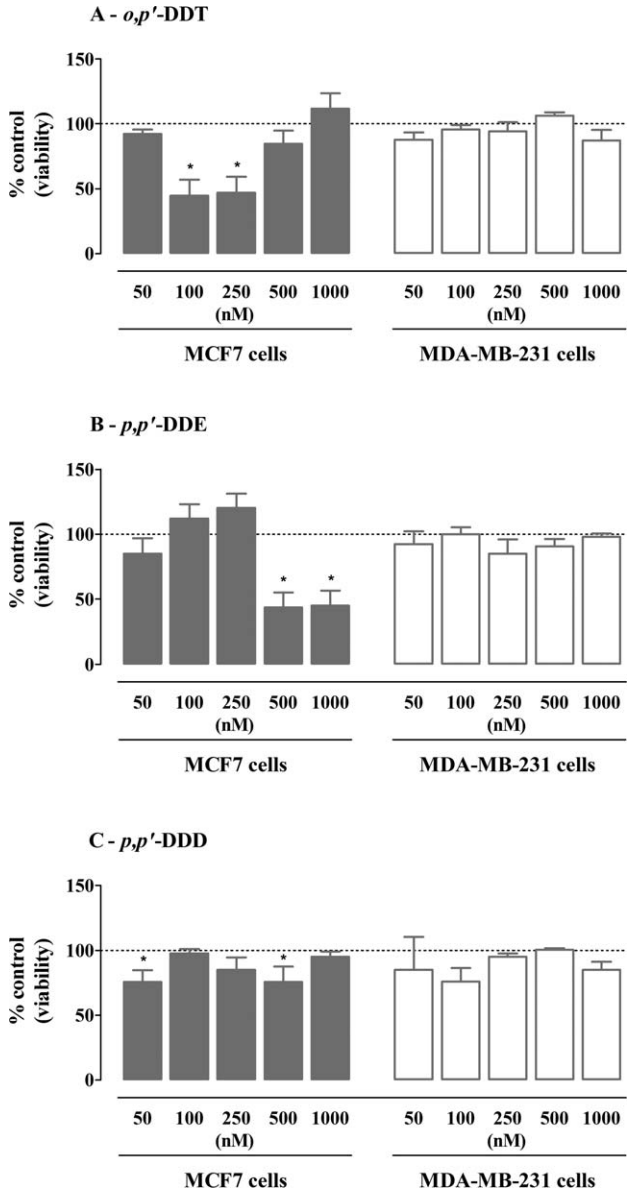

Fig. 3. Effects of (A) *o, p′*-DDT, (B) *p, p′*-DDE, and (C) *p, p′*-DDD on cell viability of two breast cancer cell lines: MCF-7 (estrogen receptor α positive) and MDA-MB-231 (estrogen receptor α negative). Cells were treated with the compounds, or vehicle, for 24 h and cellular viability was evaluated by the lactate dehydrogenase (LDH) method. Values are represented as mean of the percentage of control group ± SEM ($n \geq 6$). The absolute control values (Int/Ext LDH ratio) were 6.04 ± 0.86 and 6.43 ± 0.30 for MCF-7 and MDA-MB-231 cells, respectively. Statistical analysis with one-way ANOVA, followed by Bonferroni's multiple comparison test: *significantly different from control (vehicle), $P < 0.05$.

and 1980s, either the parent compound or the metabolites continue to be detected in a considerable amount of human samples. In our human study, results showed a relevant presence of DDT and its metabolites (Pestana et al., 2012). Furthermore, the compounds have been attributed carcinogenic effects, such as in breast cancer (Dich et al., 1997; Snedeker, 2001; Ociepa-Zawal et al., 2010; Shakeel et al., 2010).

Compounds were tested in two human breast adenocarcinoma cell lines: MDA-MB-231 and MCF-7. The major differences between these cell lines are the presence of the ERs and their invasive and metastatic potential (Aube et al.,

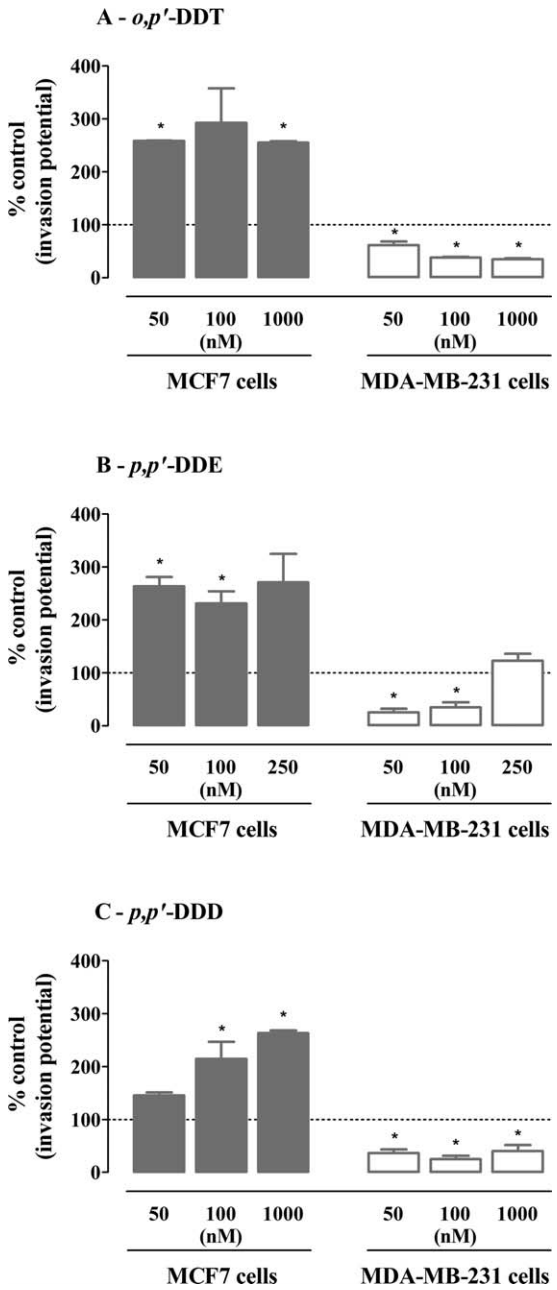

Fig. 4. Effects of (A) *o, p′*-DDT, (B) *p, p′*-DDE, and (C) *p, p′*-DDD on cell invasion potential of two breast cancer cell lines: MCF-7 (estrogen receptor α positive) and MDA-MB-231 (estrogen receptor α negative). Cellular invasion capacity was evaluated in cells treated with the compounds, or vehicle, for 24 h. Values are represented as mean of the percentage of control group ± SEM from two independent experiments. The absolute control values (Abs 480/520 nm) were 4.50 ± 1.06 and 36.00 ± 4.04 for MCF-7 and MDA-MB-231 cells, respectively. Statistical analysis with one-way ANOVA, followed by Bonferroni's multiple comparison test: *significantly different from control (vehicle), $P < 0.05$.

2011). MCF-7 is associated with a hormone-dependent stage of breast cancer and is a highly differentiated human breast adenocarcinoma cell line, strongly responsive to estradiol, insulin, and insulin-like growth factors (Brooks et al., 1973). In its turn, MDA-MB-231 is a hormone-independent cell line (ERα(−)), more invasive and metastatic than MCF-7 and is often used as a model of late-stage, hormone-independent tumors (Cailleau et al., 1978). As stated above, the use of these two cell lines gains relevance through the knowledge that estrogens are involved in carcinogenesis in the breast, predominantly in its initiation and progression (Stetler-Stevenson et al., 1993; Chaffer and Weinberg, 2011) and that DDT, DDD, and DDE possess the ability to interfere with the signalling of these hormones (Mason and Schulte, 1981; Robison et al., 1985a; Soto et al., 1995; Hanet et al., 2008).

Proliferation, viability, and invasive potential were assessed in the presence (50–1000 nM) or absence of the tested compounds. These low concentrations, detected in different human samples, may act on molecular mechanisms involved in carcinogenesis (Wolff et al., 1993; Demers et al., 2000; Cruz et al., 2003; Lopez-Cervantes et al., 2004; Zumbado et al., 2005; Silva et al., 2010; Xu et al., 2010; Pestana et al., 2012). Proliferation was evaluated by methyl-[^{3}H]-thymidine incorporation into DNA and viability through the LDH assay. Invasion potential was assessed using a commercial fluorometric cell invasion assay. Cell proliferation and viability were not equally affected by these compounds, an expected distinct response, as both the cells have differences in estrogen sensitivity and the tested compounds have distinct potencies and mechanisms of action.

Regarding MCF-7, the hormone-dependent cell model, the concentration response pattern upon cell proliferation differed between compounds, namely when comparing the inhibitory effect of middle *o,p′*-DDT concentrations ("U"-shaped response: 100–500 nM) with the marked inhibition of the lower *p,p′*-DDD concentration (50 nM) along with a concentration-dependent tendency to increase in higher concentrations. In the presence of the major and most persistent DDT metabolite, *p,p′*-DDE, proliferation was not significantly affected by none of the tested concentrations (50–1000 nM). These results seem contradictory with available data, where proliferation of MCF-7 cells appear increased in the presence of DDE (Aube et al., 2008, 2011; Bratton et al., 2012), but the main difference between those and our own results is the much lower concentration used in our study. Additionally, the concentration-response patterns were essentially maintained in the viability assay although with a more pronounced cytotoxicity found with *o,p′*-DDT (100 and 250 nM). This allows us to propose that the observed reduction in proliferation for some of the concentrations may be due to this reduction in viability. Furthermore, as opposed to proliferation, the two higher *p,p′*-DDE concentrations (500 and 1000 nM) also negatively affected cell viability, with a reduction in approximately 50%.

In contrast, MDA-MB-231 response to the treatments was quite different and less marked than in MCF-7. Neither proliferation nor viability was significantly modified by the treatments (50–1000 nM). This difference of effect is in accordance with their particular cell biology properties (Aube et al., 2011). In this regard, the differences between compounds could be related to their difference in estrogenic potencies, as *o,p′*-DDT has consistently shown a positive estrogenic response, whereas *p,p′*-DDE showed little or no response (Welch et al., 1969; Gellert et al., 1972 Chen et al., 1997). However, *p,p′*-DDE possesses a well-recognized anti-androgenic activity, inhibiting androgen binding to the androgen receptor, androgen-induced transcriptional activity, and androgen action, ultimately increasing estrogens levels (Kelce et al., 1995; Kelce and Wilson, 1997).

Cell invasion, another important property of aggressive cancer cells and metastasis, requires a change in cell state. This process includes a loss of cell adhesion and acquisition of an epithelial-to-mesenchymal transition (EMT) phenotype as a critical process for switching early stage carcinomas into invasive malignancies, which is often associated with the loss of epithelial differentiation and gain of mesenchymal phenotype, where transcription factors like Twist and Snail are known to play a part (Chaffer and Weinberg, 2011; Kong et al., 2011). The concentrations to be tested were selected in the attempt to evaluate the widest range of non-cytotoxic concentrations used in the previous experiments to, as much as possible, only evaluate cell migration. Thus, we tested the concentrations 50, 100, and 1000 nM for *o,p′*-DDT and *p,p′*-DDD, whereas for *p,p′*-DDE the highest used concentration was 250 nM.

As expected, the response to the treatments was different between cell types, but the outcome was quite curious. The less invasive and metastatic cell line MCF-7 had its invasion potential significantly increased, whereas the more invasive and hormone-independent cell line MDA-MB-231 had its invasion potential dramatically reduced after most of the treatments. It is worth noting that although incubation with the compounds reduced the invasive potential of the MDA-MB-231 cell line, absolute invasion scores were still higher than those observed for MCF-7 cells. These apparently contradicting results could also explain, at least in part, the paradox of the epidemiological data.

The pattern in MCF-7 response to compounds in the invasion assays may be considered in accordance with those seen in previous experiments, as the concentrations without significant increase in invasion in *o,p′*-DDT and *p,p′*-DDD reduced proliferation and viability. As for *p,p′*-DDE, in both cell types invasion was modified only in the presence of 50 or 100 nM of the compound, while the highest concentration (250 nM) had no effect on invasion, revealing an important effect of very low concentrations of this pesticide.

As these compounds are highly liposoluble chemicals and therefore stored in the adipose tissue, there is also a suspected association between the incidence of breast cancer in women and their levels in breast adipose tissue (Falck et al., 1992). In this sense, the increase in MCF-7 invasion potential induced by the pesticides is of extremely relevance, possibly promoting a more aggressive phenotype in a formerly less invasive cell line.

As the MDA-MB-231 cells represent a more invasive and metastatic model of late-stage carcinoma (Cailleau et al., 1978), one can conceive their interaction with these compounds in a more systemic context, and modulating their ability to metastasize. Considering that the tested concentrations did not have effects on proliferation or viability, one can speculate that the marked decrease in invasion can represent a change of cell differentiation stage and/or contribute to the establishment of a new metastasis, a challenging, and still poorly understood step when cells are in the mesenchymal phenotype (Chaffer and Weinberg, 2011; Kong et al., 2011). If on the one hand we can hypothesize that the observed MCF-7 effects could be related with the DDT and the estrogenic properties of its metabolites, the invasion potential impairment of MDA-MB-231 cells can be related with other mechanisms, for example epigenetic regulation of phenotype and EMT. Indeed, it has been advanced that epigenetic modulatory properties of the tested compounds can interfere with this tightly epigenetic regulated process (Rakitsky et al., 2000; Chaffer and Weinberg, 2011; Kong et al., 2011).

Altogether, our results showed that these compounds were able to modulate several properties related to carcinogenesis, in particular in the breast. The cell type-specific responses to nanomolar range concentrations of DDT and its metabolites that mimic those present in biological tissues are very promising, mainly because of the particular characteristics of each cell type.

While MCF-7 had all the tested properties modulated, namely, its invasion potential significantly increased, the more invasive and hormone-independent cell line MDA-MB-231 had its invasion potential dramatically reduced. Given their particular characteristics, these results seem to indicate distinct ways of action of these compounds in tumor cell biology modulation and raise different challenges. Considering their known effects, namely their estrogenic activity, further research is needed to clarify the signalling pathways involved in the effects demonstrated, to understand the specific risks of exposure to these POPs and adopt therapeutic and/or public health measures to prevent the potential health hazards that may arise from their presence human tissues.

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