Oestrogens induce G1 arrest in benzo[a]pyrene-treated MCF-7 breast cells whilst enhancing genotoxicity and clonogenic survival

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Carcinogens, such as benzo[a]pyrene (B[a]P), allow cells to evade G1 arrest (the stealth property), thus increasing the chance that DNA damage will ultimately result in transformation. In this study we have investigated the effects of B[a]P in MCF-7 cells incubated in the presence or absence of oestrogens (β-oestradiol, oestrone or oestriol). The cytokinesis block micronucleus assay was used to examine cells for chromosomal damage. Micronuclei were scored in 500 binucleate cells per treatment. Increased micronucleus formation (3-fold) occurred following 24 h treatment with 10-6 M B[a]P alone. Following co-treatment with either 10-8 M β-oestradiol, 10-8 M oestrone or 10-8 M oestriol, 2- to 3-fold increases in micronuclei were observed with 10-8 M B[a]P. When MCF-7 cells were pre-incubated for 96 h with 10-8 M β-oestradiol, 10-8 M oestrone or 10-8 M oestriol prior to the addition of B[a]P for 24 h, up to a 5-fold enhanced sensitivity to micronucleus formation was observed with β-oestradiol and oestrone, while oestriol appeared to reduce levels of micronucleus formation. B[a]P-induced decreases in cell proliferation (per cent binucleate cells) and plating efficiency were reversed by all three oestrogens. Analysis of cell cycle distributions revealed that treatment with oestrogens or B[a]P alone did not induce marked effects on cell cycle distributions. However, in combination oestrogen and B[a]P induced increases in G0/G1, decreases in S phase and increases in G2/M. This work suggests that whilst oestrogens appear to enhance carcinogen-induced DNA damage, they also appear, paradoxically, to trigger mechanisms that facilitate clonogenic survival, which may be relevant to breast cancer initiation.

Introduction

Epidemiological studies of migrant populations suggest that dietary and/or environmental exposures play a significant role in cancer incidence (Ziegler et al., 1993; Peto, 2001). Whilst breast cancer is the most common malignancy occurring in women (Higginson et al., 1992), identification of specific factors responsible for its aetiology remain obscure (Martin, 2001; Martin et al., 2001; Grover and Martin, 2002). Candidate agents that are proven rodent mammary carcinogens (El-Bayoumy, 1992) have been identified, but typical daily human exposures (Phillips, 1999) have raised arguments over their relevance to health (Ames and Gold, 2000). The only environmental exposure proven to cause breast cancer in humans is ionizing radiation (Tokunaga et al., 1987). Although increased cumulative exposures to oestrogens have been identified as risk factors for breast cancer (Feigelson and Henderson, 1996), whether oestrogens act as complete carcinogens is controversial (Joseph, 1997).

Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic products of incomplete combustion and benzo[a]pyrene (B[a]P) is a ubiquitous example. B[a]P is metabolically activated to the 7,8-diol 9,10-oxides (Sims et al., 1974) which react predominantly with the N2 position of guanine and to a lesser extent with the N6 position of adenine (Cheng et al., 1989; Rubin, 2001) to form covalent adducts.

Endogenous oestrogens (β-oestradiol, oestrone and oestriol) are essential for the maintenance of cell growth and a multitude of other normal functions (Adashi, 1992). Their production, mainly from cholesterol, is controlled primarily by pituitary gonadotropins (Lingeman, 1979). Although oestrogens, including β-oestradiol and oestrone, are multi-organ carcinogens in different animal models (Cavalieri et al., 2000), they have hitherto not tested positive in many classical bacterial and mammalian cell gene mutation assays (Roy and Liehr, 1999; Cavalieri et al., 2000). Epigenetic mechanisms, including stimulation of cell proliferation, spontaneous induction of replication errors or disruption of spindle formation and subsequent induction of aneuploidy, have been postulated to account for oestrogen carcinogenicity (Cavalieri et al., 2000; Fischer et al., 2001). There is now accumulating evidence suggesting that oestrogens may, in fact, be direct genotoxins (Yared et al., 2002).

Cell-cycle checkpoint control operates to preserve the integrity of the genome and to facilitate survival. If this cellular defence mechanism fails to arrest the progress of DNA-damaged cells through the cell cycle, then this may result in an accumulation of mutations and chromosomal aberrations in subsequent generations. It is during the G0/G1 phase that cells commit to DNA replication and to completing the cell cycle (Bartek and Lukas, 2001). The up-regulation of transcription factors such as p53, CDKN1A (also known as p21Waf1/Cip1) and pKB induces G1 arrest in DNA-damaged cells, thus delaying progression through the cell cycle (Khan and Dipple, 2000; Bartek and Lukas, 2001). This pause in the cell cycle may allow DNA repair mechanisms to correct the genome or may allow apoptosis to occur; both mechanisms are protective.

It has been shown that, following treatment with one of a range of carcinogens, including metabolites of B[a]P, a G1 arrest does not occur in MCF-7 cells or in other cell lines (Khan and Dipple, 2000; Khan and Anderson, 2001). The ability of carcinogens to induce cells to pass through the cell cycle unchecked is known as ‘the stealth property’ (Khan and Dipple, 2000). This effect of bypassing a G1 arrest occurs despite the observations that B[a]P or dibenzo[a,l]pyrene metabolites induce dose-related increases in p53 and p21Waf1/Cip1 in MCF-7 cells (Kaspian and Baird, 1996; Luch et al., 1999). Other studies have indicated that B[a]P induces...
Table I. Genotype obtained following short tandem repeat (STR) profiling of the cell line used in this study

<table>
<thead>
<tr>
<th>Locus</th>
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<td>HUMYVF3A31I/A</td>
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<td>HUMFIBRA (FGA)</td>
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</table>

Authentication of the cell line employed in this study. STR profiling was carried out using the SGMPlus Multiplex System at the Laboratory of the Government Chemist (University Diagnostics Ltd, UK). Polymorphic STR loci were amplified to give the above numerical code (Masters et al., 2001). The cell line was verified as the MCF-7 cell line.

a transient increase in human lung fibroblasts in S phase (Brinková et al., 2000).

The effects of the oestrogens, β-oestradiol, oestrone or oestriol, on the genotoxicity of B[a]P in the MCF-7 cell line, originally derived from an oestrogen receptor-positive breast carcinoma, have now been investigated. Levels of genotoxicity were measured using the cytokinesis block micronucleus assay. The effects of these combinations of treatment on mitotic rate, colony-forming ability and cell cycle distribution were also assessed.

Materials and methods

Chemicals

All chemicals, including test chemicals, were obtained from Sigma Chemical Co. (Poole, UK) unless otherwise stated. Cell culture consumables were obtained from Life Technologies (UK) unless otherwise stated.

Cell culture

The human mammary carcinoma cell line (MCF-7) (a gift from the Institute of Cancer Research, UK) was grown in Dulbecco’s modified essential medium supplemented with 10% heat-inactivated foetal calf serum, 100 µg/ml penicillin and 100 µg/ml streptomycin. MCF-7 cells were cultured routinely in 75 cm² flasks at 5% CO₂ in air and 37°C in a humidified atmosphere and sub-cultured (1:10 v/v) twice weekly.

Prior to sub-culture or incorporation into experiments, cultured cells were disaggregated, using a 0.05% trypsin, 0.02% EDTA solution, to form a single cell suspension. Additions, in dimethylsulphoxide (DMSO), were made to a maximum concentration of 1% (v/v).

Authentication of the cell line was performed using short tandem repeat (STR) profiling of a sample randomly taken during the study and sent to the Laboratory of the Government Chemist (University Diagnostics Ltd, UK) for analysis. On DNA extracted from a minimum of 10⁶ cells STR profiling using the SGMPlus Multiplex System verified that the genotype of the cell line used in this study matched the MCF-7 cell line (Table I).

The cytokinesis block micronucleus assay

MCF-7 cells were treated with or without B[a]P in the presence or absence of oestrogens (10⁻⁹ M β-oestradiol, 10⁻⁸ M oestrone or 10⁻⁸ M oestriol). Concentrations were chosen on the basis of previous work which showed that oestrogens at these concentrations induced minimal effects on mitotic rate and colony-forming ability (Yared et al., 2002).

Two different protocols were adopted in order to investigate the effects of these combinations of treatment on micronucleus formation in MCF-7 cells. For 96 h pre-incubation experiments, MCF-7 cells were seeded into 25 cm² flasks in the presence or absence of different oestrogens, as indicated, and incubated for 72 h. Following disaggregation of these cultures with trypsin/EDTA and resuspension in complete medium, 3 ml aliquots (∼1×10⁶ cells) were seeded into 30 mm Petri dishes containing 20 mm coverslips (Sarstedt, UK), again in the presence of oestrogens, as indicated. The cells were allowed to attach for 24 h prior to the addition of graded concentrations of B[a]P in DMSO. After another 24 h the medium was replaced with fresh medium without oestrogen or B[a]P but containing 2 µg/ml cytochalasin B. Cells were cultured for a further 24 h prior to removal of medium, washing of the coverslips in phosphate-buffered saline (PBS) and fixation with 70% ethanol. Cells were then stained with 5% Giemsa (in 10% DMSO) before mounting on microscope slides.

For co-incubation experiments, routinely cultured MCF-7 cells were disaggregated with trypsin/EDTA, resuspended in complete medium prior to seeding 3 ml aliquots (∼1×10⁵ cells) into 30 mm Petri dishes containing 20 mm coverslips. The cells were then allowed to attach for 24 h prior to addition of oestrogens in DMSO, as indicated, with or without graded concentrations of B[a]P. Following 24 h incubation the medium was replaced with fresh medium without oestrogen or B[a]P but containing 2 µg/ml cytochalasin B. The above method was then adopted.

For each treatment condition, micronuclei (MN) in 500 binucleate MCF-7 cells from a minimum of three experiments were scored either as micronucleated binucleate cells, total number of MN or the distribution of MN in binucleate cells. Mitotic rate was assessed as per cent binucleate cells (mean ± SD, n = 3).

The clonogenic assay

MCF-7 cells, with or without 96 h pre-incubation with oestrogens (10⁻⁹ M β-oestradiol, 10⁻⁸ M oestrone or 10⁻⁸ M oestriol), were disaggregated with trypsin/EDTA and resuspended in complete medium. Aliquots (5 ml) containing 1×10⁶ cells were seeded into 75 cm² flasks with or without graded concentrations of B[a]P in the presence or absence of oestrogens, as indicated. The cells were incubated in 5% CO₂ in air at 37°C in a humidified atmosphere for 24 h. The medium was then replaced with fresh medium. Cells were cultured undisturbed for a further 7 days prior to removal of medium, washing with PBS and fixation with 70% ethanol. Colonies were then stained with 5% Giemsa and counted, after which plating efficiencies were calculated.

Flow cytometry

Following disaggregation of routinely cultured MCF-7 cells with trypsin/EDTA and resuspension in complete medium, 10 ml aliquots containing 1×10⁶ cells were seeded into 75 cm² flasks, with or without graded concentrations of B[a]P, in the presence or absence of oestrogens, as indicated. The cells were incubated in 5% CO₂ in air at 37°C in a humidified atmosphere for 24 h. Following disaggregation with trypsin/EDTA, the cell aliquots were washed twice with PBS prior to fixation with ice-cold 70% ethanol and stored overnight at −20°C. Cell aliquots were again washed twice with PBS prior to incubation with RNase A (10 µg/ml) and propidium iodide (50 µg/ml) for 60 min at 37°C. DNA content of 10 000 events/treatment was analysed using a Becton Dickinson FACSCaliber flow cytometer and the CELLQuest software version provided by the manufacturer. Cell cycle analysis was done in ModFitLT for Mac v.2.0.

Results

Marked increases in induced MN formation in MCF-7 cells were consistently observed with 10⁻⁹ M B[a]P. The dose-related effects of B[a]P in the cytokinesis block micronucleus assay following 24 h co-incubation or 96 h pre-incubation with 10⁻⁶ M β-oestradiol, 10⁻⁸ M oestrone or 10⁻⁸ M oestriol are shown in Figures 1 and 2, respectively. Differences in oestrogen-dependent effects on the MN-forming activity of B[a]P were observed.

Following co-incubation with 10⁻⁹ M (1 nM) β-oestradiol, 10⁻⁸ M oestrone or 10⁻⁸ M oestriol, 2- to 3-fold increases in micronucleated cells were observed following treatment with 10⁻⁹ or 10⁻⁷ M B[a]P as compared to B[a]P alone (Figure 1). At these concentrations B[a]P-induced MN formation in the presence of all three oestrogens was characterized by dose-related increases in multiple micronuclei and up to 4-fold increases in total micronuclei were observed. However, the MN-forming activity of higher concentrations of B[a]P (10⁻⁶ or 10⁻⁵ M) was less profound following co-incubation in the presence of β-oestradiol or oestriol: in the presence of oestrone, reductions in MN formation were observed.

In order to determine whether susceptibility to DNA damage could be increased by oestrogens, MCF-7 cells pre-incubated with oestrogens for 96 h were treated with B[a]P. Following
pre-incubation with $10^{-9}$ M β-oestradiol or $10^{-8}$ M oestrone, 2- to 3-fold increases in micronucleated binucleated MCF-7 cells were again observed in the presence of $10^{-8}$ or $10^{-7}$ M B[a]P as compared to B[a]P alone (Figure 2). B[a]P-induced MN formation in the presence of β-oestradiol or oestrone was also characterized by dose-related increases in multiple micronuclei and, consequently, total micronuclei (~4-fold). However, the MN-forming activity of higher concentrations of B[a]P ($10^{-5}$ M) is markedly reduced after pre-incubation in the presence of β-oestradiol or oestrone. Following pre-incubation with $10^{-8}$ M oestriol, a reduction in the MN-forming activity at all concentrations of B[a]P tested was observed (Figure 2).

Figure 3 shows the effects of co-incubation or 96 h pre-incubation with $10^{-9}$ M β-oestradiol, $10^{-8}$ M oestrone or $10^{-8}$ M oestriol and treatment with graded concentrations of B[a]P on per cent binucleate MCF-7 cells following cytokinesis block. After co-incubation all three oestrogens significantly reversed the B[a]P-induced dose-related decreases in per cent binucleate cells induced by concentrations of up to $10^{-6}$ M B[a]P were again significantly reversed and were especially marked in the presence of oestriol. However, decreases in per cent binucleate cells induced by treatment with $10^{-5}$ M B[a]P were not reversed by these oestrogen-dependent effects. Pre-incubation with $10^{-8}$ M oestriol significantly enhanced the B[a]P-induced effects on per cent binucleate cells (Figure 3).

Whereas co-incubation with $10^{-9}$ M β-oestradiol, $10^{-8}$ M oestrone or $10^{-8}$ M oestriol enhanced $10^{-8}$ and $10^{-7}$ M B[a]P-induced decreases in plating efficiency, they reversed the marked reductions induced by treatment with $10^{-6}$ M B[a]P (Figure 4). These effects in $10^{-6}$ M B[a]P-treated cells were significant following co-incubation with $10^{-9}$ M β-oestradiol or $10^{-8}$ M oestriol. Approximately 2-fold increases in per cent plating efficiency were observed as compared with levels in cells treated with $10^{-6}$ M B[a]P alone. However, following pre-incubation for 96 h, all three oestrogens significantly reversed the dose-related decreases in plating efficiency induced by B[a]P, except oestriol in $10^{-8}$ M B[a]P-treated cells (Figure 4). Approximately 3-fold increases in per cent plating efficiency in MCF-7 cells were observed following oestrogen pre-incubation for 96 h and subsequent treatment
with $10^{-6}$ M B[a]P (Figure 4). Higher concentrations ($10^{-5}$ M) of B[a]P appear to be cytotoxic, as negligible colony-forming ability is observed in the presence or absence of co- or pre-incubation with oestrogens.

Table II and Figure 5 show the effects of co-incubation with oestrogens on the cell cycle distribution of MCF-7 cells following treatment with $10^{-8}$, $10^{-7}$ or $10^{-6}$ M B[a]P. In the absence of oestrogens B[a]P did not appear to induce any alterations in cell cycle distribution following 24 h treatment. Also, oestrogens alone, at the concentrations employed, did not appear to induce marked alterations in cell cycle distribution following 24 h treatment (Table II). At higher concentrations, oestradiol, oestrone and oestriol all induced reproducible increases in per cent cells in S phase in a dose-related fashion (data not shown). However, in the presence of $10^{-9}$ M oestradiol, $10^{-8}$ M oestrone or $10^{-8}$ M oestriol significant increases in per cent cells in G2/M were observed at all concentrations of B[a]P tested. Figure 5 shows representative flow cytometry analyses of these effects induced in the presence of oestrogens in $10^{-8}$ M B[a]P-treated MCF-7 cells. Similar alterations in cell cycle distributions were observed following pre-incubation of MCF-7 cells with $10^{-9}$ M oestradiol, $10^{-8}$ M oestrone or $10^{-8}$ M oestriol prior to subsequent treatment with B[a]P (data not shown).

**Discussion**

Chemical carcinogens probably elicit their effects by altering DNA structure, perhaps through the formation of bulky DNA adducts, which ultimately result in sequence alterations and mutations. In order for such genetic alterations to become permanent, cell division is required. In order to maintain genomic integrity, eukaryotic cells have developed a complex network of DNA repair mechanisms and cell cycle checkpoints (Bartek and Lukas, 2001). Checkpoints are cellular defence mechanisms which may arrest cells with compromised genomes before they progress towards DNA replication (G1 arrest) or cell division (G2 arrest) (Shackelford et al., 1999). These controls are regulated by a complex network of transcription factors that include p53 and p21Waf1/Cip1 (Bartek and Lukas, 2001). In the event of irreparable DNA damage, such checkpoint control removes cells from the system either by permanent cell cycle arrest or programmed cell death (apoptosis).

Whilst there is clear epidemiological evidence for a role for chemical carcinogens in the aetiology of breast cancer...
Oestrogen-induced B[a]P effects in breast cells

Fig. 3. Effects of B[a]P on mitotic rate in MCF-7 cells following co- or pre-incubation with different oestrogens. After treatment, cells were blocked at cytokinesis by the addition of fresh medium containing 2 µg/ml cytochalasin B as described in Materials and methods. Cells were cultured for a further 24 h prior to fixation and staining. Mitotic rate was estimated as per cent binucleate MCF-7 cells from the mean ± SD of three separate counts.

*P < 0.05, **P < 0.005, ***P < 0.0005 (treatment versus control) as determined by an unpaired t-test with Welch’s correction.

Fig. 4. Effects on per cent plating efficiency in MCF-7 cells following co- or pre-incubation for 96 h in the presence of 10^{-9} M β-oestradiol, 10^{-8} M oestrone or 10^{-8} M oestriol prior to treatment with graded concentrations of B[a]P. Cells (1×10^3 cells) were seeded into 25 cm² flasks and incubated for 24 h, as described in Materials and methods. Following addition of fresh medium, in the absence of treatment, cells were cultured undisturbed at 37°C and 5% CO₂ in a humidified atmosphere for 7 days. Surviving colonies were fixed and stained and per cent plating efficiency was calculated by estimating the percentage of colonies counted over the number of cells initially seeded.

*P < 0.05, **P < 0.005 (treatment versus control) as determined by an unpaired t-test with Welch’s correction.

(Ziegler et al., 1993; Martin et al., 2001; Peto, 2001), the concentrations required to induce tumours in rodent bioassays are several orders of magnitude greater than human exposure levels. Consequently, questions have been raised as to the relevance of such environmental and/or dietary exposures (Ames and Gold, 2000). Even the concentrations required to induce genotoxic effects in short-term in vitro assays are questionable in terms of human exposure levels (Martin et al., 1999, 2000; Pfau et al., 1999). The notion that oestrogens, acting as modulators of carcinogen-induced DNA damage, are factors underlying the aetiology of breast cancer remains to be investigated.

The present study has investigated whether oestrogens may alter the susceptibility of breast carcinoma MCF-7 cells to the DNA-damaging effects of B[a]P. Oestrogens are necessary for cell turnover and proliferation but, paradoxically, cumulative oestrogen exposure enhances the risk of developing breast cancer (Feigelson and Henderson, 1996; Roy and Liehr, 1999). There is accumulating evidence that oestrogens themselves may also be direct genotoxins (Yared et al., 2002). Such mechanisms by which oestrogens may induce DNA damage which could include DNA adduct formation by oestrogen metabolites, free radical generation by redox cycling of oestrogens through microsomal, mitochondrial or nuclear processes or oxidative stress catalysed by the presence of Cu²⁺ or Fe²⁺ (Roy and Liehr, 1999; Cavalieri et al., 2000). However, in the complex milieu of the in vivo situation, target breast cells are exposed to carcinogens (Martin et al., 1997) in the presence of fluctuating levels of circulating endogenous oestrogens (Adashi, 1992). Hence, we have investigated the effects of co- or pre-incubation with oestrogens on the levels of B[a]P-induced genotoxicity in MCF-7 cells, as measured in the cytokinesis block micronucleus assay.

Both co- and pre-incubation with oestrogens result in marked increases in MN formation induced by B[a]P concentrations 10- to 100-fold lower than the concentrations required to induce similar increases in their absence (Figures 1 and 2). This enhanced induction of MN formation is observed following co-incubation with any of the three oestrogens investigated in this study (Figure 1). Enhanced MN formation is also observed to correlate with a reversal of the B[a]P-induced reduction in
mitotic rate that occurs in the absence of oestrogen (Figure 3). Whilst pre-incubation with either β-oestradiol or oestrone gave rise to similar increases in MN formation, what appears to be a protective effect was observed following pre-incubation with oestriol (Figure 2). Paradoxically, pre-incubation with oestriol also induced the most marked increases in mitotic rate seen under these conditions, whereas pre-incubation with oestrone induced levels markedly lower than those observed in cells treated with B[a]P alone (Figure 3). This suggests that enhanced proliferation does not necessarily result in an enhanced susceptibility to micronucleus formation, an observation previously noted (Yared et al., 2002). β-Oestradiol interferes with the control of apoptosis (Leung and Wang, 1999). Oestriol may induce programmed cell death and these data support observations that oestriol appears to be protective in rodent bioassays (Lemon et al., 1999). Oestriol may induce programmed cell death and these data support observations that oestriol appears to be protective in rodent bioassays (Lemon et al., 1999). Oestriol may induce programmed cell death and these data support observations that oestriol appears to be protective in rodent bioassays (Lemon et al., 1999).

Effects of oestrogens (10⁻⁸ M β-oestradiol, 10⁻⁸ M oestrone or 10⁻⁸ M oestriol) on the cell cycle distribution of MCF-7 cells with or without treatment with 10⁻⁸ M B[a]P were measured. DNA content of 10,000 events/treatment was analysed using a Becton Dickinson FACSCaliber flow cytometer as described in Materials and methods. Treatment of cell cultures with 5 nM actinomycin D, as a positive control, resulted in 76.15 ± 4.72% of cells in G0/G1, 9.34 ± 3.0% in S and 14.52 ± 6.44% in G2/M.

<table>
<thead>
<tr>
<th>Oestrogen status</th>
<th>G0/G1 (%)</th>
<th>+ B[a]P</th>
<th>S (%)</th>
<th>+ B[a]P</th>
<th>G2/M (%)</th>
<th>+ B[a]P</th>
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<tr>
<td>10⁻⁸ M B[a]P</td>
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<td>11.24 ± 1.75b</td>
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</table>

**Table II.** Oestrogen effects on cell cycle distribution of MCF-7 breast cells in the presence or absence of B[a]P

Effects of oestrogens (10⁻⁸ M β-oestradiol, 10⁻⁸ M oestrone or 10⁻⁸ M oestriol) on the cell cycle distribution of MCF-7 cells with or without treatment with 10⁻⁸, 10⁻⁷ or 10⁻⁶ M B[a]P were measured. DNA content of 10,000 events/treatment was analysed using a Becton Dickinson FACSCaliber flow cytometer as described in Materials and methods. Treatment of cell cultures with 5 nM actinomycin D, as a positive control, resulted in 76.15 ± 4.72% of cells in G0/G1, 9.34 ± 3.0% in S and 14.52 ± 6.44% in G2/M.

*P < 0.005 (treatment versus control) as determined by an unpaired t-test with Welch's correction.

*P < 0.05 (treatment versus control) as determined by an unpaired t-test with Welch's correction.

*P < 0.0005 (treatment versus control) as determined by an unpaired t-test with Welch’s correction.

![Fig. 5. Effects of oestrogens (10⁻⁸ M β-oestradiol, 10⁻⁸ M oestrone or 10⁻⁸ M oestriol) on the cell cycle distribution of MCF-7 cells with or without treatment with 10⁻⁸ M B[a]P. The percentage of cells in G0/G1, S and G2/M were measured. DNA content of 10,000 events/treatment was analysed using a Becton Dickinson FACSCaliber flow cytometer as described in Materials and methods.](image-url)
subsequent generations. The ability to evade the cellular defence mechanism of G1 arrest (the stealth property) has been observed in MCF-7 cells and is believed to be a general characteristic observed following treatment with certain potent chemical carcinogens (Khan and Dipple, 2000). Oestrogens appear to enhance the genotoxic effects of B[a]P in MCF-7 cells (Figures 1 and 2) in the presence of an increased mitotic rate (Figure 3), whilst appearing to enhance clonogenic survival (Figure 4). In contrast to these observations, the presence of oestrogens also induces a significant G1 arrest in B[a]P-treated cells while also significantly reducing the percentage of cells in S phase (Table II and Figure 5). MCF-7 cells express wild-type p53 and its induction by PAHs has been demonstrated (Kaspin and Baird, 1996; Luch et al., 1999). The reasons for these oestrogen-induced effects on cell cycle distributions following treatment with B[a]P are yet to be ascertained. Firstly, oestrogens may be factors, hitherto missing, that are required for the proper stimulus of G1 arrest in carcinogen-treated MCF-7 cells. Secondly, despite activation of G1 arrest, oestrogens may still push B[a]P-treated cells through the cell cycle, albeit at a slower rate, thus allowing cells with damaged genomes to undergo cell division. If so, such a scenario would significantly increase the risk of malignant transformation following carcinogen treatment. Finally, induction of G1 arrest in the presence of oestrogens may allow repair processes to occur but, because levels of carcinogen-induced DNA damage are so high, more cells divide unchecked, thus accounting for enhanced clonogenic survival. However, the mechanisms underlying these paradoxical observations remain to be elucidated.

The results of these investigations suggest that a complex interaction between oestrogens and B[a]P in MCF-7 cells gives rise to an enhanced susceptibility to genotoxicity despite induction of a significant G1 arrest. Further studies are required to elucidate the mechanisms involved and such investigations may include further investigations into cell cycle distributions and expression of transcription or metabolic factors. However, this study shows for the first time that B[a]P is capable of inducing a G1 arrest in MCF-7 cells under the correct conditions.

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