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DNA content and chromatin texture of human breast epithelial cells transformed with 17-beta-estradiol and the estrogen antagonist ICI 182,780 as assessed by image analysis

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Abstract

The immortalized human breast epithelial MCF-10F cell line, although estrogen receptor α negative, develops cell proliferating activities and invasiveness indicative of neoplastic transformation, after treatment with 17- β -estradiol (E-2). These effects are similar to those produced by benzo[a]pyrene (BP). Since we have previously reported changes in the nuclear parameters accompanying BP-induced tumorigenesis in MCF-10F cells, we have examined whether similar alterations occur in E-2-treated cells. We therefore studied DNA amounts and other nuclear parameters in Feulgen-stained MCF-10F cells after treatment with various concentrations of E-2, BP, the estrogen antagonist ICI 182,780, and E-2 in the presence of ICI 182,780. E-2 caused a certain loss of DNA and changes in the nuclear size and chromatin supraorganization of MCF-10F cells. Many of these changes were similar to those produced by BP and were indicative of neoplastic transformation. More intense chromatin remodelling was seen with 70 nM E-2. Since these changes were not abrogated totally or partially by ICI 182,780, the neoplastic transformation of MCF-10F cells stimulated by E-2 involved a process that was independent of estrogen α -receptors. The changes produced by ICI 182,780 alone were attributed to effects other than its well-known anti-estrogenic activity.

Keywords

human breast epithelial cells; 17- β -estradiol; benzo[a]pyrene; ICI 182; 780; DNA content; chromatin supraorganization

1. Introduction

Treatment of MCF-10F immortalized human breast epithelial cells with 17- β -estradiol (E-2) twice a week for two weeks results in cell growth characteristics and chromosomal changes indicative of neoplastic transformation, that are similar to the effects caused by the carcinogen benzo[a]pyrene (BP) [1,2]. These changes include anchorage-independent growth, colony formation in agar-methocel, and loss of heterozygosity (LOH) in chromosomes 13 and 17 [1–3]. The effects elicited in the MCF-10F cells by the E-2, in terms of increased colony

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formation in agar-methocel and cell proliferation, as well as the induction of cell transformation and of invasiveness in matrigel, are not abrogated by the estrogen antagonist ICI 182,780 [4]. This antagonist is a selective inhibitor of intracellular estrogen α -receptors but can also inhibit BKCa channels in vascular endothelial cells via a mechanism unrelated to its anti-estrogenic activity [5]. It is particularly interesting that MCF-10F cells do not have α -receptors for estrogen [4,6,7].

We have previously shown that the BP-induced tumorigenesis in MCF-10F cells is accompanied by gradual changes in cell nuclear parameters as assessed by image analysis, indicating loss of DNA and an increase in higher order packing states of chromatin [8]. A tendency of DNA amount distribution and nuclear size restoration to values seen in non-transformed cells has been demonstrated in BP-transformed and tumorigenic MCF-10F cells after the microcell-mediated transfer of a normal chromosome 17 [9].

The metabolism of E-2 involves the cytochrome P450 enzyme isoforms CYP1A1 and CYP1B1 [10–12]. These enzymes can be activated by BP [12–14]. The cytochrome P450-mediated activation of estrogen has been reported to elicit a direct genotoxic effect by increasing the rate of mutation [15]. Changes in CYP expression can affect BP metabolism and alter the levels of BP metabolites that can bind to DNA [16]. BP can activate estrogen receptors either directly or indirectly by producing estrogenic metabolites. When estrogen receptors are activated by BP, cell proliferation is induced in estrogen-sensitive cells such as the human breast carcinoma MCF-7 cells; this effect is estrogen receptor-dependent since it is blocked by ICI 182,780 [17].

Although there are similarities in the cell growth characteristics and chromosomal LOH in E-2- and BP-treated MCF-10F cells [1,4], changes in nuclear phenotypes and DNA content, whether induced by E-2 and similar to those induced by BP [7,8], have so far not been studied in cells without estrogen α -receptors including MCF-10F cells [4]. Additionally, it is not known whether ICI 182,780 affects these nuclear characteristics in MCF-10F cells in the absence or presence of E-2.

In the present study we compared DNA amounts, nuclear sizes and chromatin higher order packing states (texture) as assessed by image analysis in Feulgen-stained MCF-10F cells treated with different concentrations of E-2, BP, and ICI 182,780, and with E-2 in the presence of ICI 182,780.

2. Materials and methods

2.1. Cells and treatments

MCF-10F cells at passage 131 were cultured in DMEM:F-12 medium containing 1.05 mM calcium, antibiotics, hormones, growth factors, and equine serum, as previously described [18]. The cells were treated with different concentrations (0.007 nM, 70 nM, and 3.6 μ M) of E-2, BP, and ICI 182,780 twice a week for two weeks. The choice of these concentrations was based on a previous study [1]. Cells treated with 70 nM E-2 in the presence of 70 nM ICI 182,780 were also studied.

Control and treated cells were cultured on 9.4 cm² plastic chamber slides [19] for 96 h in hormone- or drug-free medium, fixed in an absolute ethanol-acetic acid mixture (3:1, v/v) for 1 min, rinsed in 70% ethanol for 5 min, and air dried. The preparations were then subjected to the Feulgen reaction (4 M HCl at 24°C, 75 min), and mounted in Cargille oil (Cedar Grove, NJ, USA) ($n_D = 1.54$). Three slides were used for each experimental condition.

2.2. Video image analysis

Two hundred interphase nuclei chosen at random for each experimental condition were measured using Carl Zeiss/Kontron equipment and Kontron KS400 software (Oberkochen/Munich, Germany). The microscope images were obtained with a Zeiss Axiophot 2 microscope equipped with a Neofluar 40/0.75 objective, optovar 2, 0.90 condenser, and light of $\lambda = 546$ nm. A 100-W halogen illuminator, a voltage regulator for light intensity maintained constant at point 4, filter wheels 1 and 2 rotated into position 100 (open position), and a luminous-filter diaphragm (transmitted light) at its maximal opening were used. These illumination conditions were maintained constant for all of the nuclei investigated. Images to be processed were fed from the microscope into a Pentium computer through a Sony CCD IRIS/RGB Hyper HAD color video camera. The threshold low (L) and high (H) levels were defined such that the nuclear images appeared pseudo-colored green and well separated from each other and from the background (in the present case $L = 0$ and $H = 116\text{--}120$ gray values). Under the optical conditions used, $1\text{ }\mu\text{m}$ corresponded to 7.23 pixels. The minimum area that could be measured in this apparatus corresponded to four pixels. The software provided quantitative information on nuclear area (μm^2), nuclear perimeter (μm), mean gray value per nucleus, which was subsequently converted into optical density ($\text{OD} = \text{absorbance}$), standard deviation of the total densitometric values per nucleus or absorbance variability per nucleus (SDtd), which reflects the variability in the degree of chromatin packing state per nucleus, and entropy, the number of bits necessary to store the densitometric values per nucleus image or amount of gray value variability in a nucleus [8,19]. For conversion of the mean gray values into absorbances, gray values of 0 and 255 corresponded to light transmittances of 0% and 100%, respectively. The integrated OD (IOD; in this case, Feulgen-DNA content) was equal to the absorbances multiplied by the nuclear area.

2.3. Statistical analysis

Calculations were done using the Minitab12™ software (State College, PA, USA).

3. Results

Treatment with E-2 and BP decreased the nuclear area and perimeter and the Feulgen-DNA content of MCF-10F cells (Tables 1 and 2; Figs. 1 and 2). The most significant decrease in nuclear sizes and Feulgen-DNA values was verified in cells treated with $3.6\text{ }\mu\text{M}$ E-2 (Tables 1 and 2); Figure 1 shows the distribution of Feulgen-DNA amounts in MCF-10F cells treated with E-2 and BP at this concentration. The treatment of immortalized MCF-10F cells with $3.6\text{ }\mu\text{M}$ ($1\text{ }\mu\text{g/ml}$) BP has been demonstrated as responsible for their neoplastic transformation, giving rise to several clones in which the phenotypes associated with tumorigenesis appear progressively [8,18].

The OD decreased after treatment with E-2, except for the concentration of 70 nM, for which the values were the same as for the control MCF-10F cells. In contrast, the OD increased with all concentrations of BP (Table 1). Additionally, the SDtd and nuclear entropy increased in all of the treatments with E-2 and BP (Table 2)

At a concentration of 70 nM E-2 did not alter the OD, but significantly decreased the nuclear sizes, and produced the highest SDtd values and nuclear entropy compared to control MCF-10F cells (Tables 1 and 2). The nuclear images of nontransformed, and BP- and E-2-transformed MCF-10F cells seen on the monitor of the image analyzer show differences in chromatin areas in pseudocolors, once established a same gray level threshold for segmentation (Fig. 3). Particularly in the case of the E-2-transformed cells in Figure 3d, the gray color varied in intensity.

The treatment of MCF-10F cells with ICI 182,780 generally decreased their nuclear sizes (except for nuclear area at a concentration of 3.6 μM), OD, and Feulgen-DNA amounts, but increased the SDtd values and nuclear entropy, although not as much as seen with E-2 (Tables 1 and 2, Fig. 2).

The nuclear areas and perimeters, and the Feulgen-DNA values did not differ amongst MCF-10F cells treated with ICI 182,780, E-2, and ICI 182,780 plus E-2 (70 nM), but were all lower than those for control MCF-10F cells (Fig. 2; Tables 1 and 2). In addition, the SDtd values and the nuclear entropy of the cells treated with ICI 182,780 and ICI 182,780 plus E-2 did not differ from each other; although higher than those of control MCF-10F cells, they were much lower than those of cells treated with E-2 alone (Table 2).

4. Discussion

The decrease in the DNA content of BP-treated MCF-10F cells results from a certain loss of DNA accompanying cell transformation [8,9], and a similar phenomenon probably occurred in E-2-treated cells. Indeed, there is evidence that estrogen compromises the DNA repair system [22] and induces aneuploidy through chromosomal instability and centrosome amplification, possibly via activation of the estrogen receptor [12,21,23].

In MCF-10F cells E-2 and BP induce anchorage-independent growth, colony formation in agar-methocel, and loss of ductulogenic capacity in collagen gel, all parameters indicative of cell transformation, even though these cells have no estrogen α -receptors [2]. Although BP and E-2 induce genomic alterations at the same loci (LOH at 13q12–q13 and 17q21.1–21.2), E-2 can also affect other loci (LOH at 11q23.3, 11q24.2–q25) [2,3], and this could explain the divergent effects of these two drugs on the nuclear parameters assessed. These findings confirm that E-2 may exert transforming effects by a mechanism independent of α -receptors [4].

The changes in DNA amounts with different concentrations of E-2 and BP may indicate in this aspect a hormone or drug threshold effect rather than a concentration-dependent pathway in MCF-10F cells [1]. Even so, experimental and clinical studies with other systems have revealed E-2 or BP concentration-dependent responses in other parameters. In studies of human intestinal carcinogenesis, the growth of subconfluent Caco-2 colon cells was found to be dependent on the concentration of estradiol, with maximal growth occurring at about 0.4 mM [24]. The induction of mammary carcinomas in the ACI rat model is dependent on the concentration of E-2 released from cholesterol pellets [25]. Clinical assays in older women have revealed that different estradiol doses have differential short-term effects on markers of cardiovascular disease [26]. The cell proliferation of human breast carcinoma MCF-7 cells can be stimulated by BP at concentrations ≥ 100 nM, an effect that is estrogen receptor-dependent [17]. The BP concentration of 3.6 μM used here induces neoplastic transformation in MCF-10F cells [18] and was chosen in order to allow comparison with E-2. The other E-2 concentrations tested in the MCF-10F cells have also been shown to induce colony formation in agar-methocel, tridimensional growth in a collagen matrix, and invasiveness in matrigel; a maximal number of solid masses has been observed with 70 nM E-2 [4].

The high SDtd values and nuclear entropy in MCF-10F cells treated with E-2 and BP were indicative of some chromatin structural remodelling. This was highlighted by the action of E-2, especially at 70 nM, when the most heterogeneous distribution of chromatin packing states (texture) was observed. With 70 nM E-2 the nuclear absorbances (OD) did not differ from those of control MCF-10F cells, although the nuclear size decreased. The increase in the heterogeneity of the chromatin packing state in treated cells resulted from an increase in chromatin condensation in some sectorial nuclear domains and a decrease in less packed zones; this conclusion was supported by the increased standard deviation values for the OD values

under these conditions. The highest SDtd values and nuclear entropy seen in E-2-treated cells were not associated with the most significant decrease in Feulgen-DNA content, which supports the idea that the structural reorganization of chromatin is not necessarily requiring a direct association with loss of DNA [9]. The simultaneous increase in OD and decrease in nuclear size in BP-treated cells agrees with a previous report on the increase in chromatin condensation elicited by BP [8].

The anti-estrogenic drug ICI 182,780 did not abrogate the changes in nuclear sizes and Feulgen-DNA amounts in MCF-10F cells treated with 70 nM E-2 in the combination assay. This finding agrees with the hypothesis that the E-2-induced transformation of MCF-10F cells [1,2] involves a non-estrogen α -receptor-mediated process that may be dependent on genotoxic effect of the estrogen metabolite 4-OH-E-2 [4]. ICI 182,780 attenuated the changes in SDtd and nuclear entropy produced by E-2, and reduced the nuclear absorbances (OD), indicating that the estrogen antagonist had an action of its own on MCF-10F cells. Indeed, treatment with ICI 182,780 only also reduced the DNA content, nuclear sizes and absorbances concomitant with an increase in SDtd and nuclear entropy. This effect may be related to the activation of apoptotic pathways by ICI 182,780, a phenomenon demonstrated in endothelial cells [27]. The partial suppression of the invasion phenotype generated by the E-2 treatment of MCF-10F cells by ICI 182,780 (1 μ M) may be a toxic effect of the drug [4].

The condensation of certain chromatin zones may facilitate relaxation, with increased efficiency of expression in other chromatin domains, as suggested for BP-transformed MCF-10F cells [8]. Chromatin remodeling caused by the effects of p53 on histone acetylation or phosphorylation [28] was probably not involved in the responses of E-2, BP- and ICI 182,780-treated MCF-10F cells since the participation of the p53 gene mutation occurs early in the immortalization of MCF-10F cells [29].

The present results show that E-2 can elicit changes in the DNA content, nuclear sizes and chromatin supraorganization of MCF-10F cells, in a manner similar to BP. These alterations are indicative of neoplastic transformation. The finding that the changes in nuclear parameters were not abrogated totally or partially by the anti-estrogenic drug ICI 182,780 agrees with the suggestion that the neoplastic transformation in MCF-10F cells occurs via a non-estrogen α -receptor-mediated process [4]. Our results also support the idea that ICI 182,780 has other effects in addition to accepted anti-estrogenic activity [5].

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References

1. Russo J, Lareef MH, Tahin Q, Hu YF, Slater C, Ao X, Russo I. 17 beta-estradiol is carcinogenic in human breast epithelial cells. *J Steroid Biochem Mol Biol* 2002;80:149–162. [PubMed: 11897500]
2. Russo J, Tahin Q, Lareef MH, Hu YF, Russo IH. Neoplastic transformation of human breast epithelial cells by estrogens and chemical carcinogens. *Environm Mol Mutag* 2002;39:254–263.
3. Lareef MH, Russo IH, Sheriff FS, Tahin Q, Russo J. Genomic changes induced by estrogen in human breast epithelial cells (HBEC). *Proc Am Assoc Cancer Res* 2003;44:207.
4. Lareef MH, Garber J, Russo PA, Russo IH, Heulings R, Russo J. The estrogen antagonist ICI-182-780 does not inhibit the transformation phenotypes induced by 17-beta-estradiol and 4-OH estradiol in human breast epithelial cells. *Int J Oncol* 2005;26:423–429. [PubMed: 15645127]
5. Liu YC, Lo YC, Huang CW, Wu SN. Inhibitory action of ICI-182,780, an estrogen receptor antagonist, on BKCa channel activity in cultured endothelial cells of human coronary artery. *Biochem Pharmacol* 2003;66:2053–2063. [PubMed: 14599564]

6. Soule HD, Maloney TM, Wolman SR, Peterson WD Jr, Brenz R, McGrath CM, Russo J, Pauley R, Jones RF, Brooks SC. Isolation and characterization of a spontaneously immortalized human breast epithelial-cell line, MCF-10. *Cancer Res* 1990;50:6075–6086. [PubMed: 1975513]
7. Tait L, Soule H, Russo J. Ultrastructural and immunocytochemical characterization of an immortalized human breast epithelial-cell line, MCF-10. *Cancer Res* 1990;50:6087–6099. [PubMed: 1697506]
8. Vidal BC, Russo J, Mello MLS. DNA content and chromatin texture of benzo[a]pyrene-transformed human breast epithelial cells as assessed by image analysis. *Exp Cell Res* 1998;244:77–82. [PubMed: 9770351]
9. Mello MLS, Vidal BC, Lareef MH, Hu YF, Yang X, Russo J. DNA content, chromatin texture and nuclear morphology in benzo[a]pyrene-transformed human breast epithelial cells after microcell-mediated transfer of chromosomes 11 and 17. *Cytometry* 2003;72A:70–76. [PubMed: 12655650]
10. Roy D, Strobel HW, Liehr JG. Cytochrome-b5-mediated redox cycling of estrogen. *Arch Biochem Biophys* 1991;285:331–338. [PubMed: 1897935]
11. Hammond DK, Zhu BT, Wang MY, Ricci MJ, Liehr JG. Cytochrome P450 metabolism of estradiol in hamster liver and kidney. *Toxicol Appl Pharmacol* 1997;145:54–60. [PubMed: 9221823]
12. Liehr JG. Is estradiol a genotoxic mutagenic carcinogen? *Endocrine Rev* 2000;21:40–54. [PubMed: 10696569]
13. Whitlock JP. Induction of cytochrome P4501A1. *Ann Rev Pharmacol Toxicol* 1999;39:103–125. [PubMed: 10331078]
14. Hooven LA, Mahadevan B, Keshava C, Johns C, Pereira C, Desai D, Amin S, Weston A, Baird WM. Effects of suberoylanilide hydroxamic acid and trichostatin A on induction of cytochrome P450 enzymes and benzo[a]pyrene DNA adduct formation in human cells. *Bioorg Med Chem Lett* 2005;15:1283–1287. [PubMed: 15713371]
15. Roy D, Liehr JG. Temporary decrease in renal quinone reductase-activity induced by chronic administration of estradiol to male Syrian-hamsters-increased superoxide formation by redox cycling of estrogen. *J Biol Chem* 1988;263:3646–3651. [PubMed: 2831197]
16. Guengerich FP. Forging the links between metabolism and carcinogenesis. *Mut Res* 2001;488:195–209. [PubMed: 11397649]
17. Piskova M, Vondracek J, Vojtesek B, Kozubik A, Machala M. Deregulation of cell proliferation by polycyclic aromatic hydrocarbons in human breast carcinoma MCF-7 cells reflects both genotoxic and nongenotoxic events. *Toxicol Sci* 2005;82:246–256.
18. Calaf G, Russo J. Transformation of human breast epithelial cells by chemical carcinogens. *Carcinogenesis* 1993;14:483–492. [PubMed: 8453725]
19. Mello MLS, Lareef MH, Santos AB, Russo J, Vidal BC. Nucleus image properties and cell death in MCF-10F cells grown on slide substrates differing in nature and size. *In Vitro Cell Dev Biol- Animal* 2005;41:92–96.
20. Oberholzer M, Östreicher M, Christen H, Brühlmann M. Methods in quantitative image analysis. *Histochem Cell Biol* 1996;105:333–355. [PubMed: 8781988]
21. Roy D, Liehr JG. Estrogen, DNA damage and mutations. *Mut Res* 1999;424:107–115. [PubMed: 10064854]
22. Chakravarti D, Mailander PC, Li KM, Higginbotham S, Zhang HL, Gross ML, Meza JL, Cavalieri EL, Rogan EG. Evidence that a burst of DNA depurination in SENCAR mouse skin induces error-prone repair and forms mutations in the *H-ras* gene. *Oncogene* 2001;20:7945–7953. [PubMed: 11753677]
23. Li JJ, Weroha SJ, Lingle WL, Papa D, Salisbury JL, Li SA. Estrogen mediates Aurora-A overexpression, centrosome amplification, chromosomal instability, and breast cancer in female ACI rats. *Proc Nat Acad Sci USA* 2004;101:18123–18128. [PubMed: 15601761]
24. DiDomenico M, Castoria G, Bilancio A, Migliaccio A, Auricchio F. Estradiol activation of human colon carcinoma-derived Caco-2 cell growth. *Cancer Res* 1996;56:4516–4521. [PubMed: 8813150]
25. Mesia-Vela S, Sanchez RI, Reuhl KR, Conney AH, Kauffman FC. Induction of mammary adenocarcinomas in the ACI rat by estradiol. *Proc Am Assoc Cancer Res* 2002;43:168.
26. Prestwood KM, Unson C, Kulldorff M, Cushman M. The effect of different doses of micronized 17 beta-estradiol on C-reactive protein, interleukin-6, and lipids in older women. *J Gerontol A- Biol Sci Med Sci* 2004;59:827–832.

27. Soares R, Guo SC, Russo J, Schmitt F. Role of the estrogen antagonist ICI 182,780 in vessel assembly and apoptosis of endothelial cells. *Ultrastruct Path* 2003;27:33–39. [PubMed: 12554534]
28. Allison SJ, Milner J. Remodelling chromatin on a global scale: a novel protective function of p53. *Carcinogenesis* 2004;25:1551–1557. [PubMed: 15231688]
29. Barnabas N, Moraes R, Calaf G, Estrada S, Russo J. Role of p53 in MCF-10F cell immortalization and chemically-induced transformation. *Int J Oncol* 1995;7:1289–1296.

Fig. 1. Tukey's box plots showing the effect of 3.6 μ M BP, E-2, and ICI on Feulgen-DNA content. n = 200.

Fig. 2. Tukey's box plots showing the distribution of the Feulgen-DNA content of nontransformed, and 70 nM E-2-, ICI 182,780 (ICI)-, and ICI 182,780 + E-2-transformed MCF-10F cells. n = 200

Fig. 3. Pseudocolored nuclear images of Feulgen-stained nontransformed (**b**), 70 nM BP-transformed (**c**), and 70 nM E2-transformed (**d**) MCF-10F cells as seen on the monitor of the video image analyser. The segmentation process that identified chromatin particles in magenta was based on a threshold level established for the control. Nuclear images before the thresholding are shown as an example in **a** (same view as in **b**).

TABLE 1

Nuclear phenotypes: video image geometric parameters

Cells and Treatments	Nuclear area (μm^2)		Nuclear perimeter (μm)	
	$\bar{X}$	S	$\bar{X}$	S
MCF-10F (control)	171.81 ^a	58.55	90.67 ^a	31.85
BP – 3.6 μM	95.49 ^b	34.51	47.10 ^b	12.51
BP – 70 nM	132.56 ^c	65.72	59.44 ^c	23.55
BP- 0.007 nM	99.26 ^b	33.24	47.65 ^b	13.40
E-2 – 3.6 μM	78.55 ^d	25.13	40.36 ^d	9.88
E-2 – 70 nM	152.35 ^e	59.91	67.40 ^e	32.75
E-2 – 0.007 nM	102.22 ^b	38.88	46.98 ^b	15.16
ICI – 3.6 μM	161.82 ^a	68.66	71.37 ^e	28.47
ICI – 70 nM	146.04 ^e	53.74	63.32 ^{e,f}	19.17
ICI – 0.007 nM	130.35 ^c	53.23	59.83 ^f	22.35
ICI + E-2 – 70 nM	151.36 ^e	62.23	66.58 ^e	25.99

BP = benzo[a]pyrene; E-2 = 17-beta-estradiol; ICI = ICI 182,780; n = 200; S = standard deviation; $\bar{X}$ = arithmetic mean. Different superscript letters down a queue mean differences significant at $P_{0,01}$ (Anova)

TABLE 2

Nuclear phenotypes: densitometric and textural parameters

Cells and Treatments	Absorbances(OD)		Feulgen-DNA amounts (IOD)		SDtd		Nuclear entropy	
	> $\bar{X}$	S	> $\bar{X}$	S	> $\bar{X}$	S	> $\bar{X}$	S
MCF-10F (control)	0.57a	0.03	98.64a	32.85	5.77a	1.77	4.42a	0.43
BP – 3.6 μ M	0.68b	0.06	63.80b*	20.71	7.95b	2.08	4.90b	0.34
BP – 70 nM	0.62c	0.07	79.96c	37.25	8.21b	2.79	4.91b*	0.44
BP – 0.007 nM	0.60d	0.04	59.37b	17.92	9.90c	2.60	5.18c	0.36
E-2 – 3.6 μ M	0.51e	0.05	39.55d	11.32	12.20d	3.58	5.44d	0.40
E-2 – 70 nM	0.56a	0.07	78.77c	30.19	12.72d	3.81	5.54e	0.40
E-2 – 0.007 nM	0.50e	0.04	50.73e	17.26	10.26c	2.73	5.25f	0.35
ICI – 3.6 μ M	0.48f	0.03	76.39c	30.76	8.45b,e	2.82	4.96b,g	0.42
ICI – 70 nM	0.49f	0.05	83.04c	31.11	8.95e	2.57	5.07g	0.38
ICI – 0.007 nM	0.54h	0.07	69.52f*	26.10	9.04e	2.97	5.04g	0.46
ICI + E-2 – 70 nM	0.54h	0.04	82.24c	34.76	8.69b,e	2.18	5.05g	0.36

BP = benzo[a]pyrene; E-2 = 17-beta-estradiol; ICI = ICI 182,780; n = 200; S = standard deviation; $\bar{X}$ = arithmetic mean. Different superscript letters down a queue mean differences significant at P_{0,01} or P_{0,05}(*)(Anova)