Int. J. Cancer: **70,** 188–193 (1997)

Publication of the International Union Against Cancer
Publication de l'Union Internationale Contre le Cancer

17β-ESTRADIOL, DIETHYLSTILBESTROL, TAMOXIFEN, TOREMIFENE AND ICI 164,384 INDUCE MORPHOLOGICAL TRANSFORMATION AND ANEUPLOIDY IN CULTURED SYRIAN HAMSTER EMBRYO CELLS

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To examine the ability of estrogens and anti-estrogens to induce cellular transformation and genetic effects, Syrian hamster embryo (SHE) cells were treated with estrogens, 17β-estradiol (E_2) or diethylstilbestrol (DES), or with anti-estrogens, tamoxifen (TAM), toremifene (TOR) or ICI 164,384. Treatment with each substance for 1–3 days suppressed cellular growth in a dose-dependent manner. Colony-forming efficiency (CFE) increased following treatment of cells with E_2 or DES for 48 hr at 3 or 10 μM but decreased at 20 or 30 μM. In contrast, CFE was increased by treatment with TAM, TOR or ICI 164,348 over the concentration range examined (1–30 μM). Treatment with each chemical at 1–30 μM for 48 hr caused morphological transformation of SHE cells in a dose-related fashion. The highest frequency was exhibited in SHE cells treated with DES at 20 μM and was 2 times higher than that induced by treatment with benzo[α]pyrene (B[α]P) at 4 μM. Transformation frequencies induced by other substances (E_2, TAM, TOR and ICI 164,348) did not exceed that induced by the B[α]P treatment. TOR showed a higher transforming ability over all concentrations examined when compared to the other anti-estrogens (TAM and ICI 164,348). No significant increases in the frequencies of chromosomal aberrations were observed in SHE cells that were treated with any of the chemicals. However, treatment of SHE cells with each chemical induced a dose-dependent increase of aneuploid cells in the near diploid range. Our results indicate that the ability of the estrogens and anti-estrogens to induce numerical chromosomal abnormality may be involved in their cell transformation activity and potential carcinogenicity. ***Int. J. Cancer, 70: 188–193, 1997.***

Many estrogenic chemicals are carcinogenic in different species. Carcinogenicity of the synthetic estrogen diethylstilbestrol (DES) is demonstrated in humans and in rodents (IARC, 1979). The natural steroidal estrogen 17β-estradiol (E_2) also exhibits carcinogenic activity in a number of experimental animals (IARC, 1979). The mechanism of carcinogenesis by estrogens has not been fully elucidated, but strong evidence exists to support the hypothesis that estrogens are epigenetic carcinogens acting *via* a promoting effect related to cellular proliferation mediated by the estrogen receptor (ER) (Sheehan *et al.,* 1982). In contrast, experimental support for another mechanism, related to genotoxicity, has been provided in many reports for estrogen-induced carcinogenesis.

DES has been shown to induce genotoxic effects in cultured mammalian cells with exogenous metabolic activation and in cells with possible endogenous activation capacity for DES (Tsutsui *et al.,* 1986). We have studied the cell transforming activity and genotoxicity of DES and E_2 using Syrian hamster embryo (SHE) cells, which do not express measurable levels of the ER (Barrett *et al.,* 1981; Tsutsui *et al.,* 1983, 1986, 1987). The SHE cell transformation assay measures the induction of pre-neoplastic cells by scoring morphologically altered colonies of cells that form 7–8 days after exposure to chemical/physical carcinogens (Tsutsui *et al.,* 1983). Treatment of SHE cells with DES or E_2 induces cellular transformation without measurable gene mutations, unscheduled DNA synthesis (UDS) or structural chromosomal aberrations (Barrett *et al.,* 1981; Tsutsui *et al.,* 1983, 1987). Under the same conditions, both estrogens induce a specific type of genetic change, *i.e.,* aneuploidy. Chromosome losses and gains are induced (Tsutsui *et al.,* 1983, 1987), suggesting a non-disjunctional mechanism involved in the transforming activity. However, UDS and gene mutation at the Na^+/K^+ATPase locus are elicited when SHE cells are treated with DES in the presence of a rat liver post-mitochondrial supernatant metabolic activation system. Enhancement of the transformation frequencies is accompanied with this treatment (Tsutsui *et al.,* 1986). Thus, we have proposed 2 potential mechanisms for estrogen-induced cellular transformation: one does not involve direct DNA damage and the other one is associated with DNA damage.

Cellular DNA damage induced by chemicals can be examined by detection of DNA adduct formation through covalent modification of DNA. Liehr *et al.* (1986) demonstrated the presence of covalent DNA adducts in pre-malignant lesions in the kidneys of Syrian hamsters treated chronically with estrogens using a ^{32}P-post-labeling assay. In addition, exposure of SHE cells to DES and E_2 leads to covalent DNA adduct formation, corresponding to induction of cellular transformation (Hayashi *et al.,* 1996).

The triphenylethylene non-steroidal tamoxifen (TAM), which is a structural analogue of DES (Fig. 1), exerts anti-estrogenic, mixed or partial agonist/antagonist effects depending upon tissue, dose and species (Kendall and Rose, 1992). TAM has been widely used in the treatment of all stages of breast cancer and as an adjuvant treatment for operable breast cancer. TAM is a complete carcinogen capable of both initiation and promotion in the liver of female rats. TAM has multiple effects on the genetic apparatus, *i.e.,* DNA adduct formation, mitotic spindle disruption, numerical and structural chromosomal abnormality and micronucleus (MN) induction (Han and Liehr, 1992; Sargent *et al.,* 1994; Styles *et al.,* 1994). Metabolic activation seems to be required to exert its genotoxic activity (Styles *et al.,* 1994). Metzler and Schiffmann (1991) reported that TAM and its major metabolite, 4-hydroxy TAM, but not 3-hydroxy TAM, give rise to SHE cellular transformation, suggesting that metabolic pathways are important for cellular transformation and that certain structural features of the molecule are necessary for the transforming capability.

Toremifene (TOR) is a new triphenylethylene non-steroidal anti-estrogen, the molecular structure of which closely resembles that of TAM (Fig. 1). TOR is not hepatocarcinogenic in rats, though its tumor-promoting activity has been demonstrated in a 2-stage hepatocarcinogenesis model with rats (Dragan *et al.,* 1995). The mechanisms of the difference in hepatocarcinogenic potential between TAM and TOR are not clear. As TAM and TOR display comparable anti-estrogenic/estrogenic effects in the rat liver (Kendall and Rose, 1992), hormonal action is not likely the basis for the liver carcinogenicity of these compounds. TOR induces MN in human lymphoblastoid cell lines with monooxygenase activity, though the response is less potent than TAM (Styles *et al.,* 1994). ^{32}P-Post-labeling studies show that TOR causes DNA adducts in human lymphocytes in the presence of human or rat microsomes, though the level of DNA adducts is considerably lower than that induced

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Received 11 June 1996; revised 17 September 1996.

by TAM (Hemminki *et al.,* 1995). Davies *et al.* (1995) reported that the extent of DNA adduct formation caused by TAM and TOR was similar when calf thymus DNA was treated with these compounds in the peroxidase/H_2O_2 enzyme system. This suggests that (*i*) TOR is predominantly metabolized by peroxidase activation to the reactive intermediate(s), leading to DNA adduct formation, and (*ii*) the absence of peroxidase activation of TOR in rat or human liver is attributed to the negative responses in genotoxicity tests (Carthew *et al.,* 1995).

The 7α-alkylamide analogue of E_2, ICI 164,384, is a new steroidal anti-estrogen with the complete/pure antagonistic properties. ICI 164,348 is a very potent growth inhibitor of ER-positive human breast cancer cells (MCF-7) (Thompson *et al.,* 1989). As far as we know, there are no reports on the genotoxicity and carcinogenicity of ICI 164,384.

As described above, DES (Barrett *et al.,* 1981; Tsutsui *et al.,* 1983), E_2 (Tsutsui *et al.,* 1987) and TAM (Metzler and Schiffmann, 1991) have been demonstrated to induce transformation of SHE cells. These substances are capable of microtubule disruption or improper mitotic spindle formation in certain experimental conditions (Tucker and Barrett, 1986). In addition, aneuploidy induction and/or MN formation have been recognized as properties of DES, E_2, TAM and TOR (Tsutsui *et al.,* 1983, 1987; Styles *et al.,* 1994). This indicates a possible involvement of chromosomal mutations, *e.g.,* aneuploidy induced by perturbation of mitotic apparatus, in cellular transformation and carcinogenesis by estrogens and anti-estrogens.

In this study, we examined the ability of estrogens (DES and E_2) and their anti-estrogens (TAM, TOR and ICI 164,348) to induce cellular transformation and genetic effects in the same cells to clarify the participation of chromosomal mutations by these substances in carcinogenesis. All substances tested induced morphological transformation of SHE cells with no increases in the frequencies of chromosomal aberrations. Numerical chromosomal changes, however, were elicited in the near diploid range by all substances, corresponding to their transforming abilities. Aneuploidy induction by these substances may be involved in their transformation activity and potential carcinogenicity.

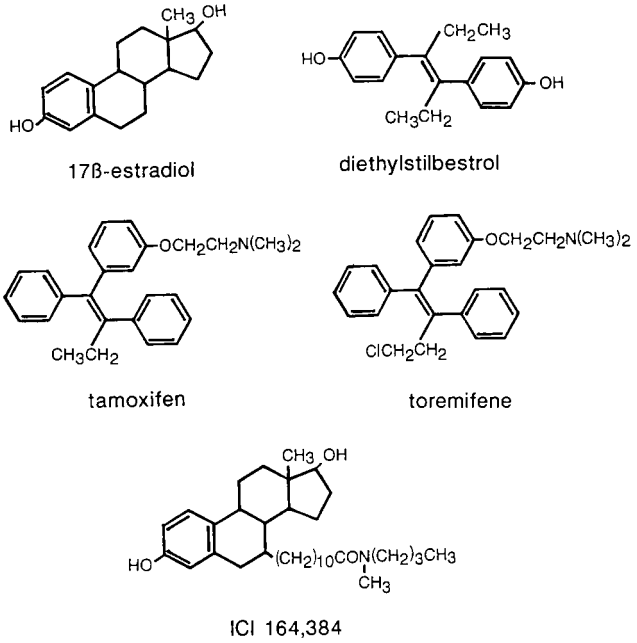

FIGURE 1 – Chemical structures of E_2, DES, TAM, TOR and ICI 164,384.

MATERIAL AND METHODS

Cells and chemicals

SHE cell cultures were established from 13-day gestation hamster fetuses and grown as previously described (Tsutsui *et al.,* 1983). E_2, DES and TAM were purchased from Sigma (St. Louis, MO). TOR and ICI 164,384 were generously provided by Dr. A.R. Imondi (Adria, Dublin, OH) and Dr. A.E. Wakeling (ICI Pharmaceuticals, Macclesfield, UK), respectively. The chemical structures are shown in Figure 1. E_2, DES, TAM and TOR were dissolved in DMSO at 10 mM, and ICI 164,384 was dissolved in 99.5% ethanol at 10 mM. Solutions were diluted with complete medium to the final concentrations. Benzo[α]pyrene (B[α]P) (Aldrich, Milwaukee, WI) was obtained from Sigma and dissolved in DMSO at 4 mM. DMSO or 99.5% ethanol was added to control cultures at a final concentration of 0.3%.

Growth curve

Cells (3×10^4) in logarithmic growth phase were plated on 35-mm dishes (Falcon, Oxnard, CA). After overnight incubation, cells were treated with test substances at various concentrations for 1–3 days. The number of cells per 35-mm dish was determined after trypsinization. Cell counts are presented as mean ± SD from 4 dishes per counting point (1, 2 and 3 days after start of treatment).

Cellular transformation

Cells (2.5×10^5) were plated into 75-cm^2 flasks (Falcon), incubated overnight and treated with test substances for 48 hr. After trypsinization, 2,000 cells were replated on 100-mm dishes (20 dishes for each group) and incubated for 7 days to form colonies. Cells were fixed with absolute methanol and stained with a 10% aqueous Giemsa solution. The number of surviving colonies with >50 cells and morphologically transformed colonies were scored by previously established criteria (Tsutsui *et al.,* 1983).

Chromosomal aberrations and chromosome number

SHE cells were plated into 75-cm^2 flasks at 1.3×10^5 cells/flask for the 72-hr treatment group, 2.5×10^5 cells/flask for the 48-hr treatment group and 5×10^5 cells/flask for the 24-hr treatment group. After overnight incubation, cells were treated with test substances for 24–72 hr. Three hours before the end of the treatment time, Colcemid (GIBCO, Grand Island, NY) was administered at 0.2 μg/ml and metaphase chromosomes were prepared as described previously (Tsutsui *et al.,* 1983). After trypsinization, cells were treated with 0.9% sodium citrate at room temperature for 13 min, fixed in Carnoy's solution (methanol:acetic acid, 3:1) and spread on glass slides by the air-drying method. Specimens were stained with a 3% Giemsa solution in 0.07 M phosphate buffer (pH 6.8) for 7 min. For determination of both chromosomal aberrations (gaps, breaks, exchanges, dicentrics, o-rings and fragmentations) and chromosome number, 100 metaphases per experimental group were scored. Achromatic lesions greater than the width of the chromatid were scored as gaps unless there was displacement of the broken piece of chromatid. If there was displacement, these were recorded as breaks.

RESULTS

The effects on cellular growth of treatment with varying concentrations of E_2, DES, TAM, TOR or ICI 164,384 for 1–3 days were examined (Fig. 2). Exposure of cells to each estrogen or anti-estrogen at 3 μM had little effect on cellular growth. However, at concentrations of ≥10 μM, each substance suppressed cellular growth in a dose-dependent manner. As compared with the growth-inhibitory effect caused by exposure to these substances at 20 μM, TAM was the most cytotoxic, then DES followed by TOR.

Colony-forming efficiencies (CFEs) and frequencies of morphological transformation of SHE cells following treatment with test substances for 48 hr are shown in Table I. Treatment of SHE cells with E_2 or DES at 3 or 10 μM increased the CFEs of the cells over the control level. However, exposure to the estrogens at 20 or 30

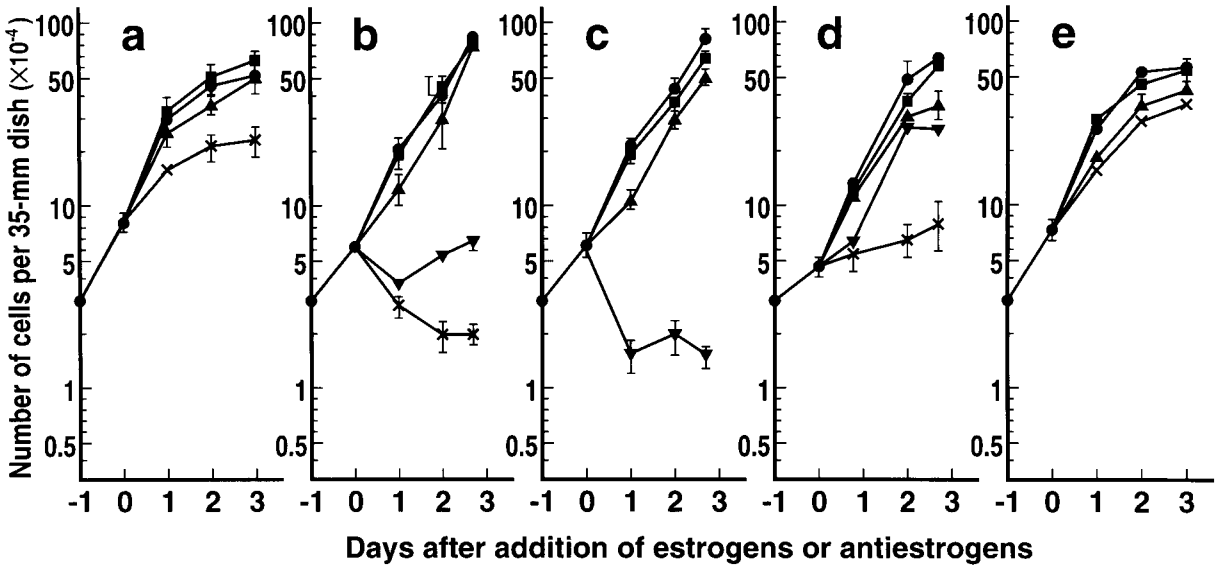

FIGURE 2 – Growth of SHE cells treated with estrogens or anti-estrogens. SHE cells were plated, in quadruplicate, on 35-mm dishes at a density of 3×10^4 cells/dish. After overnight incubation, cells were treated with (*a*) E_2, (*b*) DES, (*c*) TAM, (*d*) TOR and (*e*) ICI 164,384 at concentrations of 0 (●), 3 (■), 10 (▲), 20 (▼) and 30 (×) μM for 1, 2 and 3 days. Bars denote SD. When not indicated, SD are within symbols.

TABLE I – MORPHOLOGICAL TRANSFORMATION OF SHE CELLS INDUCED BY ESTROGENS OR ANTI-ESTROGENS

Chemicals	Dose (μM)	Relative plating efficiency (%)	Number of morphologically transformed colonies/number of colonies scored	% Transformation
Control (DMSO)	0[1]	100	2/7,494[3]	0.03
Control (ethanol)	0[2]	100	2/6,154[6]	0.03
17β-estradiol	3	113**	3/4,450[4]	0.08
	10	111**	17/4,404[4]	0.39**
	30	60**	16/2,267[5]	0.71**
Diethylstilbestrol	3	111**	5/4,414[4]	0.11
	10	133**	10/5,256[4]	0.19*
	20	18**	10/705[4]	1.42**
Tamoxifen	3	102	3/4,050[4]	0.07
	10	102	7/7,807[6]	0.09
	20	141**	19/5,265[4]	0.36**
Toremifene	3	111**	25/8,551[6]	0.29**
	10	126**	45/9,712[6]	0.46**
	20	112**	46/8,601[6]	0.53**
	30	157**	34/12,042[6]	0.28**
ICI 164,384	1	114**	1/3,585[6]	0.03
	3	136**	18/8,347[6]	0.22**
	10	156**	19/9,303[3]	0.20**
	30	197**	20/5,055[4]	0.40**
B[α]P	4	75**	21/2,975[4]	0.71**

[1]Actual plating efficiency was 9.61 ± 0.53% (SD).–[2]Actual plating efficiency was 8.96 ± 0.58%.–[3]Thirty-nine dishes were scored.–[4]Twenty dishes were scored.–[5]Nineteen dishes were scored.–[6]Forty dishes were scored.

*Statistically different from each control culture ($p < 0.05$, χ^2 test).–**Statistically different from each control culture ($p < 0.01$, χ^2 test).

μM decreased the CFEs to 18–60% relative to control cells. In contrast, CFEs were significantly increased in a dose-related fashion when SHE cells were treated with their anti-estrogens (TAM, TOR or ICI 164,384) over the concentration range examined (1–30 μM).

Treatment with the estrogens or anti-estrogens caused morphological transformation of SHE cells (Table I). Exposure to E_2 at 10 or 30 μM and DES at 10 or 20 μM resulted in a significant increase in the transformation frequencies in a dose-related manner. When SHE cells were treated with TAM at 3–20 μM, morphological transformation was significantly induced in cells treated at the highest concentration examined. A dose-dependent, but not statistically significant, increase in the transformation frequencies was observed in SHE cells exposed to TAM at 3 or 10 μM. Treatment with TOR at 3–30 μM induced a statistically significant increase in the frequencies of morphological transformation, though the frequency was decreased in cells treated at the highest concentration. A dose-dependent increase in the frequencies of morphological transformation was also observed when SHE cells were treated with ICI 164,384 at 1–30 μM. Comparing the transformation frequencies induced by the estrogens or anti-estrogens, the highest frequency was elicited in cells treated with DES at 20 μM, which was 2-fold higher than that induced by treatment with 4 μM B[α]P used as a positive control. Transformation frequencies induced by other substances (E_2, TAM, TOR and ICI 164,384) did not exceed that induced by B[α]P treatment. Exposure of cells to TOR resulted in high transformation frequencies over all concentrations examined compared with those induced by other anti-estrogens (TAM and ICI 164,384).

To examine the ability of these estrogens and anti-estrogens to induce chromosomal abnormalities, SHE cells treated with the substances were analyzed for structural and numerical abnormalities of chromosomes. No significant increases in the frequencies of chromosomal aberrations were observed in cultures treated with each substance at 3–30 μM for 24 hr (data not shown). Because treatment at high concentrations with substances can induce mitotic delay as suggested by growth curves (Fig. 2), experiments with prolonged treatment times were performed. As shown in Table II, none of the substances, which were given to the cells for 48 or 72 hr, increased the levels of chromosomal aberrations, which were observed in 0–2% of the metaphases of both control and treated cells. In contrast, the percentage of cells with numerical chromosome changes was increased by treatment with the estrogens or anti-estrogens (Table II). The majority (94–95%) of the metaphases from control cultures had a diploid ($2n = 44$) number of chromosomes, and 4–6% were in the polyploid range. None or a small percentage (1%) of the metaphases in control cultures had an aneuploid number of chromosomes, which was near the diploid number.

Treatment of cells with E_2 at 3–30 μM for 48 hr increased the percentage of aneuploid cells with a near diploid number of

TABLE II – DISTRIBUTION OF THE CHROMOSOME NUMBER PER METAPHASE OF CULTURED SHE CELLS TREATED WITH ESTROGENS OR ANTI-ESTROGENS

Chemicals	Treatment time (hr)	Dose (μM)	Number of chromosomes									% Diploid cells	% Heteroploid cells		Chromosomal aberrations
			<41	42	43	44	45	46	47	48	>80		Tetraploid and near tetraploid	Near diploid[1]	% Aberrant metaphases
Control (DMSO)	48	0				94					6	94.0	6.0	0	1.0
	72	0				95	1				4	95.0	4.0	1.0	2.0
Control (ethanol)	48	0				94					6	94.0	6.0	0	2.0
E_2	48	3			2	89	4				5	89.0	5.0	6.0*	ND
		10	2		3	85	3				7	85.0	7.0	8.0*	2.0
		30			2	90	5	1			2	90.0	2.0	8.0*	2.0
	72	30		1	8	77	8				6	77.0	6.0	17.0**	1.0
DES	48	3			2	89	4				5	89.0	5.0	6.0*	0
		10			3	85	4	1			7	85.0	7.0	8.0*	1.0
		20			5	82	7	1			5	82.0	5.0	13.0**	0
		30	Few metaphases												
	72	20			6	17	3	1	1		72	17.0	72.0**	11.0**	2.0
		30	Few metaphases												
TAM	48	3				93					7	93.0	7.0	0	ND
		10				93	3				4	93.0	4.0	3.0	1.0
		20			2	91	4				3	91.0	3.0	6.0*	2.0
	72	20		1	1	95	1				2	95.0	2.0	5.0	1.0
TOR	48	3			1	96	1				2	96.0	2.0	2.0	1.0
		10			2	88	1				9	88.0	9.0	3.0	0
		20			4	86	5	1			4	86.0	4.0	10.0**	0
		30			2	91	5				2	91.0	2.0	7.0*	0
	72	20			2	92	4				2	92.0	2.0	6.0	1.0
		30			2	90	3	1			4	90.0	4.0	6.0	0
ICI	48	3	1		2	87	3	1			6	87.0	6.0	7.0*	1.0
		10	ND[2]												
		30			5	84	5				6	84.0	6.0	10.0**	1.0

[1]Aneuploid cells with a chromosome number within 1 to 3 of the diploid chromosome number (2n = 44).–[2]Not done.
*Statistically different from untreated cultures ($p < 0.05$, χ^2 test).–**Statistically different from untreated cultures ($p < 0.01$, χ^2 test).

chromosomes to 6–8%. The percentage was increased to 17% by exposure to E_2 at 30 μM for 72 hr. No significant increases in the percentage of heteroploid cells with tetraploid and near tetraploid numbers of chromosomes were observed when the cells were treated with E_2 at 3–30 μM for 48 and 72 hr. A dose-dependent increase in the percentage of aneuploid cells in the near diploid range was demonstrated in cells treated with DES at 3–20 μM for 48 hr. A similar level of aneuploidy induction in the near diploid range was observed in both cultures that were treated with DES at 20 μM for 48 and 72 hr. On the contrary, the percentage of heteroploid cells with a tetraploid or near tetraploid number of chromosomes was increased to as high as 72% following treatment of cells with DES at 20 μM for 72 hr.

Exposure of cells to TAM, TOR or ICI 164,384 resulted in dose-dependent increases in the percentage of aneuploid cells with a near diploid number of chromosomes. These anti-estrogens, however, did not cause a statistically significant increase in the percentage of polyploid cells.

DISCUSSION

We have shown here that estrogens (E_2 and DES) and anti-estrogens (TAM, TOR and ICI 164,384) induce morphological transformation of SHE cells. All substances failed to induce chromosomal aberrations but caused numerical chromosome changes in the same cells, nearly corresponding to the induction of cellular transformation. The present study provides evidence that TOR and ICI 164,384 induce morphological transformation and numerical chromosomal abnormalities in SHE cells. E_2 and DES have been found to bind and disrupt polymerization of microtubules in cultured mammalian cells (Tucker and Barrett, 1986). Functional or conformational changes in microtubule organization could lead to chromosomal non-disjunction and aneuploidy induction. Although both E_2 and DES result in DNA adduct formation and aneuploidy in SHE cells, it is not clear which cytogenetic end-point is more correlated as a causal basis with the estrogen-induced transformation. In addition, other effects of estrogens, *e.g.*, covalent binding to microsomal proteins (Haaf and Metzler, 1985) and generation of reactive oxygen species (Liehr, 1990), could participate in inducing transformation of cells. Furthermore, we cannot rule out the possibility that multiple effects of estrogens act together to cause genetic alterations leading to cellular transformation.

TAM binds to calmodulin, an intracellular Ca^{2+}-binding protein, and acts as a calmodulin antagonist (Edwards *et al.,* 1992). Sargent *et al.* (1994) have reported that both unipolar spindles and incompletely elongated spindles were observed in cultured hepatocytes from rats treated with TAM. Calmodulin is associated with the spindle pole body and possibly plays an important part in the proper function of mitotic spindles. TAM-induced MN and aneuploidy may be due to the inhibitory effect of calmodulin by TAM.

TAM and TOR are possibly relevant to human carcinogenesis due to their similarities with DES. Unlike TAM, TOR is non-carcinogenic to rodents under conditions in which TAM induces liver tumors in rats (Han and Liehr, 1992). TOR and its metabolites, however, are predicted to be carcinogenic in rodents by an analysis of the chemical structure (Cunningham *et al.,* 1996). TOR induces MN and DNA adduct formation in human cells with monooxygenase activity, though the responses are less potent than those with TAM (Styles *et al.,* 1994; Hemminki *et al.,* 1995). The extent of TOR-induced DNA adduct formation is enhanced in both *in vivo* and *in vitro* systems with peroxidase activity (Davies *et al.,* 1995). The frequencies of transformation and aneuploidy in SHE cells induced by TOR were higher than those induced by TAM (Tables I, II). This can be attributed to the high level of peroxidase activity found in SHE cells (Degen *et al.,* 1983). A chlorine atom at the end

of the ethyl side chain in TOR may contribute to the peroxidative activation that leads to the cellular transformation activity of TOR.

Treatment with ICI 164,384 induced morphological transformation of SHE cells and aneuploidy in the near diploid range in the same cells, suggesting that ICI 164,384 may affect the mitotic spindles.

The percentage of heteroploid cells with a tetraploid or a near tetraploid chromosome number was increased following treatment of SHE cells with DES at 20 μM for 72 hr. Exposure to the same dosing regimen of E_2 failed to increase the percentage of heteroploid cells in the tetraploid or near tetraploid range. However, high levels of polyploidy induction have been observed when SHE cells were treated with E_2 at doses that caused growth arrest. Polyploidy induction was not demonstrated in the present study following treatment of SHE cells with TAM or TOR even at doses causing growth arrest. This suggests that, despite similar effects on aneuploidy induction in the near diploid range, these 2 chemicals appear to act by mechanisms different from E_2 or DES.

Although treatment with E_2, DES, TAM, TOR or ICI 164,384 over the dose range examined did not stimulate the growth of SHE cells, significant increases in the CFEs of SHE cells were elicited following treatment with each substance for 48 hr, except for treatment with E_2 or DES at the highest doses, which are cytotoxic. This suggests that reduction in cellular growth by these substances is not due to the killing effect but to growth arrest, probably resulting from the functional damage of the mitotic apparatus. In addition, enhancement of CFEs following treatment may indicate the acquisition of growth-stimulating activity of the cells. Estrogens (E_2 and DES) and a partial antagonist to the ER (TAM) stimulate insulin-like growth factor-I gene expression or activate the phosphorylation of insulin-like growth factor-I receptors in their target organs for cancer, corresponding to cellular proliferation (Chen and Roy, 1995). This is, however, unlikely the basis for the CFE stimulation observed in this study because ICI 182,780, a 7α-alkylamide analogue of E_2 with pure anti-estrogenic activity, like ICI 164,384, is found to be opposite from the stimulative effects seen with E_2, DES or TAM. The CFE promoting activity of these estrogens and anti-estrogens remains to be clarified.

Estrogen replacement therapy has been associated with an increase in endometrial carcinoma. Women taking TAM for therapeutic or prophylactic purposes of breast cancer have an increased risk of developing endometrial carcinoma as well (Fisher *et al.,* 1994). Although serum concentrations of estrogens in women during normal menstrual cycles are 0.1–1 nM (Murad and Haynes, 1985), which are much below the estrogen concentrations used in the present study, the levels of estrogens could vary in various tissues depending on their accumulation and metabolic fate. Serum levels of TAM at steady state in patients on adjuvant treatment of breast cancer are 0.1–1 μM (Lerner and Jordan, 1990). In humans, the concentrations of TAM and its metabolites are 10- to 60-fold higher in tissues than in serum (Lien *et al.,* 1991). In chronically treated rats, the liver levels are 100–200 times greater than the serum levels for TAM and its metabolites, while uterine levels are 400–500 times greater (Robinson *et al.,* 1991).

In conclusion, both estrogens (E_2 and DES) and anti-estrogens (TAM, TOR and ICI 164,384) induce morphological transformation of SHE cells in a dose-related fashion. These substances did not cause increases in chromosomal aberrations in SHE cells but induced numerical chromosome changes in the near diploid range, corresponding to transforming activity. Although we cannot neglect the involvement of DNA damage in SHE cellular transformation by these substances, induction of numerical chromosome changes, aneuploidy, could be a causal mechanism of estrogen- and anti-estrogen-induced cellular transformation and carcinogenesis. We are studying the induction of DNA adduct formation in SHE cells treated with these substances using the ^{32}P-post-labeling method.

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