# Effects of Estrogens on Microtubule Polymerization *in Vitro*: Correlation with Estrogenicity


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Several estrogens of diverse classes, namely stilbene estrogens, steroidal estrogens, phytoestrogens, mycoestrogens, lignans, and bisphenol A, were studied for their ability to interfere with the assembly of microtubules under cell-free conditions. Inhibition of microtubules in intact cells may lead to the induction of micronuclei and aneuploidy and thereby contribute to estrogen-mediated carcinogenesis. Diethylstilbestrol and various other stilbene estrogens, as well as bisphenol A, were good inhibitors of microtubule polymerization whereas the other tested compounds were devoid of this activity. Therefore, the ability of estrogenic substances to interact with microtubules does not correlate with the hormonal activity. — Environ Health Perspect 103(Suppl 7):21–22 (1995)



Key words: microtubules, stilbene estrogens, steroidal estrogens, phytoestrogens, mycoestrogens, lignans, bisphenol A


## Introduction

Both natural estrogens, e.g., 17β-estradiol ($E_2$), and synthetic estrogens, e.g., diethylstilbestrol (DES), are associated with human neoplasia (*1*). In experimental animals $E_2$ and DES as well as several of their analogs induce tumors in various tissues, e.g., the kidney of the male Syrian golden hamster (*1,2*). Moreover, primary cells such as Syrian hamster embryo (SHE) fibroblasts are morphologically and neoplastically transformed by $E_2$ and DES (*3–5*). In short-term assays, carcinogenic estrogens fail to induce gene mutations and DNA damage but lead to numerical and structural chromosomal aberrations (*6,7*). In particular, nonrandom aneuploidies were observed in DES-transformed SHE cells (*8*). Several laboratories have reported that DES has colchicinelike effects, causing inhibition of microtubule (MT) polymerization under cell-free conditions as well as disruption of the mitotic spindle and the cytoplasmic MT complex in mammalian cells (*9–16*). To clarify the question whether interaction with MTs is a property shared by all estrogens, we have studied several estrogens of diverse classes, namely stilbene estrogens, steroidal estrogens, phytoestrogens, mycoestrogens, and lignans, for their ability to interfere with MT assembly. Bisphenol A, used for the manufacture of polycarbonate plastics and recently identified as an environmental estrogen, has also been included in this study.


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Abbreviations used: $E_2$, 17β-estradiol; DES, diethylstilbestrol; SHE, Syrian hamster embryo; MT, microtubule.

## Methods

The MT polymerization assay used in our laboratory has been described before (*17*). Briefly, the test compound dissolved in ethanol or dimethyl sulfoxide was added to 0.5 ml (final volume) of 10 μM MT proteins from bovine brain in assembly buffer; concentrations of the estrogens varied between 10 and 100 μM; the concentration of the organic solvent was 2%. Following an incubation period of 20 min at 37°C, MT assembly was started by adding 0.5 mM guanosine triphosphate, and the increase in turbidity was measured at 350 nm for 30 min. The control incubation containing all components except the test compound was used as reference (100% assembly). Depolymerization at 4°C was carried out to confirm MT formation and detect aggregation.

## Results

The result of our study is summarized in Table 1. All stilbene estrogens tested at 50-μM concentrations caused a 10 to 40% inhibition of MT assembly. The data are published in detail elsewhere (*17*). The plastic estrogen bisphenol A proved to be a powerful inhibitor, exhibiting about half the activity of DES (*18*). In contrast, none of the tested steroidal estrogens showed a detectable inhibitory effect on MT polymerization even at 100-μM concentrations. The same lack of effect was observed for the phytoestrogens, mycoestrogens, and

**Table 1.** Ability of various estrogens[a] to inhibit microtubule (MT) polymerization under cell-free conditions.

| Inhibitors of MT assembly | No inhibition of MT assembly[b] |
|---|---|
| E-DES | Estrone, $E_1$ |
| Z-DES | 2-Hydroxy-$E_1$ |
| 3,3′-DES | 4-Hydroxy-$E_1$ |
| 3,3′,5,5′-Tetrafluoro-DES | 17β-Estradiol, $E_2$ |
| E,E-Dienestrol | 2-Hydroxy-$E_2$ |
| Z,Z-Dienestrol | 4-Hydroxy-$E_2$ |
| Indenestrol A | 17α-Ethinyl-$E_2$ |
| Indenestrol B | 11β-Methoxy-17α-ethinyl-$E_2$ |
| Indanestrol | Coumestrol, 50 μM |
| Erythro-hexestrol | Genistein, 50 μM |
| Threo-hexestrol | Daidzein, 50 μM |
| Bisphenol A | Zearalenone, 50 μM |
| | Zeranoe, 50 μM |
| | Enterolactone |
| | Enterodiol |
| | Equol |

[a]Nomenclature: DES, 3,4-di(4′-hydroxyphenyl)-hex-3-ene; 3,3′-DES, 3,4-di(3′-hydroxyphenyl)-hex-3-ene; 3,3′,5,5′-tetrafluoro-DES, 3,4-di(3′,5′-difluoro-4′-hydroxyphenyl)-hex-3-ene; dienestrol, 3,4-di(4′-hydroxyphenyl)-hexa-2,4-diene; indenestrol A, 1-ethyl-2-(4′-hydroxyphenyl)-3-methyl-5-hydroxyindene; indenestrol B, 1-methyl-2-(4′-hydroxyphenyl)-3-ethyl-6-hydroxyindene; indanestrol, 1-ethyl-2-(4′-hydroxyphenyl)-3-methyl-5-hydroxyindane; hexestrol, 3,4-di(4′-hydroxyphenyl)-hexane; bisphenol A, 2,2-di(4′-hydroxyphenyl)-propane. [b]At 100-μM concentration except when noted otherwise.

lignans listed in Table 1. However, several of these compounds could not be tested at 100-μM concentrations due to limitations in solubility. The highest concentrations tested without precipitation of the estrogens are given in Table 1.

## Discussion

When the inhibitory effects of the various compounds for MT assembly are compared with their known estrogen receptor binding affinities, it becomes obvious that no correlation between these two properties exists. Stilbene estrogens with very low estrogen receptor binding, such as Z,Z-dienestrol, indanestrol, or threo-hexestrol (*19*), inhibit MT polymerization as well as the strong estrogens DES, E,E-dienestrol, or erythro-hexestrol. On the other hand, powerful steroidal estrogens such as $E_2$, 17α-ethinyl-$E_2$, and 11β-methoxy-17α-ethinyl-$E_2$ (moxestrol) fail to inhibit MT assembly, as do the moderately strong phytoestrogens and mycoestrogens coumestrol, genistein, and zearalenone.

Recent studies on the mechanism of the DES-mediated inhibition of MT polymerization have shown that DES has two binding sites on the tubulin heterodimer (*20*), which is the major constituent of MT proteins. One of the binding sites of DES is related to that of colchicine, as both compounds compete for this site. Binding to this site probably causes the inhibition of MT assembly. The other DES binding site seems to have no relevance for MT polymerization, as it also binds $E_2$, which displays no inhibitory effect.

It should be pointed out that the effect of a compound on MT assembly observed under cell-free conditons may be different in intact cells where metabolism of the estrogen can either diminish or enhance the propensity for tubulin binding. An increased inhibition of MT assembly might be expected for all compounds capable of quinone formation, e.g., DES, indenestrol A, the catechol estrogens 2- and 4-hydroxy-$E_1$ and $E_2$, and possibly coumestrol, as these reactive intermediates should covalently bind to thiol groups of tubulin essential for MT polymerization (*21*). Furthermore, mechanisms other than inhibition of MT formation may be employed by certain estrogens to cause chromosomal nondisjunction or chromosomal breakage. Examples are $E_2$ and coumestrol (*22*): $E_2$ appears to damage the kinetochore and thereby impair the attachment of the mitotic spindle to the chromatids, whereas coumestrol seemingly has clastogenic effects in mammalian cells. Therefore, the noncovalent interaction with tubulin and subsequent inhibition of MT assembly is only one among several mechanisms whereby estrogens can cause chromosomal damage. This study has shown that the ability of an estrogenic substance to bind tubulin does not correlate with the hormonal activity.

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