Review Article

17β-Estradiol epoxidation as the molecular basis for breast cancer initiation and prevention

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Epidemiological and animal studies have indicated that 17β-estradiol (E_2) is involved in breast cancer; however, the mechanism is unclear. We found that E_2 could be activated by epoxidation, resulting in its ability to inhibit nuclear DNA-dependent RNA synthesis, and to bind DNA, forming DNA adducts both *in vitro* and *in vivo*. Because epoxidation is required for the activation of many chemical carcinogens, including benzo(a)pyrene, 7,12-dimethylbenz(a)anthracene and aflatoxins, we proposed previously that E_2 epoxidation is the underlying mechanism for the initiation of breast cancer. The first part of this review is to present the experimental evidence obtained from this laboratory in support of this hypothesis. Based on these newly discovered insights on E_2 epoxidation and its initiation role in breast cancer carcinogenesis, a method to screen chemopreventive agents against breast cancer has been developed. This constitutes the second part of the review. Two examples will be used to illustrate the utility of this screening technique. The effect of fat on breast cancer has been a longstanding but unresolved issue. Epidemiological studies provide conflicting results regarding the association of dietary fat and breast cancer. Because vegetable oils contain various amount of mono- and polyunsaturated fatty acids, they are potential antioxidants. Data are presented to show that commercial vegetable oils, independent of their mono- or polyunsaturated fatty acid content, are all able to prevent the formation of E_2 epoxide, as measured by the loss of the ability of E_2 to inhibit nuclear RNA synthesis *in vitro*. Tamoxifen (TAM), an anti-estrogen used for breast cancer treatment, has recently been found to have a strong breast cancer preventive effect. The mechanism for this is unknown. Using the same screening technique, we found that when incubated together with E_2 for epoxidation, TAM was able to prevent the formation of E_2 epoxide, as evidenced by both the loss of the ability of E_2 to inhibit nuclear RNA synthesis and the reduced binding of [^{3}H]-labelled E_2 to nuclear DNA in a dose-dependent manner. These experimental results suggest that the breast cancer preventive effect of TAM is to prevent the formation of E_2 epoxide through a competitive epoxidation mechanism with E_2.

Key words: **17β-estradiol, 17β-estradiol epoxide, breast cancer, chemopreventive agent screening, DNA adduct, RNA synthesis, tamoxifen, vegetable oil.**

Introduction

Breast cancer is the most common form of cancer among US women, with an estimated 183 000 new cases each year, and is the second leading cause of cancer deaths estimated at 41 000 per year.[1] Animal and epidemiological studies have indicated that oestrogens are involved in uterine[2,3] and breast[4–6] cancers. However, their mechanisms are still not well understood.[7] Basically, there are two ways to fight and win the war on cancer. One is to find a means of preventing the disease and the other is to find a way to successfully treat it. Studies indicate that as many as 80% of all cancers are related to environmental or external factors and are therefore, in theory, preventable.[8,9] However, at present, because our understanding of cancer is still very limited, we cannot prevent cancers from occurring. In addition, there are social, political, economical and personal factors that may prevent the application of our knowledge in cancer prevention. An additional complication in breast cancer prevention is that 17β-estradiol (E_2) and estrone (E_1) are endogenous hormones. Furthermore, oestrogens (either natural or synthetic) are widely used in a variety of clinical conditions from oestrogen replacement therapy to cancer treatment, irrespective of the fact that they are known to be carcinogens.[2–6] Earlier studies have clearly demonstrated that the synthetic oestrogen diethyl-stilbestrol (DES), used with the goal of stabilising pregnancies, has been associated with an increased risk of breast cancer in those women who take it,[10] and of vaginal adeno-carcinoma in their daughters.[11] Results from oestrogen replacement studies have indicated that although exposure to exogenous oestrogen for less than two years does not increase the risk of breast cancer, extended periods of use lasting more than 10 years may increase the risk by 25–30%.[12] Data from recent prospective case-control studies have clearly shown that there is a positive association between blood levels of E_2 and E_1 and the risk of breast cancer in postmenopausal women.[4–6] Clearly, the basic understanding of the molecular

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mechanism of these carcinogens is fundamentally important for the proper design of breast cancer prevention strategies and treatment of the disease.

17β-estradiol epoxidation as the underlying mechanism of breast cancer initiation

Several years ago, we found that E_1 and E_2 could be activated by the epoxide-forming oxidant dimethyldioxirane (DMDO). This resulted in the inhibition of rat liver nuclear and nucleolar RNA synthesis *in vitro* (Fig. 1).[13] Because epoxidation is required for the activation of many well-known chemical carcinogens (e.g., benzo(a)pyrene, 7,12-dimethylbenz(a)anthracene and aflatoxins),[14–23] we proposed that oestrogen epoxidation is the underlying mechanism for the initiation of breast cancer (Fig. 2).[13]

Chemical carcinogenesis is a multistage process that includes initiation, promotion and progression.[14–19] Initiation, the first critical and irreversible step in carcinogenesis, requires the covalent binding of a carcinogen to DNA.[14–19] For this reason, one of the basic tests of our hypothesis was to determine whether E_1 and E_2 are able to bind to DNA after epoxide activation. In support of our hypothesis, we found
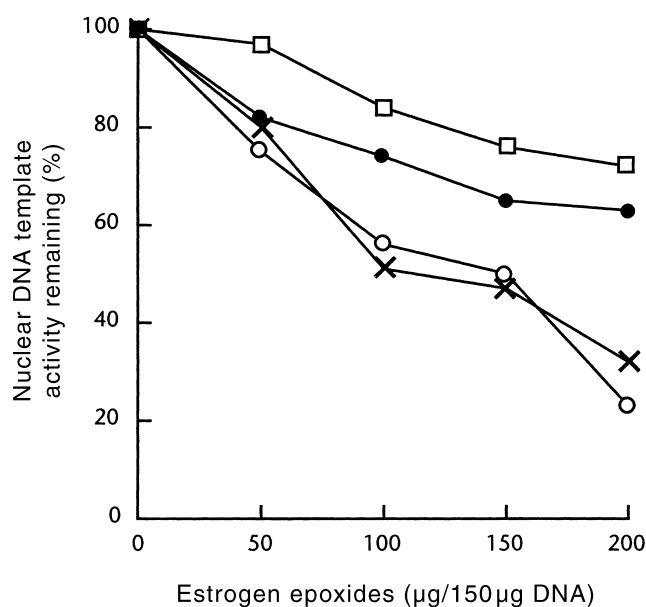
that [^{3}H]-labelled E_1 and E_2 are able to bind to DNA only after epoxide activation using several different DNA templates (Fig. 3).[24,25] The covalent binding nature of E_1 and E_2 to DNA was further confirmed by [^{32}P]-post-labelling analysis (Fig. 4).[24–26]

However, as these results were obtained mainly from *in vitro* experiments, it is important to show that oestrogen DNA adducts are also formed *in vivo*. A recent report of female ACI rats showed that when a continuous treatment of E_2 was delivered through Silastic tubing implants containing 27.5 mg crystalline E_2, 100% of the rats developed mammary tumours within a year.[27] Using the same strain of

Figure 3. Binding of [^{3}H]-labelled 17β-estradiol (E_2) epoxide to (○–○) calf thymus DNA, (●–●) poly(●(A-T)) and (×–×) poly(●(G-C)). Values given are the average of 2–3 separate experiments. (Reproduced, with permission, from reference 14.)

Figure 1. Dose–response inhibition curves of (×–×) dimethyldioxirane (DMDO)-activated estrone (E_1), (○–○) 17β-estradiol (E_2), (□–□) diethylstilbestrol (DES) and (●–●) tamoxifen (TAM) on rat liver nuclear RNA synthesis *in vitro*. Values given are the mean of 2–4 separate experiments. (Reproduced, with permission, from reference 13.)
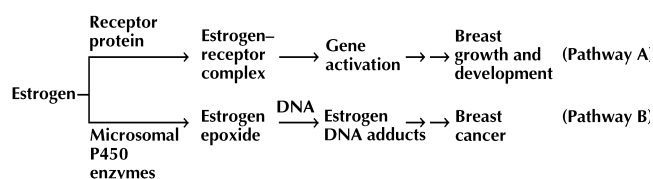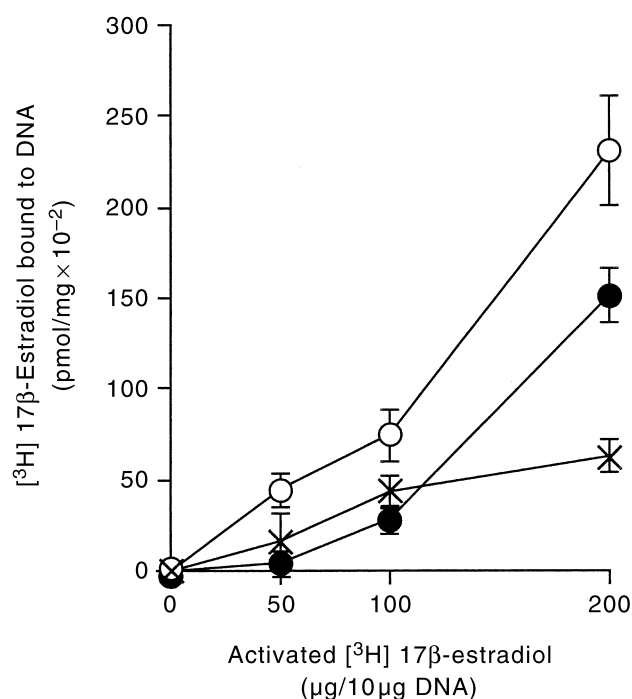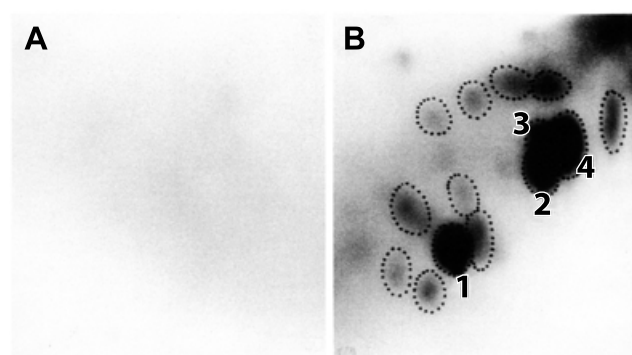
Figure 2. Epoxidation of estrogen as the basis for carcinogenesis.

Figure 4. ^{32}P-postlabelling maps of the 17β-estradiol (E_2) and dimethyldioxirane (DMDO)-activated E_2-treated calf thymus DNA. (a) Control group; (b) E_2 epoxide group. (Reproduced, with permission, from reference 25.)

rats, we found that when the female ACI rats were given intramammillary injections of E_2 or DMDO-activated E_2 (i.e., E_2 epoxide), identical DNA adducts were formed *in vivo*, and the E_2 epoxide was at least 25 000 time more active than E_2 in the formation of DNA adducts in mammary glands (Figs 5,6).[28–30] Therefore, these *in vitro* and *in vivo* experiments have provided critical evidence in support of our proposed hypothesis of oestrogen epoxidation and the initiation of oestrogen carcinogenesis.[13]

17β-estradiol epoxidation as a molecular basis for breast cancer prevention

It is clear that in order to properly prevent a disease, it is necessary to know the cause of the disease. In terms of breast cancer, our findings that E_2 could be activated by epoxidation[13] and was consequently able to bind DNA, forming DNA adducts, *in vitro* and *in vivo*[24–26,28–30] have provided a strong molecular basis for an initiatory role of E_2 in breast cancer aetiology.[13] Based on this new insight, we
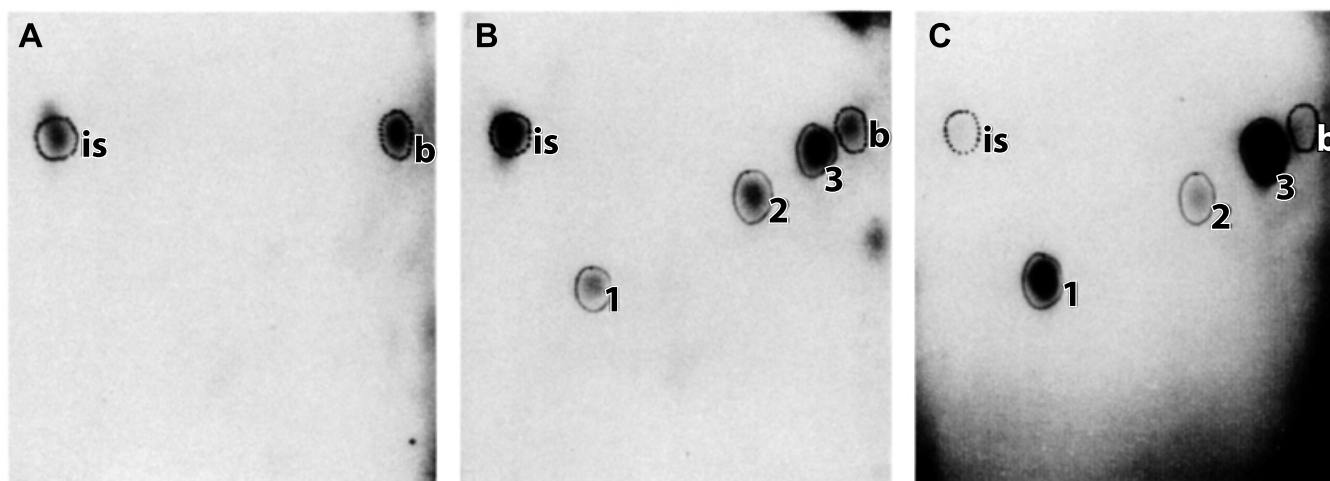

Figure 5. *In vivo* detection of 17β-estradiol (E_2)-DNA adducts in the mammary glands of female ACI rats after intramammillary injections of E_2 or E_2 epoxide. (a) Control group: only the solvent, 20% DMSO in corn oil, was injected. (b) E_2 group: single injection of 250 μg/mammary gland per day for three consecutive days. (c) E_2 epoxide group: single injection of 1 μg/mammary gland. (Reproduced, with permission, from reference 30.)
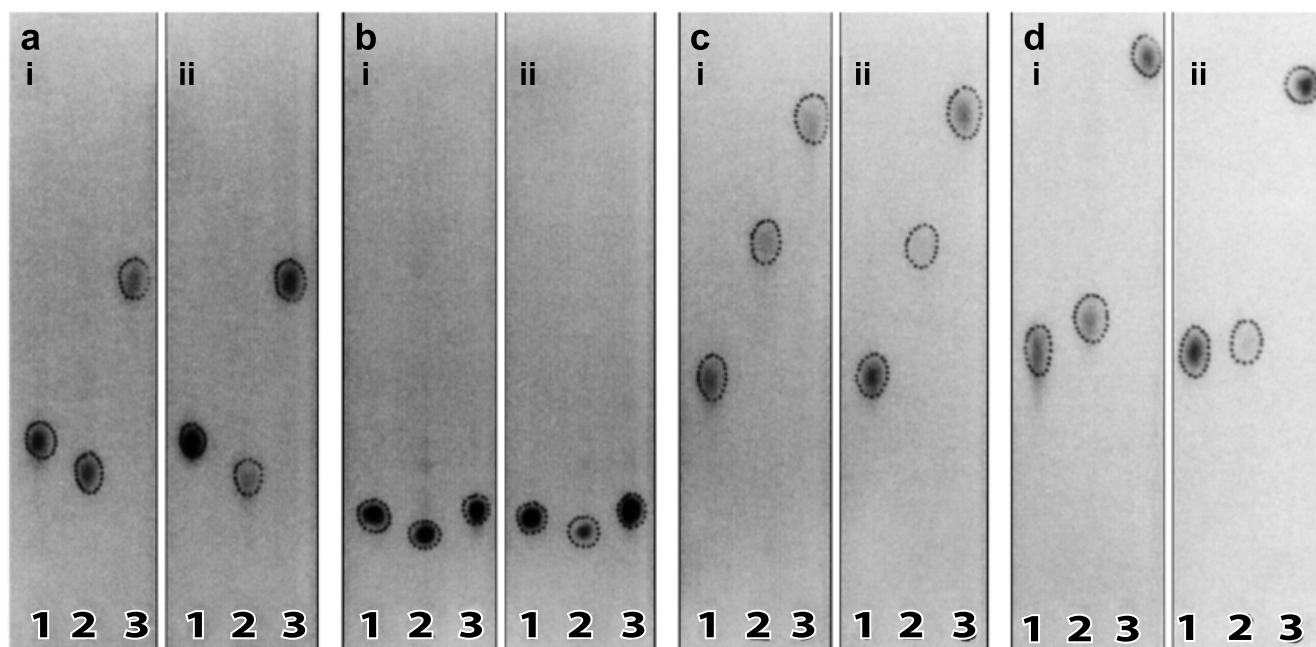

Figure 6. *In vivo* evidence for the formation of identical 17β-estradiol (E_2)-DNA adducts in the mammary glands of female ACI rats given intramammillary injections of E_2 or E_2 epoxide. The major DNA adducts, namely 1, 2 and 3, from both E_2 and E_2 epoxide groups were excised, eluted, concentrated and analysed by thin-layer chromatography under four different solvent systems. (a) 0.4 M Tris-HCl, 0.4 M H_3BO_3, 8 mM EDTA, 1.04 M NaCl, and 6.4 M urea (pH 8); (b) Isopropanol: 4 N NH_4OH (1:1, v/v); (c) 0.56 M LiCl, 0.24 M NaH_2PO_4, 0.4 M Tris-base, and 6.8 M urea (pH 4.5); (d) 0.64 M NaH_2PO_4, 0.4 M Tris-HCl and 6.8 M urea (pH 8.0). (i) 1, 2 and 3 are the three major DNA adducts from the E_2 group, as shown in Figure 5. (ii) 1, 2 and 3 are the three major DNA adducts from the E_2 epoxide group, as shown in Figure 5. (Reproduced, with permission, from reference 30.)

have developed a technique to screen potential chemo-preventive agents at the initiation step of breast cancer carcinogenesis. This screening test determines whether a chemical agent is able prevent the formation of E_2 epoxide (i.e., prevention at the initiation step), as measured by both the loss of the ability of E_2 to inhibit nuclear DNA-dependent RNA synthesis[13,24,25] and the ability of [³H]-labelled E_2 to bind to nuclear DNA.[24–26,28–30] The following two examples are used to illustrate the utility of this screening technique.

1. Evidence for the potential of vegetable oils in breast cancer prevention

The effect of dietary fat on breast cancer has been a long-standing and unresolved issue.[31–33] Although it is a popular belief that monounsaturated fat (e.g., olive oil) protects and polyunsaturated fat (e.g., linoleic acid) promotes breast cancer carcinogenesis,[33–35] results from recent large-scale epidemiological studies have found no evidence that the intake of either total fat or specific subtypes of fat were associated with breast cancer risk.[36–38] There are at least two basic reasons why this issue has not been resolved for so long: (i) Epidemiological studies measuring dietary intake do not take into consideration other lifestyle risk factors (e.g., obesity, physical activity and other eating habits) that may contribute to the final outcome of the disease; (ii) Chemical carcinogenesis is a multistage process[14–19] and epidemiological studies are not able to differentiate the beneficial or harmful effects of dietary fat at a defined stage during the multistage process of carcinogenesis. Based on the above analyses, it is clear that in order to have a better understanding of the effect of dietary fat on breast cancer, it is necessary to dissect and study the effect of dietary fat at the individual steps of the multistage process of chemical carcinogenesis.

Because vegetable oils contain various amounts of mono- and polyunsaturated fatty acids, they are potential anti-oxidants. The results from our studies, as shown in Fig. 7, indicate that commercial vegetable oils, independent of their mono- or polyunsaturated fatty acid content, are all able to prevent the formation of E_2 epoxide, as measured by the loss of the ability of E_2 to inhibit nuclear RNA synthesis in vitro.

These are very dramatic findings. Basically, these results confirm our belief that vegetable oils are effective anti-oxidants and are able to prevent the formation of E_2 epoxide in vitro. However, because vegetable oils are heated in cooking (except when used in salad dressing), and because heating may cause oxidation of the unsaturated fatty acids in the vegetable oil, possibly causing them to lose their protective effect against E_2 epoxidation, it is important to know whether heating will abolish the protective effect of the vegetable oils. As indicated in Table 1, heating the vegetable oils at 200°C for 5 min did not reduce this preventive effect.

2. Prevention of E_2 epoxide formation through competitive epoxidation as the mechanism for tamoxifen in breast cancer prevention

Tamoxifen has been used for adjuvant therapy in breast cancer treatment since the early 1970s. Recent large clinical trials indicate that TAM is also an effective chemopreventive agent for breast cancer.[39] Because TAM is known to block the binding of E_2 to its receptor, this anti-estrogen action is believed to be the underlying mechanism for the efficacy of
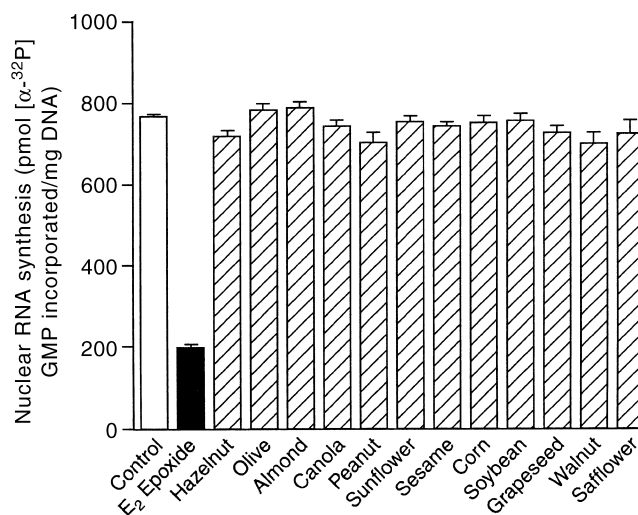
Figure 7. The preventive effect of vegetable oils on the epoxide formation of 17β-estradiol (E_2) in vitro. (□), Control group, 1 mg E_2 + 0.5 mL acetone; (■), E_2 epoxide group, 1 mg E_2 treated with 0.5 mL dimethyldioxirane (DMDO); and (▨), vegetable oil group, 1 mg E_2 + 20 μL vegetable oil treated with 0.5 mL DMDO. Values given are the mean of 4–6 independent experiments. GMP, guanosine 5-monophosphate.

Table 1. Preventive effect of vegetable oil on the inhibition of nuclear RNA synthesis by 17β-estradiol (E_2) epoxide in vitro after heating

Group	Nuclear RNA synthesis (pmol [³²P]-GMP incorporated/mg DNA)			
	Before heating	%	After heating	%
Control	768 ± 9	100	768 ± 9	100
Olive oil	768 ± 10	100	745 ± 15	97
Sunflower oil	750 ± 16	98	691 ± 7	90
Corn oil	791 ± 32	103	714 ± 8	93
Soybean oil	742 ± 18	97	722 ± 15	94
Grapeseed oil	745 ± 43	97	752 ± 8	98

Values given are the mean of 2–3 independent experiments. GMP, guanosine 5-monophosphate.

TAM in breast cancer therapy. However, this single mode of action of TAM is inadequate in explaining the fact that TAM is also known to induce endometrial[39–42] and possibly other[43–45] cancers. Recent studies indicate that after metabolic activation, TAM is able to bind to DNA, forming DNA adducts.[45] These results strongly suggest that TAM is not only a carcinogen but, more specifically, an initiating carcinogen. Based on these facts, it is believed that TAM has at least two opposing mechanisms of action: (i) competing with E_2 at the receptor level and blocking the promotional role of E_2 in breast cancer; and (ii) binding to DNA after metabolic activation and initiating carcinogenesis. However, this dual mechanism of TAM action is still not able to explain how it is able to prevent breast cancer.[39]

17β-Estradiol requires activation by epoxidation to bind to DNA and form DNA adducts,[24–26,28–30] as does TAM[13] (Tables 2,3). This raises the possibility that TAM, as an effective competitor for epoxidation, may act indirectly by preventing the formation of E_2 epoxide and, consequently, breast cancer. Our recent studies (Figs 8,9) have indeed shown that when incubated together with E_2 for epoxidation, TAM is able to dramatically reduce the formation of E_2 epoxide, as measured by both the loss of the ability of E_2 to inhibit nuclear RNA synthesis and the reduced binding of [³H]-labelled E_2 to nuclear DNA. Identical results were obtained when TAM and E_1 were used. These results strongly suggest that the prevention of E_2 epoxide formation through competitive epoxidation is the underlying mechanism used by TAM for its preventive effect against breast cancer.

Conclusions

Evidence has been presented to show that after activation by epoxidation, E_2 is able to inhibit DNA-dependent RNA synthesis and bind to DNA, forming DNA adducts, both *in*

Table 2. Inhibition of 17β-estradiol (E_2) epoxide on nuclear RNA synthesis *in vitro*

Group	Nuclear RNA synthesis (pmol [³²P]GMP incorporated/mg DNA)	%
Control	768 ± 9	100
E_2	829 ± 15	108
E_2 epoxide	200 ± 8	26

Values given are the mean of 3–4 independent experiments. GMP, guanosine 5-monophosphate.

Table 3. Effect of tamoxifen (TAM) and TAM epoxide on nuclear RNA synthesis *in vitro*

Group	Nuclear RNA synthesis (pmol [³²P]GMP incorporated/mg DNA)	%
Control	610 ± 16	100
TAM	491 ± 18	81
TAM epoxide	427 ± 18	70

Values given are the mean of 3–4 independent experiments. GMP, guanosine 5-monophosphate.

vitro and *in vivo*. These experimental results not only lend strong support to our hypothesis regarding E_2 epoxidation and the initiation of breast cancer, but also provide a molecular basis to screen potential chemopreventive agents against breast cancer. As shown in screening for the preventive potentials of vegetable oils and in deciphering the underlying mechanism of TAM for its preventive effect against breast cancer, the basic screening test determines whether a chemical agent is able to prevent the formation of E_2 epoxide (i.e., prevention at the initiation step), as measured by both the loss of the ability of E_2 to inhibit nuclear
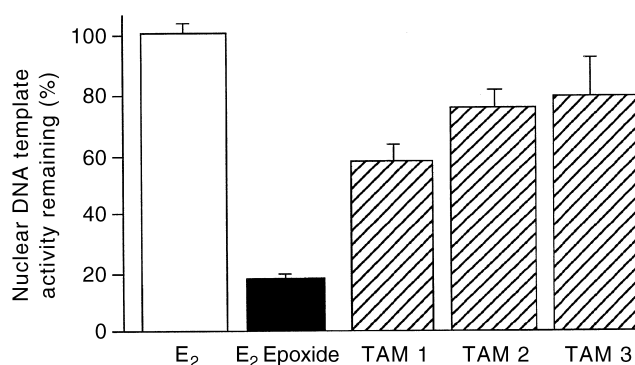

Figure 8. Preventive effect of tamoxifen (TAM) on the epoxide formation of 17β-estradiol (E_2) *in vitro*, reflected in the loss of inhibition of RNA synthesis. (□), Control group, 1 mg E_2 + 0.5 mL acetone; (■), E_2 epoxide group, 1 mg E_2 treated with 0.5 mL dimethyldioxirane (DMDO); and (▨), tamoxifen (TAM) groups, 50 μg (TAM1), 100 μg (TAM2) or 250 μg (TAM3) TAM. Values given are the mean of 3–4 independent experiments.
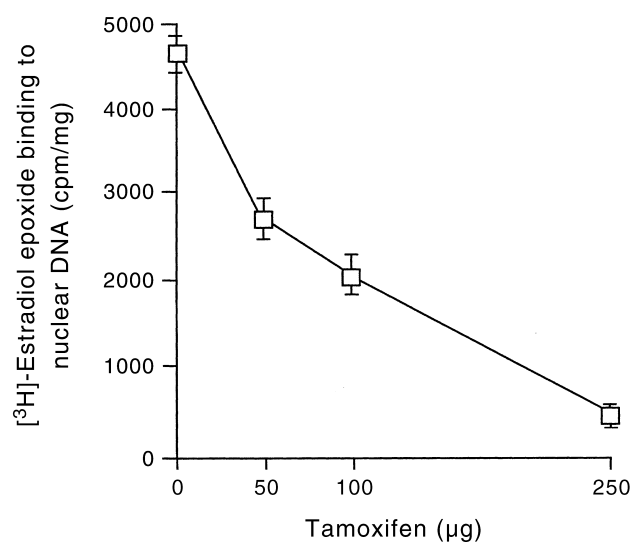

Figure 9. Preventive effect of tamoxifen (TAM) on the epoxide formation of 17β-estradiol (E_2) *in vitro*, reflected in the reduced binding of [³H]-labelled E_2 to nuclear DNA. (□), Control group, 1 mg E_2 (containing 10 μCi [³H]-labelled E_2) + 0.5 mL acetone; (■), E_2 epoxide group, 1 mg E_2 (containing 10 μCi [³H]-labelled E_2) + 0.5 mL dimethyldioxirane (DMDO); and (▨), tamoxifen (TAM) groups, 50 μg (TAM1), 100 μg (TAM2) or 250 μg (TAM3) TAM. Values given are the mean ± SE of 2–3 independent experiments.

DNA-dependent RNA synthesis and the ability of [^{3}H]-labelled E$_2$ to bind to nuclear DNA. We believe that this screening technique will provide a fast and economical way to identify a wide variety of potential chemopreventive agents for further *in vivo* animal testing.

References

1. Greenlee RT, Murray T, Bolden S, Wingo PA. Cancer statistics. CA Cancer J Clin 2000; 50: 7–33.
2. Mcgonigle KF, Karlan BY, Barbuto DA, Leuchter RS, Lagasse LD, Judd HL. Development of endometrial cancer in women on estrogen and progestin hormone replacement therapy. Gynecol Oncol 1994; 55: 126–132.
3. Grady D, Gebretsakid T, Kerlikowske K, Ernster V, Petitti D. Hormone replacement therapy and endometrial cancer risk: a meta-analysis. Obstet Gynecol 1995; 85: 304–313.
4. Toniolo PG, Levitz M, Zeleniuch-Jacquotte A, Banerjee S, Koenig KL, Shore RE, Strax P, Pasternack BS. A prospective study of endogenous estrogens and breast cancer in postmenopausal women. J Nat Can Inst 1995; 87: 190–197.
5. Colditz GA. Relationship between estrogen levels, use of hormone replacement therapy, and breast cancer. J Natl Cancer Inst 1998; 90: 814–823.
6. Hankinson SE, Willett WC, Manson JE, Colditz GA, Hunter DJ, Speigelman D, Barbieri RL, Speizer FE. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 1998; 90: 1292–1299.
7. Feigelson HS, Henderson BE. Estrogens and breast cancer. Carcinogenesis 1996; 17: 2279–2284.
8. Doll R, Peto R. The causes of cancer: quantitative estimates of available risks of cancer in the United States today. J Natl Cancer Inst 1981; 66: 1191–1308.
9. Weinstein IB. Cancer prevention: recent progress and future opportunities. Cancer Res 1991; 51: 5080S–5085S.
10. Greenberg ER, Barnes AB, Resseguie Z, Barrett JA, Burnside S, Lanza LL, Neff RK, Stevens M, Young RH, Colton T. Breast cancer in mothers given diethylstilbestrol in pregnancy. N Engl J Med 1984; 311: 1391–1398.
11. Herbst AL, Ulfelder H, Poskauzer DC. Adenocarcinoma of the vagina: association of maternal stilbestrol therapy with tumor appearance in young women. N Engl J Med 1971; 284: 878–881.
12. Harris RE, Namboodiri KK, Wynder EL. Breast cancer risk: effects of estrogen replacement therapy and bodymass. J Natl Cancer Inst 1992; 84: 1575–1582.
13. Yu F-L, Bender W. Activation of 17β-estradiol and estrone by dimethyldioxirane and inhibition of rat liver nuclear and nucleolar RNA synthesis in vitro. Carcinogenesis 1996; 17: 1957–1961.
14. Miller EC, Miller JA. Searches for ultimate chemical carcinogens and their reactions with cellular macromolecules. Cancer 1981; 47: 2327–2345.
15. Weisburger EK. Metabolism and activation of chemical carcinogens. Mol Cell Biochem 1980; 32: 95–104.
16. Farber E. Cellular biochemistry of the stepwise development of cancer with chemicals: GHA Clowes memorial lecture. Cancer Res 1984; 44: 5463–5474.
17. Dipple A. DNA adducts of chemical carcinogens. Carcinogenesis 1995; 16: 437–441.
18. Harris CC. Chemical and physical carcinogenesis: advances and perspective for the 1990s. Cancer Res 1991; 51: 5023S–5044S.
19. Hemminki K. DNA adducts, mutations and cancer. Carcinogenesis 1993; 14: 2007–2012.
20. Yu F-L, Bender W, Hutchroft A. Studies on the binding and transcritional properties of aflatoxin B$_1$-8,9-epoxide. Carcinogenesis 1994; 15: 1737–1741.
21. Yu FL, Cahill JM, Lipinski LJ, Dipple A. Effect of aflatoxin B$_1$-8,9-epoxide-DNA adducts on transcription of a *supF* gene fragment. Cancer Lett 1996; 109: 77–83.
22. Park KK, Surh YJ, Stewart BC, Miller JA. Synthesis and properties of vinyl-carbamate epoxide, a possible ultimate electrophilic and carcinogenic metabolite of vinyl carbamate and ethyl carbamate. Biochem Biophys Res Commun 1990; 169: 1094–1098.
23. Park KK, Sohn Y, Liem A, Kim HJ, Stewart BC, Miller JA. The electrophilic and tumorigenic activities of phenol and 4-nitrophenyl vinyl ethers and their epoxide metabolites. Carcinogenesis 1997; 18: 431–437.
24. Yu FL, Wang MY, Li DH, Bender W, Zheng WY. Evidence for the DNA binding and adduct formation of estrone and 17β-estradiol after dimethyldioxirane activation. Chemico–Biol Interacts 1998; 110: 173–187.
25. Yu F-L, Bender W, Zheng WY, Wang MY. The transcriptional effects and DNA-binding specificities of 17β-estradiol after dimethyldioxirane activation. Carcinogenesis 1998; 19: 1127–1132.
26. Yu FL, Zheng WY, Wang MY, Bender W, Cheerva A, Miller J. The effect of 17β-estradiol-DNA adducts on the replication of exon #5 of the human tumor suppressor gene p53. FEBS Lett 1999; 454: 7–10.
27. Shull JD, Spady TJ, Synder MC, Johansson SL, Pennington KL. Ovary-intact, but not ovariectomized female ACI rats reacted with 17β-estradiol rapidly develop mammary carcinoma. Carcinogenesis 1997; 18: 1595–1601.
28. Yu FL, Wang MY, Bender W. A hypothesis on breast cancer. In: Ioannidou-Mouzaka L, Agnantis NJ, Lopez DM, eds. 22nd Congress of the International Association for Breast Cancer Research, 24–28 September, 1998, Athens, Greece. Bologna: Monduzzi Editori, 1998; 19–24.
29. Yu FL, Wang MY, Bender W. Evidence for an initiation mechanism of 17β-estradiol carcinogenesis. 90th Annual Proceedings of the American Association for Cancer Research, 10–14 April, 1999, Philadelphia. Linthicum: Cadmus Journal Services, 1999; 40: 45.
30. Yu FL, Wang MY, Bender W. Evidence for an initiation mechanism of 17β-estradiol carcinogenesis. In: Li JJ, Daling J, Li SA, eds. Hormonal Carcinogenesis, III. New York: Springer-Verlag, 2000; 444–450.
31. Greenwald P. Role of dietary fat in the causation of breast cancer: Point. Cancer Epidemiol Biomarkers Prev 1999; 8: 3–7.
32. Hunter DJ. Role of dietary fat in the causation of breast cancer: Counterpoint. Cancer Epidemiol Biomarkers Prev 1999; 8: 9–13.
33. Wynder EL, Cohen LA, Muscat JE, Winters B, Dwyer JT, Blackburn G. Breast cancer: Weighing the evidence for a promoting role of dietary fat. J Natl Cancer Inst 1997; 89: 766–775.
34. Wolk A, Bergstrom R, Hunter D, Willett W, Ljung H, Holmberg L, Bergkvist L, Bruce A, Adami H. A prospective study of association of monounsaturated fat and other types of fat with risk of breast cancer. Arch Intern Med 1998; 158: 41–45.
35. Trichopoulou A, Katsouyanni K, Struver S, Tzala L, Gnardellis C, Rimm E, Trichopoulos D. Consumption of olive oil and specific food groups in relation to breast cancer risk in Greece. J Natl Cancer Inst 1995; 87: 110–116.
36. Willett WC, Hunter DJ, Stampfer MJ, Colditz G, Manson JE, Spiegelman D, Rosener B, Hennekens CH, Speizer FE. Dietary fat and fiber in relation to risk of breast cancer: An 8-year follow-up. JAMA 1992; 268: 2037–2044.
37. Holmes MD, Hunter DJ, Colditz GA, Stampfer MJ, Hankinson SE, Speizer FE, Rosner B, Willett WC. Association of dietary intake of fat and fatty acids with risk of breast cancer. JAMA 1999; 281: 914–920.
38. Velie E, Kulldorff M, Schairer C, Block G, Albanes D, Schatzkin A. Dietary fat, fat subtypes, and breast cancer in postmenopausal women: a prospective cohort study. J Natl Cancer Inst 2000; 92: 833–839.

39. Fisher B, Constantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM, Vogel V, Robidoux A, Dimitrov N, Atkins J, Daly M, Wieand S, Tan-Chiu E, Ford L, Wolmark N. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst 1998; 90: 1371–1388.

40. Van Leeuwen FE, Benraadt J, Coebergh JWW, Kiemeney LALM, Gimbrere CHF, Otter R, Schouten LJ, Damhuis RAM, Bontenbal M, Diepenhorst FW, van den Belt-Dusebout AW, van Tinteren H. Risk of endometrial cancer after tamoxifen treatment of breast cancer. Lancet 1994; 343: 448–452.

41. Fornander T, Hellstom AC, Moberger B. Descriptive clinopathologic study of 17 patients with endometrian cancer during or after adjuvant tamoxifen in early breast cancer. J Natl Cancer Inst 1993; 85: 1850–1855.

42. Kedar RP, Bourne TH, Powles TJ, Collins WP, Ashely SE, Cosgrove DO, Campell S. Effects of tamoxifen on uterus and ovaries of postmenopausal women in a randomised breast cancer prevention trial. Lancet 1994; 343: 1318–1321.

43. Rutquist LE, Johanson H, Signomlao T, Johanson T, Wilking N. Aduvant tamoxifen therapy for early stage breast cancer and second primary malignancies. J Natl Cancer Inst 1995; 87: 645–650.

44. Curtis RE, Boice JD, Shriner DA, Hankev BF, Fraumeni FF. Second cancers after adjuvant tamoxifen therapy for breast cancer. J Natl Cancer Inst 1996; 88: 832–834.

45. Phillips DH. Understanding the genotoxicity of tamoxifen? Carcinogenesis 2001; 22: 839–849.