4-(Hydroxyphenyl)retinamide selectively inhibits the development and progression of ductal hyperplastic lesions and carcinoma in situ in mammary gland

Albert Green, Anne Shilkaitis and Konstantin Christov

Department of Surgical Oncology, University of Illinois at Chicago College of Medicine, 840 South Wood Street (M/C 820), Chicago, IL 60612, USA

To whom correspondence should be addressed
Email: christov@uic.edu

In previous most chemoprevention studies on inhibition of mammary carcinogenesis, the formation of palpable tumors has been used as an end-point. Little is known about whether chemopreventive agents may similarly or selectively suppress hyperplastic and premalignant stages of the neoplastic process. In this study, we evaluated the effect of 4-(hydroxyphenyl)retinamide (4-HPR) on the development and progression of hyperplastic lesions and carcinoma in situ (CIS) in the N-methyl-N-nitrosoamide (MNU) mammary carcinogenesis model in rats. 4-HPR was used as the chemopreventive agent because of its proven inhibitory effect on both the early and late phases of mammary carcinogenesis. Treatment with 4-HPR (2.0 mM/kg diet), beginning 2 days after MNU administration and administered continuously for 10 weeks, suppressed all mammary gland lesions (hyperplasia, CIS and invasive carcinoma) in 35% of animals. In the remaining 65%, 4-HPR allowed the development of hyperplastic lesions, alone or combined with CIS, and/or invasive carcinomas (CA). 4-HPR also increased by 2-fold the ratio between CIS and CA (0.75 per animal in control versus 1.5 in 4-HPR-treated animals), suggesting that it may also suppress the transition of CIS into CA. 4-HPR, when administered beginning 4 weeks after MNU administration [when hyperplastic and premalignant (CIS) lesions are present in the mammary gland], inhibited the frequency of terminal end bud hyperplasia (TEBH) and CA but did not significantly suppress ductal hyperplasia, ductal alveolar hyperplasia, alveolar hyperplasia and CIS. In these animals, 4-HPR induced partial disintegration of mostly peripheral areas of lesions, including carcinomas. Taken together, our data indicate that 4-HPR selectively suppresses the development and progression of hyperplastic lesions and CIS in TEBs. Furthermore, it appears that, in addition to mammary carcinomas, TEBH and CIS could also be used as end-point biomarkers in breast cancer chemoprevention studies.

Introduction

The N-methyl-N-nitrosoamide (MNU) mammary carcinogenesis model in rats has been widely used in various breast cancer chemoprevention studies (1–4). Most MNU-induced mammary carcinomas are of ductal origin and, in morphology and endocrine status, appear similar to human breast cancer (5,6). Rats treated with MNU develop not only mammary carcinomas, but also various hyperplastic and premalignant lesions that in morphology resemble ductal hyperplasia (DH) and carcinoma in situ (CIS) of human breast (7–9). Most of these lesions occur prior to mammary carcinomas and their number exceeded by three to five times that of carcinomas (10,11). Little is known of the biology of these lesions and their potential for progression and malignant transformation or for regression and disintegration when treated with chemopreventive agents.

Previous studies showed that 4-(hydroxyphenyl)retinamide (4-HPR) is an effective inhibitor of mammary carcinogenesis both in animal models (12,13) and in premenopausal women (14). Recently, 9-cis-retinoic acid (15) and, particularly, Targretin (16) have also demonstrated inhibitory effects on mammary carcinogenesis as well as on the growth of established mammary tumors (17). In all these studies, palpable mammary tumors have been used as end-point biomarkers.

The main goal of the present study was to assess whether 4-HPR might selectively suppress the development and progression of various ductal hyperplastic and premalignant lesions and whether some of these lesions could be used as potential end-point biomarkers in chemoprevention studies. 4-HPR was used as the chemopreventive agent because of its low toxicity and proven inhibitory effect on both the early and late stages of mammary carcinogenesis (13). We hypothesize that 4-HPR may selectively suppress various hyperplastic lesions and CIS and thus stop the neoplastic process in mammary gland. Here, we report that: (i) 4-HPR given for 10 weeks after MNU administration suppressed all stages of the neoplastic process in mammary gland (hyperplastic, premalignant and malignant) in 35% of the animals, however, in the other 65% 4-HPR was less effective and selectively inhibited terminal end bud hyperplasia (TEBH) and ductal alveolar hyperplasia (DAH), as well as the transition of CIS into invasive carcinoma (CA); (ii) a 6 week treatment with 4-HPR, started 4 weeks after MNU administration (when hyperplastic and premalignant lesions are present in mammary gland) reduced TEBH and CA but had little effect on DH, DAH, alveolar hyperplasia (AH) and CIS.

Material and methods

Animals

Female, virgin Sprague–Dawley [Hsp: (SD/BR)] rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN) at 35 days of age and, after 1 week of quarantine, were randomized by weight and injected with MNU twice, at 43 and 50 days of age. The animals were fed 4% Purina Chow diet (Teklad, Madison, WI) ad libitum and had free access to water. The weight of the animals was measured once a week. Beginning 3 weeks after MNU administration, the animals were palpated weekly, monitoring for mammary tumor development.

Chemical carcinogen

MNU was obtained from Ash Stevens (Detroit, MI), dissolved in sterile acidified saline (pH 5.0) and injected i.p. (50 mg/kg body wt) twice as indicated above. Previous studies have shown that the i.p. injection of MNU is as effective as i.v. injection in inducing mammary carcinomas (18). In order
to increase the number of hyperplastic and premalignant lesions, we used two
doses of MNU given at 1 week intervals (19).

4-(Hydroxyphenyl)retinamide

4-HPR was obtained from R.W. Johnson Pharmaceutical Research Institute
(Spring House, PA) and was administered at 2 mM/kg diet, starting either 2
days or 4 weeks after the second dose of MNU (Figure 1). Placebo diet
containing the 4-HPR vehicle only was given to the control animals.

Fig. 1. Experimental design and groups of animals. The animals were
injected i.p. with two doses of MNU at the ages of 43 and 50 days. In a
control group (line A), animals were killed 4 and 10 weeks after MNU
administration. In experiment B, 4-HPR was given in the diet starting 2
weeks after MNU administration and continued for 10 weeks. In experiment
C, the animals were treated with 4-HPR between weeks 4 and 10 after
MNU administration.

Whole mount of mammary gland

For identification of hyperplastic and premalignant lesions in mammary gland,
which are small in size and impalpable, the whole mount procedure was used
(20). Palpable tumors or tumors visible at autopsy in all mammary glands
were also examined (Table I).

Histomorphology

On the basis of their origin and morphology, various hyperplastic lesions were
separated into four categories: (i) TEBH, when the hyperplastic process was
predominantly localized in terminal end buds (TEBs); (ii) DH, when the
hyperplastic process occupied ductal structures only; (iii) DAH, comprising
ductal and alveolar structures; (iv) AH, involving alveolar structures only.
Lesions with characteristics of CIS were also identified.

The incidence and frequency of various hyperplastic lesions and CIS were
evaluated in abdominal (#4) and inguinal (#5 and #6) mammary glands only
(Table II). The cervical (#1) and thoracic (#2 and #3) glands were not used
because of the small number of hyperplastic lesions and CIS in the former
and the difficulties in identifying and separating lesions from the latter. The
thoracic glands overlap and interdigitate with the pectoral muscle, which
makes difficult the assessment of frequency of small hyperplastic lesions.
Mammary CA were evaluated in all mammary glands and abdominal glands
concomitant with hyperplastic lesions and CIS. CA were classified as suggested
by Russo et al. (21).

Statistical analysis

Differences in the incidence of mammary gland lesions among various groups
were evaluated by ANOVA. Differences in the average numbers of tumors
were determined by ANOVA followed by unpaired Student’s t-test. SAS
software was used for statistical analysis.

Results

In order to assess the effect of 4-HPR both on the development and
progression of hyperplastic lesions and CIS, two sets of

<p>| Table I.  Experimental groups, incidence and frequency of mammary carcinomas a |
|---------------------------------|---------|---------|----------------|---------|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>No. animals</th>
<th>4-HPR (weeks) treatment</th>
<th>Sacrifice (weeks)</th>
<th>Animal wt (g)</th>
<th>Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Incidence (%)</td>
</tr>
<tr>
<td>MNU</td>
<td>10</td>
<td></td>
<td>4</td>
<td>210.3 ± 6.5</td>
<td>20.0</td>
</tr>
<tr>
<td>MNU</td>
<td>23</td>
<td></td>
<td>10</td>
<td>234.9 ± 15.8</td>
<td>95.4</td>
</tr>
<tr>
<td>MNU + 4-HPR</td>
<td>20</td>
<td></td>
<td>10</td>
<td>228.6 ± 10.3</td>
<td>45.0</td>
</tr>
<tr>
<td>MNU + 4-HPR</td>
<td>16</td>
<td></td>
<td>6</td>
<td>231.6 ± 17.3</td>
<td>87.5</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-------------</td>
<td>-------------------------</td>
<td>------------------</td>
<td>--------------</td>
<td>--------</td>
</tr>
<tr>
<td>a Tumors from all mammary glands (cervical, thoracic, abdominal and inguinal) evaluated at the tissue level by the whole mount procedure and histomorphology.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b Significant differences as estimated by ANOVA followed by unpaired Student’s t-test (P &lt; 0.05) between MNU- and MNU+4-HPR-treated animals.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c Significant difference (P &lt; 0.05) between MNU- and 4-HPR-treated (10 and 6 weeks/animal). MNU (50 mg/kg body wt) was injected i.p. twice, when the animals were 43 and 50 days old; 4-HPR (2 mM/kg diet) was given in the diet for 10 or 6 weeks.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<p>| Table II. Effect of 4-HPR on the incidence and frequency of hyperplastic lesions, CIS and CA in abdominal and inguinal mammary glands |
|---------------------------------|---------|--------|---------|--------|--------|--------|--------|</p>
<table>
<thead>
<tr>
<th>Lesion</th>
<th>MNU (no. 23) b</th>
<th>MNU+4-HPR (no. 20) a,b</th>
<th>MNU+4-HPR (no. 16) a,b</th>
<th>Incidence (%)</th>
<th>Frequency (no.)</th>
<th>Incidence (%)</th>
<th>Frequency (no.)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No lesion</td>
<td>4.3</td>
<td>1/23</td>
<td>35</td>
<td>7/20</td>
<td>0.02</td>
<td>13.0</td>
<td>2/16</td>
<td>n.s.</td>
</tr>
<tr>
<td>TEBH</td>
<td>91.3</td>
<td>1.4</td>
<td>50</td>
<td>0.6</td>
<td>0.001</td>
<td>50.0</td>
<td>0.6</td>
<td>0.001</td>
</tr>
<tr>
<td>DH</td>
<td>73.9</td>
<td>1.2</td>
<td>60</td>
<td>0.8</td>
<td>n.s.</td>
<td>56.2</td>
<td>0.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>DAH</td>
<td>47.6</td>
<td>0.9</td>
<td>15</td>
<td>0.2</td>
<td>0.008</td>
<td>62.5</td>
<td>0.9</td>
<td>n.s.</td>
</tr>
<tr>
<td>AH</td>
<td>21.7</td>
<td>0.3</td>
<td>20</td>
<td>0.2</td>
<td>n.s.</td>
<td>25.0</td>
<td>0.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>CIS</td>
<td>82.6</td>
<td>1.2</td>
<td>45</td>
<td>0.6</td>
<td>0.005</td>
<td>68.7</td>
<td>0.9</td>
<td>n.s.</td>
</tr>
<tr>
<td>CA</td>
<td>91.3</td>
<td>1.6</td>
<td>30</td>
<td>0.4</td>
<td>0.0001</td>
<td>75.0</td>
<td>1.0</td>
<td>0.05</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-------------</td>
<td>-------------------------</td>
<td>-------------------------</td>
<td>--------------</td>
<td>----------------</td>
<td>--------------</td>
<td>----------------</td>
<td>---</td>
</tr>
<tr>
<td>a The animals were killed 10 weeks after the first dose of MNU and various mammary gland lesions were evaluated in abdominal (4) and inguinal (5 and 6) mammary glands only.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b 4-HPR was given in the diet for 10 weeks starting 2 days after the second dose of MNU.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c 4-HPR treatment started 4 weeks after MNU administration.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d Values between the frequency of lesions of control (MNU) animals and animals treated for 6 weeks with 4-HPR. P: values of differences between the frequency of lesions; n.s., non-significant difference (P &gt; 0.05).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
experiments were performed (Figure 1). (i) 4-HPR was given in the diet starting 2 days after the second dose of MNU and continued for 10 weeks (Figure 1, line B). We used this protocol to evaluate the effect of 4-HPR on the development of mammary gland lesions. (ii) 4-HPR was given for 6 weeks starting 4 weeks after the second dose of MNU (Figure 1 line C). In this second experiment, we addressed questions related to the effect of 4-HPR on the progression of already established mammary lesions. When all mammary glands were examined for palpable tumors, we observed in both experiments a significant reduction in the frequency of mammary carcinomas (Table I).

4-HPR selectively suppresses the development of hyperplastic lesions and CIS in mammary gland

In MNU-treated controls, 22 of 23 rats developed mammary hyperplasia, CIS and/or CA (Table I). In contrast, in rats treated with 4-HPR beginning 2 days after MNU administration, seven of 20 animals were free of mammary gland lesions (Table II). Thus, among the animals treated with 4-HPR, two groups were identified: one (35% of the animals) where 4-HPR was highly efficacious (at least within the first 10 weeks) and suppressed all stages of the neoplastic process and a second one (65% of the animals) where 4-HPR was less efficacious, allowing the development of hyperplastic lesions alone or combined with CIS and CA (Figure 2).

The total number of hyperplastic lesions (TEBH, DH, DAH and AH) in MNU-treated animals was 3.1 times higher than that of CIS (87 versus 28) and 2.4 times higher than that of CA (87 versus 37) (Table II). In the animals treated with 4-HPR, a significant decrease in TEBH, DAH, CIS and CA was found (Table II). The frequency of DH and AH remained unchanged (Figure 2). These data suggest that the origin (TEBs, ducts and alveoli) of mammary lesions may modulate their response to 4-HPR.

We also compared the ratio between hyperplastic lesions as a whole, CIS and/or CA in MNU– and MNU+4-HPR-treated
A Green, A Shilkaitis and K Christov

Table III. Relationship between frequency of ductal hyperplastic lesions, CIS and CA

<table>
<thead>
<tr>
<th>Lesion</th>
<th>MNU</th>
<th>MNU+4-HPR$^a$</th>
<th>MNU+4-HPR$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperplasia$^c$ versus CIS</td>
<td>2.91</td>
<td>2.66</td>
<td>2.44</td>
</tr>
<tr>
<td>Hyperplasia versus CA</td>
<td>2.20</td>
<td>4.00$^d$</td>
<td>2.25</td>
</tr>
<tr>
<td>TEBH versus CIS</td>
<td>1.16</td>
<td>1.00</td>
<td>0.66</td>
</tr>
<tr>
<td>TEBH versus CA</td>
<td>0.87</td>
<td>1.50$^d$</td>
<td>0.62</td>
</tr>
<tr>
<td>CIS versus CA</td>
<td>0.75</td>
<td>1.50</td>
<td>0.90</td>
</tr>
</tbody>
</table>

$^a$ Animals treated for 10 weeks with 4-HPR.
$^b$ Animal treated for 6 weeks with 4-HPR, starting 4 weeks after MNU administration.
$^c$ Hyperplasia involves TEBH, DH and DAH.
$^d$ P < 0.05 as compared with MNU-treated animals.

Table IV. Incidence and frequency of mammary gland lesion 4 weeks after MNU administration

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Incidence (%)</th>
<th>Frequency (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEBH</td>
<td>80</td>
<td>1.6</td>
</tr>
<tr>
<td>DH</td>
<td>80</td>
<td>1.7</td>
</tr>
<tr>
<td>DAH</td>
<td>60</td>
<td>1.3</td>
</tr>
<tr>
<td>CIS</td>
<td>40</td>
<td>0.5</td>
</tr>
<tr>
<td>CA</td>
<td>10</td>
<td>0.2</td>
</tr>
</tbody>
</table>

$^a$ Ten animals were killed 4 weeks after i.p. injection of two doses of MNU (see Materials and methods). The whole mount procedure and histomorphology were used for evaluation of various lesions in abdominal and inguinal mammary glands. No. no. of lesions/treated animal.

animals and found that 4-HPR did not significantly change the ratio between hyperplastic lesions (TEBH, DH and DAH) and CIS, but increased almost 2-fold the ratio between hyperplastic lesions separately and CA, as well as between CIS and CA (bold text, Table III).

4-HPR selectively suppresses the progression of hyperplastic lesions towards CIS and CA

In the second experiment (Figure 1, line C), we assessed the effect of 4-HPR on already established hyperplastic and premalignant mammary lesions. To make sure that 4 weeks after MNU administration hyperplastic lesions and CIS do occur in mammary gland, 10 animals were killed at that point and the incidence and frequency of mammary gland lesions was evaluated by the whole mount procedure and histomorphology (Table IV). In these animals, ductal hyperplastic lesions were most frequent, although a small number of CIS and microcarcinomas (not palpable) were also found. Six weeks of treatment with 4-HPR significantly decreased the frequency of TEBH and CA, but not of DH, DAH, AH and CIS (Figure 2). From these data we concluded that 4-HPR, when initiated 4 weeks after MNU administration, is still efficacious in inhibiting mammary carcinogenesis and that, among various hyperplastic lesions, TEBH is the most sensitive to 4-HPR.

4-HPR affects the morphology of mammary gland lesions

In the animals treated with 4-HPR starting 2 days after MNU administration and continuing for 10 weeks, the histomorphology of hyperplastic lesions, CIS and CA were similar to those of the animals treated with MNU only (Figure 3c) with two exceptions: in one carcinoma, we found squamous metaplasia and in another, tumor cells deeply infiltrated the surrounding stroma. However, when 4-HPR was started 4 weeks after MNU administration and continued for 6 weeks, in most lesions (Figure 3e), including carcinomas, tissue disintegration and development of cystic formations were observed. These changes were most prominent in the peripheral areas of the lesions (Figure 3d).

Discussion

In contrast to most previous studies where palpable tumors have been used as end-points in breast cancer chemoprevention studies, in this study we addressed questions mainly related to the effect of 4-HPR on hyperplastic and premalignant mammary gland lesions which precede and/or accompany the occurrence of carcinomas. The study was designed to determine whether 4-HPR, which is effective in preventing development of mammary carcinoma, may be more or less effective in altering the development and progression of hyperplastic lesions and CIS in mammary gland (Figure 1, lines B and C). As expected, 4-HPR, when given for 10 weeks after MNU administration, decreased the incidence of mammary carcinomas by >50% (from 95.6% in control to 45% in 4-HPR-treated animals) and tumor multiplicity by >68%, in the range of previously published results (1,12,13,18). Moreover, in 35% of the animals, all stages of the neoplastic process (hyperplastic, premalignant and malignant) were inhibited. Since, as shown in previous studies (1,12,13), 4-HPR also extends the latency of tumors, some of the animals without lesions 10 weeks after initiation of 4-HPR administration will probably develop hyperplastic lesions, CIS and carcinomas later.

Among various hyperplastic lesions, 4-HPR was particularly effective in suppressing the occurrence of TEBH and DAH, but had minimal effect on DH and AH. The differential effect
of 4-HPR on the frequency of various lesions increased by almost twice the ratio between hyperplastic lesions (TEBH, DH and DAH) as a whole and CA, as well as between CIS and CA, suggesting that 4-HPR can selectively suppress the progression of ductal hyperplasia and CIS towards CA. The main objective of the second experiment was to determine the response to 4-HPR of already existing hyperplastic and premalignant lesions in mammary gland. We found that 4-HPR, when initiated 4 weeks after MNU administration, significantly reduced the frequency of TEBH and CA but was less efficacious in inhibiting DH, DAH and AH (Table II and Figure 3). Since most CIS and CA arise from TEBH, inhibition of the development and progression of TEBH is probably the main avenue of suppression of mammary carcinogenesis by 4-HPR (Figure 4). The resistance of established DH, DAH and AH to 4-HPR could be associated with the low proliferative activity of mammary epithelial cells (MECs) in these lesions and/or with other factors, as yet unknown, that may affect cell differentiation or apoptotic cell death in these lesions. In a recent study (K.Christov, unpublished data), we found data supporting the above hypothesis: in TEBH, the bromodeoxyuridine labelling index (BrdU) was 21.8 ± 7.4%, in contrast to 7.0 ± 3.1% in DAH and to 5.3 ± 1.4% in AH. Earlier publications of Russo and Russo (23,24), as well as our recent studies on the role of cell proliferation and apoptosis in mammary carcinogenesis (25), have shown that MECs in TEBs have higher proliferative activity than those in ductal and alveolar structures; this might also affect the resistance of the latter structures to MNU as well as to the chemopreventive efficacy of 4-HPR. Data have also been reported that 4-HPR suppresses alveolar differentiation of ductal epithelial cells, which might also contribute to the small frequency of DAH in the animals treated with 4-HPR (1,13,26).

We also found that 4-HPR can modulate the morphology of various lesions, including carcinomas. Cell loss and disintegration of cellular structures was mostly found in the periphery of lesions, suggesting a higher concentration of 4-HPR or its metabolites in the peripheral tumor areas or that 4-HPR is most effective in the areas of high proliferation (tumor periphery). On the basis of these data, we proposed the following hypothetical model of inhibition of mammary carcinogenesis by 4-HPR (Figure 4). This model suggests three potential levels of inhibition of mammary carcinogenesis. (i) 4-HPR suppresses the occurrence of hyperplastic lesions (TEBH and DH) and thus prevents the development of the neoplastic process. If this hypothesis is true, then in the animals without mammary carcinomas we should not expect hyperplastic lesions and CIS. This was found in 35% of the animals, supporting the above hypothesis. (ii) 4-HPR inhibits the progression of TEBH, DH and DAH into CIS and/or CA. The increased ratio between hyperplastic lesions and CIS supports this hypothesis. However, we did not find an increase in the ratio between hyperplastic lesions and CIS, suggesting that some CA may arise from hyperplastic lesions (vertical dashed lines) without involvement of CIS as an intermediate endpoint. (iii) 4-HPR suppresses the progression of CIS towards CA. This hypothesis is supported by the increased ratio between CIS and CA in the animals treated with 4-HPR (from 0.75 in MNU-treated to 1.5 in 4-HPR-treated animals). Thus, the present study provides data indicating that 4-HPR may interrupt the neoplastic process in the mammary gland very early, at the level of hyperplastic lesions, or later at the level of progression of hyperplastic lesions and CIS towards CA. TEBH was most sensitive to 4-HPR, which significantly decreased in both experiments.

In conclusion, in this study we obtained data indicating that 4-HPR suppresses the development of all stages (hyperplastic, premalignant and malignant) of mammary carcinogenesis or might selectively reduce the incidence and frequency of TEBH, DAH and CIS. 4-HPR might also suppress the progression of already established TEBH into CIS and CA. The consistent inhibition of TEBH and CIS by 4-HPR suggests that, in addition to mammary carcinomas, TEBH and CIS could be used as end-point biomarkers in breast cancer chemoprevention studies.

Acknowledgements

We thank Dr Richard Moon, who encouraged this study, and Dr Ronald Luber from NCI for reading the manuscript and valuable suggestions. The authors also wish to thank Kevin Grandfield for editing the manuscript and Lilia Hristova from the Public Health Institute, Chicago, for statistical work. This work was supported by grants from the University of Illinois at Chicago Campus Research Board and the Cancer Research Foundation of America (to K.C.). Part of this work was presented at the 89th Meeting of the AACR, New Orleans, 1998.

References


Received October 6, 1998; revised February 16, 1999; accepted March 3, 1999