Chemoprevention of Mammary Carcinogenesis in the Rat: Combined Use of Raloxifene and 9-cis-Retinoic Acid

Mario A. Anzano, Christopher W. Peer, Joseph M. Smith, Larry T. Mullen, Mark W. Shrader, Daniel L. Logsdon, Craig L. Driver, Charles C. Brown, Anita B. Roberts, Michael B. Sporn*

It is imperative to develop new agents for the prevention of breast cancer, considering the incidence of this disease and its associated morbidity and mortality (1). Tamoxifen, a nonsteroidal estrogen receptor agonist, is the hormonal therapy of choice for the treatment of breast cancer and for prevention of the recurrence of disease (2,3). The rationale for clinical use of tamoxifen in chemoprevention is that, in eight randomized controlled trials, it reduced by one third the incidence of contralateral tumors in women with breast cancer (4,5). Although tamoxifen is well tolerated and generally free of serious side effects (6), its long-term administration has been shown to increase the risk of endometrial cancer (7). In animals, tamoxifen reduced the incidence of breast cancer induced by chemical carcinogens (8,9), but it also induced highly stable DNA adducts in two rodent species (10) and, at very high doses, induced liver tumors in rats (11).

One strategy for lessening these deleterious effects of tamoxifen is to use it at reduced concentrations in conjunction with another chemopreventive agent such as a vitamin D analogue (12) or 9-cis-retinoic acid (9cRA) (13). Another strategy is to use other estrogen response modifiers that are not uterotrophic.

In the present study, the combination of 9cRA and raloxifene (LY156758) (formerly called keoxifene), which has a high binding affinity for the estrogen receptor and a low level of estrogenic activity and does not promote growth of uterine epithelium (14-16), was evaluated for its chemopreventive activity in the well-accepted rat model of breast cancer induced by the carcinogen nitrosomethyleneurea (NMU) (17). A previous study showed that raloxifene alone, given by injection in peanut oil, had some preventive activity in this model, although it was less potent than tamoxifen (8).

Three hundred virgin female Sprague-Dawley rats were obtained from Taconic Farms, Germantown, NY, and 264 rats were used in two separate experiments (84 rats in experiment 1 and 180 rats in experiment 2) with identical design. At 55 days of age, the rats were anesthetized by metofane inhalation and were given a single intravenous injection of NMU (50 mg/kg body weight) as previously described (12). One week later, the animals were randomized to one of six experimental groups (Table 1).

Raloxifene was provided by Eli Lilly and Co. (Indianapolis, IN), and 9cRA was obtained from Kuraray Company (Osaka, Japan). Raloxifene (Ral Hi = 60 mg raloxifene per kilogram diet; Ral Lo = 20 mg raloxifene per kilogram diet) and/or 9cRA (60 mg/kg diet) were blended into the diets as described previously (12) and were fed ad libitum alone or in combination for the duration of the experiment.

Rats were observed for gross evidence of toxicity, weighed, and palpated for tumor formation twice per week. They were killed by carbon dioxide inhalation, and tumors were confirmed and weighed at autopsy. National Institutes of Health guidelines for proper and humane use of animals were observed.

The following statistical procedures were used to compare the differences between treatment groups: 1) the Fisher exact test (18) for comparing incidence rates, 2) a nonparametric test proposed by Mantel (19) for comparing average numbers of tumors per animal, and 3) the Wilcoxon rank test (18) for comparing tumor burdens. All P values are two-sided.

At both high and low doses, raloxifene alone and, most notably, in combination with 9cRA was found to be an effective agent for prevention of mammary tumors (Fig. 1, Table 1). If one combines the data from both experiments, 52 of 54 control animals given vehicle alone had mammary tumors (96% tumor incidence) at autopsy, with an average of 3.2 tumors and an average tumor burden of 12.6 g. Rats treated with Ral Hi or with Ral Lo had significant decreases in the incidence and weight of breast tumors, as measured by multiple parameters: 55% (P<.0001) and 57% (P<.0001) tumor incidence, respectively; 0.8 (P<.0001) and 1.0 (P<.0001) tumors per rat, respectively; and average tumor burdens of 2.2 g (P<.0001) and 2.3 g (P<.0001), respectively. Rats treated with 9cRA alone had 79% tumor incidence (P = .02), with an average of two tumors per rat (P = .0005) and an average tumor burden of 8.8 g (P = .007). Treatment with the combination of Ral Hi + 9cRA or Ral Lo + 9cRA showed that the addition of 9cRA to either raloxifene treatment markedly decreased the tumor incidence, the number of tumors, and the average tumor burden; P values for these parameters for the combination treatment were all <.0001 when treated animals were compared with untreated (vehicle) control animals. The addition of 9cRA to a raloxifene regimen pro-

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donna peptide.
chemopreventive agents.

Nitrosomethylurea (NMU) (50 mg/kg) was injected into controls.

Experiment 1:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of tumor-free rats/total No. of rats</th>
<th>Average No. of tumors</th>
<th>ATB†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>1/24</td>
<td>3.0</td>
<td>10.6</td>
</tr>
<tr>
<td>Ral Hi</td>
<td>4/12 (.03)</td>
<td>1.1 (.0006)</td>
<td>3.2 (.0006)</td>
</tr>
<tr>
<td>Ral Lo</td>
<td>2/12 (.26)</td>
<td>1.7 (.01)</td>
<td>2.4 (.002)</td>
</tr>
<tr>
<td>9cRA</td>
<td>3/12 (.1)</td>
<td>1.7 (.02)</td>
<td>9.0 (.08)</td>
</tr>
<tr>
<td>Ral Hi + 9cRA</td>
<td>9/12 (&lt;.0001; 1)</td>
<td>0.3 (&lt;.0001; .04)</td>
<td>0.3 (&lt;.0001; .03)</td>
</tr>
<tr>
<td>Ral Lo + 9cRA</td>
<td>7/12 (.01; .09)</td>
<td>0.5 (&lt;.0001; .03)</td>
<td>0.6 (&lt;.0001; .06)</td>
</tr>
</tbody>
</table>

Experiment 2:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of tumor-free rats/total No. of rats</th>
<th>Average No. of tumors</th>
<th>ATB†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>1/30</td>
<td>3.3</td>
<td>14.2</td>
</tr>
<tr>
<td>Ral Hi</td>
<td>15/30 (.0002)</td>
<td>0.6 (&lt;.0001)</td>
<td>1.8 (&lt;.0001)</td>
</tr>
<tr>
<td>Ral Lo</td>
<td>16/30 (.0001)</td>
<td>0.7 (&lt;.0001)</td>
<td>2.3 (&lt;.0001)</td>
</tr>
<tr>
<td>9cRA</td>
<td>6/30 (.1)</td>
<td>2.1 (.01)</td>
<td>8.7 (.02)</td>
</tr>
<tr>
<td>Ral Hi + 9cRA</td>
<td>23/30 (&lt;.0001; .06)</td>
<td>0.3 (&lt;.0001; .05)</td>
<td>0.4 (&lt;.0001; .03)</td>
</tr>
<tr>
<td>Ral Lo + 9cRA</td>
<td>24/30 (&lt;.0001; .05)</td>
<td>0.3 (&lt;.0001; .03)</td>
<td>1.8 (&lt;.0001; .05)</td>
</tr>
</tbody>
</table>

* Doses used were as follows: 60 mg raloxifene per kilogram diet (Ral Hi), 20 mg raloxifene per kilogram diet (Ral Lo), and 60 mg 9cRA per kilogram diet. All animals (55 days old) were given an injection of 50 mg nitrosomethylurea per kilogram body weight 1 week before starting the feeding of chemopreventive agents.

† P1 is the value for the comparison of rats treated with chemopreventive agents with control rats treated with vehicle alone; P2 is the value for the comparison of rats treated with 9cRA + raloxifene with rats treated with raloxifene alone.

‡ ATB = average tumor burden; average weight in grams of a rat's tumor at autopsy.

§ In experiment 1, chemopreventive agents were fed for 5 months.

In experiment 2, chemopreventive agents were fed for 4.5 months.

References:


Notes

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