

Chemoprevention by Lipoxygenase and Leukotriene Pathway Inhibitors of Vinyl Carbamate-induced Lung Tumors in Mice

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Abstract

5-Lipoxygenase pathway inhibitors, Accolate, MK-886, and Zileuton, were evaluated as chemopreventive agents in female strain A mice. The mice were administered by injection vinyl carbamate (2 × 16 mg/kg) to induce lung tumors. Two weeks later, they received in their diet Accolate (270 and 540 mg/kg), MK-886 (30 mg/kg), Zileuton (600 and 1200 mg/kg), or combinations containing the lower concentration of two agents. Thirteen weeks later, Accolate, Zileuton (only the high concentration), and combinations of Zileuton with either Accolate or MK-886 reduced lung tumor multiplicity by 37.8, 29.5, and 28.1%, respectively. They also decreased the size of the tumors and the yield of carcinomas. These results demonstrate that leukotriene inhibitors prevent lung tumors and slow the growth and progression of adenomas to carcinoma; leukotriene inhibitors warrant further consideration for potential use in humans.

Introduction

The purpose of this study was to evaluate the efficacy of three 5-lipoxygenase pathway inhibitors (Accolate, MK-886, and Zileuton) to prevent lung tumors in mice. The potential use of leukotriene inhibitors for cancer prevention has been reviewed recently (1). Inhibitors of the 5-lipoxygenase pathway have been reported to prevent lung cancer in mice and to slow the growth of lung cancer cells (1–4). The 5-lipoxygenase inhibitor, acetylsalicylic acid, was more efficacious in reducing lung tumor multiplicity by 37.8, 29.5, and 28.1%, respectively. They also decreased the size of the tumors and the yield of carcinomas. These results demonstrate that leukotriene inhibitors prevent lung tumors and slow the growth and progression of adenomas to carcinoma; leukotriene inhibitors warrant further consideration for potential use in humans.

Materials and Methods

Chemicals. Vinyl carbamate (purity ≥99%) was purchased from Toronto Research Chemicals (North York, Ontario, Canada). MK-886 was acquired from the DCPC Repository (c/o McKesson Bioservices, Rockville, MD). Accolate as 20-mg tablets (lot FAD721) was from Zeneca Pharmaceuticals (Wilmington, DE). Zileuton as 600-mg Zyflo tablets (lot 52019AF21) was from Abbott Laboratories (North Chicago, IL).

Animals. Female A/J mice (5–6 weeks of age) were purchased from The Jackson Laboratory (Bar Harbor, ME). The mice were housed in our American Association of Laboratory Animal Care-accredited laboratory animal facility. Mice were housed in polycarbonate solid-bottom, shoebox-type cages (height, 13 cm; width, 18 cm; length, 28 cm) with Anderson Bed-o-Cob 1/8 bedding (Anderson’s, Maumee, OH). The mice were quarantined for 2 weeks before the bioassay. The environment in the animal rooms was maintained at a temperature of 72 ± 2°C, relative humidity of 40–60%, at least 10–15 air changes/h, 30 foot candles of light (cage level), and a light cycle of 12 h on/12 h off. The diet was a semipurified AIN-76A diet containing 20% casein, 0.3% in-methionine, 52% corn starch, 13% dextrose, 5% corn oil, 5% Alphacel fiber, 3.5% AIN mineral mixture, 1.0% AIN vitamin mixture, and 0.2% choline bitartrate (Dyets, Inc., Bethlehem, PA). The leukotriene inhibitors were mixed into the AIN-76A diet. The diet and drinking water were provided ad libitum.

When the mice were 7–8 weeks of age, they were administered the first of two i.p. injections of vinyl carbamate of 16 mg/kg each and 7 days apart. Two weeks after the second dose of vinyl carbamate, the mice received the leukotriene inhibitors in their diet. Accolate (270 or 540 mg/kg), Zileuton (600 or 1200 mg/kg), and MK-886 (30 mg/kg) was provided at the indicated mg/kg concentrations in the diet. The doses selected for this study were based upon reports published previously (2, 3, 12); the high dose of Accolate was 100 times the Food and Drug Administration-approved dose for humans to counteract decreased bioavailability when provided in the animal diet. Binary combinations containing 600 mg/kg Zileuton with 270 mg/kg Accolate or 30 mg/kg MK-886 and 270 mg/kg Accolate with 30 mg/kg MK-886 were also provided. Mice were weighed weekly through the first 6 weeks of exposure to the leukotriene inhibitors. After which, they were then weighed every 2–4 weeks until sacrificed. Mice were sacrificed by carbon dioxide asphyxiation after 13 and 43 weeks of exposure to the drugs. The lungs were harvested, fixed overnight in formalin, transferred to 70% alcohol, and evaluated for tumors before embedding in paraffin for histology.

Histopathological Evaluation. Lung tumors were classified as being solid/alveolar adenomas, papillary adenomas, and undifferentiated carcinomas. The criteria used for solid/alveolar adenoma classification required well-differentiated, cuboidal-shaped cells obliterating at least three contiguous alveolar

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3 The abbreviation used is: NNK, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone.
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Results and Conclusions

The dose levels of the three leukotriene inhibitors and of the combinations were not toxic. Reported adverse effects in humans for the two Food and Drug Administration-approved drugs (Accolate and Zileuton) are potential elevations of liver enzymes and hypersensitivity reactions to the drugs or their active ingredients. Although Phase 1 human trials were discontinued for lack of efficacy in asthmatics, adverse effects of MK886 in humans have not been reported. During the course of the study, the mice did not exhibit any clinical sign of toxicity, nor were body weight gains affected by treatment. In fact, mice administered Accolate and MK-886 gained more weight than mice administered the control diet (data not shown). Also, none of the leukotriene inhibitors or their combinations altered the liver or spleen: body weight ratios.

The ability of the leukotriene inhibitors to prevent vinyl carbamate-induced lung tumors is presented in Table 1. After 13 weeks of exposure, the yield of lung tumors was significantly decreased by both dose levels of Accolate (270 and 540 mg/kg), the high dose of Zileuton (1200 mg/kg), and the combinations containing 600 mg/kg Zileuton with either Accolate or MK-886. The efficacy of the combination containing Zileuton and Accolate to prevent lung tumors was not significantly different from the efficacy of either inhibitor administered alone. Although when administered alone at the dose level in their combination, neither Zileuton or MK-886 prevented lung tumors; the combination containing them did significantly prevent tumors. In contrast, the combination containing Accolate and MK-886 did not reduce the yield of tumors, whereas Accolate administered alone did significantly reduce the yield of tumors.

After 13 weeks of drug exposure, the results from the mice demonstrated that two leukotriene inhibitors, Accolate and Zileuton, when administered starting 2 weeks after vinyl carbamate were capable of preventing lung tumors during the promotional phase of carcinogenesis. Two of the three binary combinations of the leukotriene inhibitors also demonstrated the ability to prevent lung tumors. However, the binary combinations did not demonstrate a consistent advantage for using more than one agent. The combination of Zileuton and Accolate was no more efficacious than either agent alone, of Zileuton and MK-886 was more efficacious than either agent, and of Accolate and MK-886 was less efficacious than one of the agents (Accolate).

With the exception of MK-886, there was no significant difference between the various chemopreventive agent treatment groups regarding efficacy of lung tumor inhibition.

After 43 weeks of drug provided in the diet, all three leukotriene inhibitors significantly reduced the yield of lung tumors (Table 1). MK-866 was the most efficacious, reducing tumor yield by 37.8%, followed by Accolate (29.5%) and Zileuton (28.1%). There was no significant difference of lung tumor multiplicity between the three leukotriene inhibitor treatment groups. The leukotriene inhibitors reduced the size of the tumors (Fig. 1). The tumors were divided into three size categories, <1, 1–3, and >3 mm. Most of the tumors, regardless of treatment, were 1–3 mm in diameter. All three lipoygenase inhibitors decreased the number of lung tumors of this size.

The histological morphology of the tumors is presented in Fig. 2. The tumors were classified as being solid adenomas, papillary adenomas, or adenocarcinomas. In mice that received the control diet, carcinomas were the most common tumor type, comprising 44% of the tumors. All three leukotriene inhibitors significantly decreased the yield of carcinomas.

Table 1 Yield of vinyl carbamate-induced lung tumors

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>n*</th>
<th>Week 13</th>
<th>n</th>
<th>Week 43</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control diet⁶</td>
<td>31</td>
<td>17.0 ± 0.74⁴</td>
<td>27</td>
<td>27.8 ± 1.17</td>
</tr>
<tr>
<td>1200 Zileuton</td>
<td>20</td>
<td>13.0 ± 1.39⁴</td>
<td>20</td>
<td>20.0 ± 1.42²⁴</td>
</tr>
<tr>
<td>600 Zileuton</td>
<td>20</td>
<td>14.7 ± 1.10⁴</td>
<td>NA*</td>
<td>NA</td>
</tr>
<tr>
<td>30 MK-886</td>
<td>20</td>
<td>19.6 ± 1.94⁴</td>
<td>19</td>
<td>17.3 ± 1.15²³⁴</td>
</tr>
<tr>
<td>540 Accolate</td>
<td>19</td>
<td>12.8 ± 1.30⁴</td>
<td>19</td>
<td>19.6 ± 1.51²³⁴</td>
</tr>
<tr>
<td>270 Accolate</td>
<td>20</td>
<td>12.4 ± 1.29⁴</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>600 Zileuton + 30 MK-886</td>
<td>20</td>
<td>11.4 ± 1.25⁴</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>600 Zileuton + 270 Accolate</td>
<td>20</td>
<td>11.4 ± 0.97²⁴</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>270 Accolate + 30 MK-886</td>
<td>20</td>
<td>13.7 ± 1.22</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Untreated control</td>
<td>11</td>
<td>0 ± 0²⁴</td>
<td>31</td>
<td>0.11 ± 0.11²⁴</td>
</tr>
</tbody>
</table>

* The number of mice at sacrifice.

All the mice of the study received the vinyl carbamate except for the untreated control group. The mg/kg concentration of the leukotriene inhibitor in the diet is indicated in front of the drug.

The results are mean ± SE.

²⁴ Significant difference from the control diet group by ANOVA followed by a Tukey test with P < 0.05.

NA, not applicable, because no animals were in these treatment groups.
nomas. The yield of solid and papillary adenomas was not significantly decreased by the leukotriene inhibitors, except for solid adenomas by MK-886 and papillary adenomas by Accolate. The results demonstrate that the leukotriene inhibitors decreased the growth and progression of adenomas to carcinomas.

Leukotriene inhibitors A-79175 and MK-886 have been shown previously to reduce the multiplicity of NNK-induced lung tumors in mice (2). Our data demonstrate that one of these drugs, MK-886, and two other leukotriene inhibitors, Accolate and Zileuton, prevent lung tumors when administered during the promotional phase of carcinogenesis, i.e., after the carcinogen. Thus, drugs that inhibit the leukotriene pathway by three different mechanisms: (a) an inhibitor of LTA₄ (Accolate); (b) a 5-lipoxygenase-activating protein inhibitor (MK-886); and (c) a 5-lipoxygenase inhibitor (Zileuton), all prevented lung tumors. Because the dose levels of the drugs that were used are comparable with human doses currently used to treat asthma, our data demonstrate that lipoxygenase inhibitors may be useful as potential cancer chemopreventive compounds, as suggested previously in the literature (12, 14). Combinations of these lipoxygenase inhibitors with different mechanisms did not appear to have a significantly greater chemopreventive activity than the individual drugs. Thus, our results do not support the use of combinations of leukotriene inhibitors to obtain additive or synergistic prevention of lung tumors. However, our evaluation was too limited to exclude that possibility. The ability of the leukotriene inhibitors to slow the neoplastic progression to cancer was indicated by the decrease in both the size of the tumors and the yield of carcinomas. The leukotriene inhibitors were administered starting 2 weeks after the vinyl carbamate, i.e., during the promotional phase of carcinogenesis, so that they appear to slow the progression to cancer by inhibiting this phase of carcinogenesis. Thus, it would appear warranted to further evaluate and develop leukotriene inhibitors as chemopreventive drugs for potential use in humans.

References


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