Abstract. Curcumin (diferuloylmethane), a phenolic compound from the plant Curcuma longa (Linn.) has been shown to exhibit antitumor activity and apoptosis in many human cancer cell lines including that of lung and liver cancer. In this study, curcumin was evaluated in BALB/c mice for its ability to inhibit pulmonary and liver adenoma formation and growth after they were orally treated with N-bis(2-hydroxypropyl)nitrosamine (DHPN). Animals were treated with DHPN in water for approximately 14 days before multiple doses of curcumin were given intraperitoneally. It was found that 200 μM curcumin reduced lung and liver tumor multiplicity by 37% (p<0.05) and 30% (p<0.05) respectively. The results indicated that curcumin significantly inhibited pulmonary and liver adenoma formation and growth in BALB/c mice. The precise mechanism by which curcumin inhibits lung and liver tumorigenesis remains to be elucidated. Thus, curcumin appears to be a promising new chemotherapeutic and preventive agent for lung and liver cancer induced by DHPN.

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Key Words: Curcumin, DHPN (N-bis(2-hydroxypropyl)nitrosamine, tumorigenesis BALB/c mice, in vivo.
Materials and Methods

Materials and reagents. Curcumin, DHPN and olive oil were obtained from Sigma (MO, USA). RPMI-1640, fetal bovine serum, penicillin-streptomycin and glutamine were obtained from Gibco BRL (Grand Island, NY, USA).

BALB/c mice. Male BALB/c mice (approximately 22-28 g) were obtained at the age of 5 weeks from the Laboratory Animal Center, National Taiwan University College of Medicine (Taipei, Taiwan). Animals were quarantined for 1 week and housed with woodchip bedding in environmentally controlled cages, with a 12-hour light–dark cycle and 50% relative humidity. Drinking water and diet were supplied ad libitum. The animals were maintained at the Animal Center of the China Medical University for 2 weeks under animal guidelines before the grouping and experiments were performed.

Tumor induction and curcumin treatment. Seventy-five BALB/c mice were split into 5 groups. Four groups of BALB/C mice, at the age of 6 weeks, were given drinking water containing 0.1% DHPN for 2 weeks, while group 1 received water only. From the age of 7 weeks, treatment groups of animals received corn oil alone or containing 0, 10, 100, or 200 μM curcumin in 0.2 ml corn oil. Noncurcumin-treated animals receiving corn oil alone acted as positive control.

Five groups of BALB/C mice, at the age of 5 weeks, were given drinking water containing 0.1% DHPN [N-bis(2-hydroxypropyl) nitrosamme] for 2 weeks, for initiation except group 1 which received water only. From the age of 7 weeks, treatment groups of animals received corn oil alone or containing 0, 10, 100, or 200 μM curcumin in 0.2 ml corn oil. Noncurcumin-treated animals receiving corn oil alone acted as positive control.

Table I. Experimental designs of curcumin affect lung and liver cancer in BALB/C mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Experimental time (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>0---2------------------------------------------22</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>Water</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>0.1% DHPN for two weeks then 0 μmoles curcumin in 0.2 ml corn oil</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>0.1% DHPN for two weeks then 10 μmoles curcumin in 0.2 ml corn oil</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>0.1% DHPN for two weeks then 100 μmoles curcumin in 0.2 ml corn oil</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1% DHPN for two weeks then 200 μmoles curcumin in 0.2 ml corn oil</td>
</tr>
</tbody>
</table>

Results

In this study, BALB/c mice were used as test animals, curcumin as an antitumor agent and DHPN as an inducer of lung and liver tumorigenesis in vivo. This protocol is designed to determine any suppressing effects on the stages of lung and liver tumorigenesis (promotion and progression).

The effects of curcumin on final liver and lung weight of mice treated with DHPN. The animals in the control and corn oil-treated positive control groups showed no signs of gross toxicity or loss of body weight during the experiment (data not shown). However, the control and DHPN-treated groups showed significant differences (p<0.05) (Tables II and III), but the 10 μM curcumin treated mice were not significantly different from these of the positive control (Tables II and III). The effects of 100 and 200 μM curcumin treatment were significantly different from those of the positive control (Tables II and III) (p<0.05; p<0.01).

The effects of curcumin on DHPN-induced liver and lung tumorigenesis in BALB/c mice. The i.p. administration of curcumin reduced tumor numbers. The treatment of curcumin (10, 100 and 200 μM) led to a decrease of lung tumor number by approximately 15%, 36% and 37%, and of liver tumor number by approximately 5%, 23% and 32%, respectively when compared to the positive controls (Figure 1A and B).
It is well known that antitumor agents can block tumor initiation by blocking carcinogen activation, scavenging reactive carcinogens, enhancing DNA damage repair, inducing apoptosis of tumor initiative cells, or by suppressing the progression of initiated cells. Aiming to identify novel chemotherapy agents for lung and liver cancer, we have determined the efficacy of curcumin in blocking lung and liver tumorigenesis induced by DHPN in mice. We found that curcumin was an effective chemopreventive agent in our mouse model of lung cancer at doses (10-200 μM) that caused no significant toxicity. Such mice have been used widely in experimental research. In 1978, it was reported that oral treatment with DHPN led to the development of lung and liver cancer (14). DHPN is a very potent mutagen and a wide-
spectrum carcinogen in rodents. DHPN causes carcinomas of the lung, thyroid and kidney in F344 and Wistar rats (16-19). It was also reported that DHPN induced lung and liver cancer in ddY mice (20). DHPN acting as a tumor initiator is a useful tool for screening chemopreventive as well as tumorigenic activities of chemicals (21). In the present study, we also confirmed that DHPN induced lung and liver cancer in BALB/c mice. Curcumin is a naturally occurring compound present in turmeric which possesses both anti-inflammatory and antioxidant properties, and has been tested for its chemopreventive properties in colon carcinogenesis in vivo (22), as well as in skin and forestomach (23, 24). Curcumin has been demonstrated to prevent chemically induced cancer in several different animal tumor bioassay systems, such as the inhibition of benzo[a]pyrene (BaP*)-induced forestomach tumorigenesis in A/J mice, N-ethyl-N-nitro-N-nitrosoguanidine- duodenal tumorigenesis in C57BL/6 mice and azoxymethane-induced colon carcinogenesis in CF-1 mice (25-27). Curcumin co-inhibited COX-2 and EGFR expression and decreased Erk1/2 activity. This inhibition was associated with decreased survival and enhanced induction of apoptosis in lung and pancreatic adenocarcinoma cells (28).

This study demonstrated for the first time that curcumin reduced the tumor number in mice in vivo. Our data indicated that DHPN induced liver and lung cancer, in agreement with other investigators findings (29). Although curcumin appears to be safe in both large and small animal models, the systematic studies of the pharmacology and toxicology of curcumin in humans are few (30-32). Minimal toxicity of doses up to 8,000 mg have been reported in humans (29, 30), however, the maximum tolerated dose has not yet been defined. The peak plasma concentration has been identified 1 to 2 hours after single dose oral administration of 4,000 mg and higher (30). Recently, it was reported that the tolerance of curcumin (C3 Complex™, Sabinsa Corporation, Piscataway, NJ, USA) in single oral doses up to 12,000 mg appears to be excellent and warrants further investigation for its utility as a long-term chemopreventive intervention (31). Therefore, the dose of curcumin selected in this study was reasonable. In conclusion, curcumin may be a chemotherapeutic or/and preventive agent against lung and liver cancer induced by DHPN.

References


Received February 4, 2008
Revised April 14, 2008
Accepted June 30, 2008