Tetrahydrocurcumin is more effective than curcumin in preventing azoxymethane-induced colon carcinogenesis

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Tetrahydrocurcumin (THC), a major metabolite of curcumin (CUR), has been demonstrated to have potent anti-cancerogenic activity. The anti-carcinogenic effects of this compound is demonstrated by its ability to inhibit tumour initiation by azoxymethane (AOM).

The anti-inflammatory and anti-carcinogenic activities of CUR have been studied by research groups and the action mechanism of CUR is found to be complicated. Tetrahydrocurcumin, a partially reduced derivative of Curcumin found in turmeric, appears to be the major active metabolite formed when Curcumin is administered in mice. THC is hypothesized to be one of the metabolites with higher physiological and pharmacological activities than CUR in the intestine.

With this background, the present study, investigated the chemopreventive effects and underlying molecular mechanisms of dietary administration of CUR and THC in azoxymethane (AOM)-induced colon carcinogenesis in mice.

Experimental set up: Male mice at 5 weeks of age were given AOM at a dose of 5 mg/kg via an i.p. injection twice a week for 2 wk.

Treatment: CUR and THC (obtained from Sabinsa) at two doses (0.005 and 0.02%) were given orally. Diet intake was monitored everyday.

Effect on Aberrant Crypt Formation (ACF)

Colonic ACF were identified and analyzed under a light microscope after methylene blue staining. All mice developed ACF in the colon after AOM treatment. Compared with the control group with AOM treatment only, dietary THC-treated mice were lower in total number of ACF and large ACF than CUR-treated group. THC notably reduced the number of large ACF to 21±9 and 18±6 at 0.005 or 0.02%, respectively, compared with 40±7 in AOM-treated group (p<0.01).

Figure 1: Morphology features of AOM-induced colon carcinogenesis in mice. ACF were identified under light microscope (200 x magnification) with methylene blue staining. This revealed a small focus consisting of three (middle) or more than five crypts (large ACF, right).
**Effect on polyp formation**

Mice were fed CUR and THC for 23 wk, the colonic tissues were collected and tumors identified were examined by H&E staining. Histopathology of the colons and polyps were characterized and the results are shown in Fig. 2. Dietary supplementation of THC markedly inhibited polyp formation in AOM-treated mice. The results suggested that dietary consumption of THC may be more effective than CUR in preventing AOM-induced ACF and colonic polyp formation (Table 1).

![Normal crypt vs. Polyp](image)

**Figure 2:** Histopathology of colonic polyps were detected by hematoxylin and eosin stain (200x magnification).

**Table 1:** Effects of dietary CUR and THC on AOM-induced aberrant crypt foci (ACF) and polyp formation and in ICR mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>No. of ACF/colon</th>
<th>No. of polyp/colon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ACF</td>
<td>Large ACF</td>
</tr>
<tr>
<td>AOM</td>
<td>41.2 ± 2.1</td>
<td>47 ± 10</td>
<td>28 ± 9</td>
</tr>
<tr>
<td>AOM + 0.005% CUR</td>
<td>44.6 ± 1.2</td>
<td>33 ± 3</td>
<td>28 ± 12</td>
</tr>
<tr>
<td>AOM + 0.02% CUR</td>
<td>46.0 ± 0.9</td>
<td>24 ± 6</td>
<td>17 ± 5</td>
</tr>
<tr>
<td>AOM + 0.005% THC</td>
<td>44.6 ± 1.6</td>
<td>13 ± 3</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>AOM + 0.02% THC</td>
<td>44.4 ± 1.2</td>
<td>5 ± 1</td>
<td>15 ± 4</td>
</tr>
</tbody>
</table>

**Inhibition of AOM-induced iNOS and COX-2**

The inflammatory molecules iNOS and COX-2 have been considered involved in colonic carcinogenesis. Compared with CUR, dietary THC resulted in a dramatic reduction of iNOS protein level in colonic mucosa. Compared with CUR, dietary THC resulted in a dramatic reduction of COX-2 protein levels in colonic mucosa 23 weeks after AOM injection.

ERKs is another key signaling molecule involved in inflammatory response and has been reported involved in inflammation-related colon carcinogenesis. Western blot analysis has shown that AOM treatment induced a dramatic increase in the phosphorylation of ERK1/2. However, feeding with 0.02% THC markedly decreased the phosphorylation of ERK1/2 than CUR (0.02%).
**Modulation AOM-induced β-catenin signalling**

β-Catenin is a downstream effector of Wnt/APC/β-catenin signaling pathway that controls colonic epithelial cell proliferation, and commonly dysregulated in colon ACF and tumor. To determine the effect of dietary CUR and THC on β-catenin expression, the colonic tissues were collected and analyzed by IHC and Western blot analysis. IHC examination showed that cytoplasmic and nuclear β-catenin intensity was increased when mice were treated with AOM alone as evidenced by dark brown staining. In contrast, dietary THC groups had lighter nuclear staining of β-catenin than CUR in AOM-treated mice. A similar observation was made using Western blot analysis as well.
Inhibitory effects of dietary CUR and THC on AOM-upregulated Cx-43

Recent studies suggested that induction of Wnt1/b-catenin signaling pathway in a mammary epithelial cell line led to an increase in gap-junctional communication and Cx-43 protein expression. Compared with CUR, dietary THC markedly inhibited AOM-induced Cx-43 protein expression in a dose-dependent manner. This was affirmed using both IHC as well as Western blot analysis.

This is the first investigation with evidence that dietary THC has great potential as a novel chemopreventive agent than CUR to be used in the treatment of inflammation associated with tumorigenesis, especially in the prevention and treatment of colorectal cancer. These results provide evidence for the use of THC supplement as an important chemopreventive agent for colon tumorigenesis.

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