ENHANCING EFFECT OF PREADMINISTRATION OF CARBON TETRACHLORIDE ON METHYLAZOXYMETHANOL ACETATE-INDUCED INTESTINAL CARCINOGENESIS

Kazuo KATO, Toshiro KAWAI*, Masahiko FUJII**, Yasuo BUNAI, Hiroto SHIMA and Masayoshi TAKAHASHI

Department of Pathology, Gifu University School of Medicine,
Tsukasa-machi, Gifu 500, Japan
* Department of Pathology, Jichi Medical School,
Minamikawachi-machi, Kawachi-gun, Tochigi 329-04, Japan
** Tokyo Metropolitan Detection Center of Cancer,
Kandasurugadai, Chiyoda-ku, Tokyo 101, Japan

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Abstract——This study concerns the modifying effect of carbon tetrachloride (CCL,) on methylazoxymethanol acetate (MAM)-induced intestinal carcinogenesis in ACI rats of both sexes. Forty five animals were given CCl, (0.5 ml/kg body weight) through a stomach tube, followed by an i. p. injection with MAM (25 mg/kg body weight) 24 hours after CCl, treatment. The paired administrations were done once a week for 4 weeks and animals were observed until sacrifice 30 weeks later. Pretreatment with CCl, caused not only early death from chemical toxicity of MAM but also an increase in small-bowel tumors.

Key words: ACI rat, carbon tetrachloride, intestinal tumor, liver intoxication, methylazoxymethanol acetate.

INTRODUCTION

It is well recognized that the neoplastic response to a carcinogen depends largely on the physiological state of the host (Yokoro et al., 1973). Thus the organotropic spectrum of a carcinogen can be magnified or diminished by disrupting the homeostatic regulation of certain cell populations.

Cancer of the large bowel has been the subject of several epidemiological reviews (Correa, 1975: Moore and Holdeman, 1975), which suggest that dietary factors, particularly high consumption of fat and a relative lack of dietary fiber, may be important in the etiology of this cancer. However, it is scarcely known how important changes of the homeostatic regulation such as functional liver damages are in the intestinal carcinogenesis (Caselden and Shilkin, 1979; Mori et al., 1977).
There follows a report on the modifying effect of carbon tetrachloride on methyloxazomethanol acetate (MAM)–induced intestinal carcinogenesis in rats.

**MATERIALS AND METHODS**

Weanlings of inbred ACI rats of both sexes kept in our laboratory were divided at random into 3 groups. Rats of Group 1 (45 animals) received oral administrations of carbon tetrachloride (CCl₄) at a dose of 0.5 ml/kg body weight once a week for 4 weeks by means of a gastric tube into the stomach. Each administration of CCl₄ was followed by an intraperitoneal administration of MAM at a dosage of 25 mg/kg body weight 24 hours later. Thirty rats of Group 2 were given MAM alone in the same dosage schedule as those of Group 1. And 30 rats of Group 3 received CCl₄ alone at the same dosage as those of Group 1 once a week for 4 weeks. The MAM was purchased from Ash Stevens Inc., Detroit, Mich. and CCl₄ from Kishida Chemical Co. Ltd., Osaka, Japan.

All animals were weighed weekly. They were autopsied either when died, or when they were sacrificed in a moribund condition or at the end of the experiment at 30th week. Water and food (Diet CE-2; CLEA Japan Inc., Tokyo) were given ad libitum. The organs were fixed in 10% formalin and embedded in paraffin, and sections of all organs were stained with hematoxylin and eosin.

**RESULTS**

*General observation*

Manifestation of general toxicities was much more evident when both of CCl₄ and MAM were given: in this group, 20% of the rats died or had to be sacrificed till the 25th day of the experiment because of their moribund state and their mean survival period of the dead or sacrificed animals was 13.7 days. In the group treated with CCl₄ alone, 3 rats died or were sacrificed till the 42nd day (25.3 days, mean survival period of the dead or sacrificed animals). In the group treated with MAM alone, no animals died within 6 weeks but three rats died on the 112nd, the 118th and the 127th day, respectively. All remaining rats treated with MAM and/or CCl₄ survived at the termination of the experiment.

*Tumor incidence*

Tumors were induced in the intestine. Many animals which had been injected with MAM and survived longer than 112 days developed one or more tumors in the intestine. But no tumors were found either in the intestines or in the other organs of animals which were treated with CCl₄ alone. The incidence of tumors of the intestine in the CCl₄ and/or MAM administered groups is shown in Table 1, with the number of males bearing tumors 18 (94.7%), females 13 (76.5%) in Group 1, males 14 (93.3%) and females 10 (71.4%) in Group 2. The difference in the incidence of tumors is not significant between these two groups. The total number of tumors in the whole intestine was 86
in males and 30 in females of the group treated with CCI, and MAM respectively, while 43 in males and 23 in females of the group treated with MAM alone, respectively. The number of tumors in the intestine of rats of Group 1 was significantly more than the number in rats of Group 2 especially in the small intestine ($p<0.025$, t test, Table 2).

The intestinal tumors were polypoid, sessile or elevated herispherical. Fifty seven of 86 (66.3%) tumors in males and 13 of 30 (43.3%) in females of Group 1 were more than 5 mm in size (the largest diameter), while 17 of 43 (39.5%) in males and 6 of 23 (26.1%) in females of Group 2 were so (significantly different between males, $p<0.01$, $\chi^2$ test).

Histologically, the tumors were classified into 4 types: tubular adenoma, tubular adenocarcinoma, mucinous carcinoma and signet-ring cell carcinoma. The incidence of the histological types was not different between two groups.

Two female rats treated with CCI, and MAM had interstitial proliferations in the kidney, but no lesions in the kidney were found in the other groups.

**Focal proliferations in the liver**

Foci of cell proliferation were microscopically detected in the liver of the animals treated with MAM. Their characteristic features were the emptiness or the intensity of the acidophilic staining of the cytoplasm. The cells were unaltered in size or slightly enlarged as compared with normal liver cells. The foci were smaller than a lobule, but a few approached that size. Such cell foci were found in the rats of both groups treated with MAM. The two groups differed in neither the incidence nor the number of foci per unit area of the liver tissue preparations (Table 1). No liver cirrhoses were observed in the three groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of experimental animals</th>
<th>Effective No. of animals</th>
<th>Animals with intestinal tumors</th>
<th>No. of animals</th>
<th>No. of animals with foci</th>
<th>No. of foci/cm² of liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 CCI+MAM</td>
<td>M24</td>
<td>19</td>
<td>18</td>
<td>94.7</td>
<td>9</td>
<td>3.44±0.82⁴⁶</td>
</tr>
<tr>
<td></td>
<td>P21</td>
<td>17</td>
<td>13</td>
<td>76.5</td>
<td>11</td>
<td>9.09±1.48</td>
</tr>
<tr>
<td></td>
<td>Total 45</td>
<td>36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31</td>
<td>86.1</td>
<td>20</td>
<td>6.55±1.10</td>
</tr>
<tr>
<td>2 MAMalone</td>
<td>M15</td>
<td>15</td>
<td>14</td>
<td>93.3</td>
<td>10</td>
<td>7.40±2.13</td>
</tr>
<tr>
<td></td>
<td>F14</td>
<td>14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10</td>
<td>71.4</td>
<td>11</td>
<td>7.00±2.63</td>
</tr>
<tr>
<td></td>
<td>Total 29</td>
<td>29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24</td>
<td>82.8</td>
<td>21</td>
<td>7.19±1.73</td>
</tr>
<tr>
<td>3 CCI, alone</td>
<td>M 15</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>F 15</td>
<td>13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>Total 30</td>
<td>27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Survived more than 312 days after initiation of experiment
<sup>b</sup> Mean ± S.D.
<sup>c</sup> Significantly different between two values ($p<0.05$, $\chi^2$ test)
Table 2 Incidence of tumors of intestine in rats treated with MAM

<table>
<thead>
<tr>
<th>Group</th>
<th>Effective No of rats&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of rats bearing tumors&lt;sup&gt;b&lt;/sup&gt;</th>
<th>No. of tumors B/A</th>
<th>No. of rats bearing tumors&lt;sup&gt;c&lt;/sup&gt;</th>
<th>No. of tumors D/C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. (A)</td>
<td>No. (B)</td>
<td>No. (C)</td>
<td>No. (D)</td>
</tr>
<tr>
<td>CCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Male</td>
<td>19(100)&lt;sup&gt;m&lt;/sup&gt;</td>
<td>13(88.4)&lt;sup&gt;m&lt;/sup&gt;</td>
<td>44 3.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15(78.9)&lt;sup&gt;n&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>17(100)</td>
<td>10(58.8)</td>
<td>16 1.6</td>
<td>10(58.8)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>36(100)</td>
<td>23(63.9)</td>
<td>60 2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25(69.4)</td>
</tr>
<tr>
<td>MAM alone</td>
<td>Male</td>
<td>15(100)</td>
<td>10(66.7)</td>
<td>14 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13(86.7)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>14(100)</td>
<td>5(35.7)</td>
<td>5 1.0</td>
<td>10(71.4)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>29(100)</td>
<td>15(51.7)</td>
<td>19 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23(79.3)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Survived more than 112 days after initiation of experiment  
<sup>b</sup> Number of rats and percent in parenthesis  
<sup>c</sup> Significantly different between two values (p<0.025, t test)

DISCUSSION

MAM is a potent carcinogen for the digestive tract in rats, and the dose-response relationship of MAM for the development of intestinal tumors is clearly established (Casleden and Shilkin, 1977; Fushimi, 1974; Pollard and Luckert, 1981). MAM is considered to be finally converted in the liver into the methyldiazonium ion, which acts ultimately as a carcinogen (Fiala, 1975). Therefore, it was postulated that hepatotoxic agents may affect the metabolism of MAM and thereby carcinogenic activity toward the intestine (Fukushima et al., 1977). CCl<sub>3</sub> has a hepatotoxic action in rats and induced marked cirrhosis by chronic administration. However, in this experiment, liver cirrhosis was not observed, because CCl<sub>3</sub> treatment was for only 4 weeks with a long subsequent observation period.

In this study, pretreatment with CCl<sub>3</sub> caused not only early death from presumed chemical toxicity of MAM but also an increase in small-bowel tumors. Our results show that MAM and CCl<sub>3</sub> have synergistic action in intestinal tumorigenesis in rats.

However, the number of precancerous lesions in the liver was not affected by pretreatment with CCl<sub>3</sub>. This difference between in the liver and in the intestine may be due to different effects of CCl<sub>3</sub> on MAM metabolism or excretion in the liver and in the intestine. The possibility of involvement of the intestinal microflora and bile acids is not ruled out, since these factors might also be generally affected by liver damage.

Several recent studies reported that alcohol dehydrogenase can convert MAM to an electrophilic agent (Feinberg and Zedek, 1980) and pyrazole which inhibits alcohol dehydrogenase activity (Golderg and Ryberg, 1969) had a marked inhibitory effect on colon tumorigenesis in rats induced by MAM (Notman et al., 1982). Therefore, the influence of preadministration of CCl<sub>3</sub> was the exact opposite to that of pyrazole, at least suggesting that preadministration of CCl<sub>3</sub> did not inhibit MAM metabolism by interfering with alcohol dehydrogenase activity as observed in pyrazole administration. However, it should be noted that alcohol dehydrogenase is not the only enzyme system capable of catalyzing the oxidation of various alcohols (Cardemil, 1978), and
pyrazole can affect the activity of enzymes other than alcohol dehydrogenase (Evarts et al., 1980; Fiala et al., 1978; Lieber et al., 1970; Rubin et al., 1971).

**REFERENCES**


