Inhibition of Large Intestinal Cancers by Celecoxib Using a Serial Sacrifice Technique*

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Abstract. In this serial sacrifice experiment, celecoxib (C) was administered at a 0.1% dose level, in the diet of female Swiss Webster CFW outbred mice. The animals also received either 1,2-dimethylhydrazine dihydrochloride (1,2-DMH) as ten weekly subcutaneous (s.c.) injections at 20 μg/g body weight or physiological saline (PS) as ten weekly s.c. injections at 0.01 ml/g body weight. Subsequently, the mice were sacrificed at 26 weeks or 35 weeks after the first injection of 1,2-DMH or PS. The number of mice with large intestinal tumors and the total number of these tumors were: Group 1 (1,2-DMH), 29 and 438; Group 2 (C + 1,2-DMH), 18 and 64; and Group 3 (PS), 1 and 1, in the mice sacrificed at 26 weeks. The corresponding tumor incidences in the mice sacrificed at 35 weeks were: Group 1 (1,2-DMH), 30 and 323; Group 2 (C + 1,2-DMH), 23 and 134; and Group 3 (PS), 0 and 0. Histopathologically, the tumors were diagnosed as polypoid adenomas and adenocarcinomas of the cecum, colon and rectum. Celecoxib treatment inhibited the development of large intestinal cancers in mice sacrificed at 26 or 35 weeks after the first injection of the carcinogen.

In a recent study, we administered celecoxib (C), at a 0.1% dose level, in the diet of female Swiss Webster CFW outbred mice for life. The animals also received 1,2-dimethylhydrazine dihydrochloride (1,2-DMH) as ten weekly subcutaneous (s.c.) injections at 20 μg/g body weight. The administration of C reduced, in a statistically significant manner, the number of mice with large intestinal cancer and the total number of tumors (1). In the present series of experiments, essentially identical treatments were given to the same type of mice, except that the animals were sacrificed at either 26 or 35 weeks after the first injection of 1,2-DMH or physiological saline (PS).

Serial sacrifice investigations are frequently employed to disclose the time sequence of cancer induction and progression in chemical carcinogenesis and prevention studies. Additionally, the current studies were part of some immunological experimentation in which the immune phenotype, cytokine expression and the reversal of tumor-associated immune suppression were altered. The induction of large intestinal tumors was associated with a significant increase in immature myeloid suppressor cells and a significant decrease in CD+T cells in the spleen, both of which were reversed by C administration (2).

Thus, celecoxib, a non-steroidal anti-inflammatory agent selective for the cyclooxygenase-2 (COX-2) enzyme, which is essential to the synthesis of prostaglandins, was proven to prevent large intestinal carcinogenesis in experimental animals.

Materials and Methods

Female Swiss albino Webster, CWF, outbred mice (Charles River Laboratories, Wilmington, MA, USA) were used. The mice were housed, in a modified barrier facility, in groups of five in plastic micro-isolator cages on ventilated racks and provided with granular cellulose bedding. The were given a Harlan Teklad Rodent powdered diet and tap water ad libitum. The temperature was kept between 18 and 26°C, the humidity 30% and 70%, while the lighting was rotated in a 12-hour on and 12-hour off cycle.

The carcinogen used was 1,2-dimethylhydrazine dihydrochloride, symmetrical (1,2-DMH), (molecular weight, 133.02, melting point, 168°C), which was obtained from Aldrich Chemical Company, Inc., (Milwaukee, WI, USA). The1,2-DMH was dissolved in sterile physiological saline (PS). The mice were s.c. injected in the interscapular region using a tuberculin syringe with 24-gauge needle.

Celecoxib (celebrex, C) (molecular weight, 381.37, melting point, 156-158°C), was obtained from LKT Laboratories, Inc., (St. Paul, MN, USA) in powdered form mixed with the diet and given orally.

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The mice were divided into six experimental groups:

**Group 1**: Thirty mice, nine weeks old, given ten weekly injections of 1,2-DMH at 20 ìg/g body weight in 0.01 ml PS.

**Group 2**: Thirty mice, seven weeks old, given C at 0.1% w/w basis in the powdered diet. The C treatment was followed by 1,2-DMH, as described for Group 1.

**Group 3**: Thirty mice, nine weeks old, given PS as 10 weekly injections of 0.01 ml/g body weight.

**Group 4**: Identical treatment as Group 1.

**Group 5**: Identical treatment as Group 2.

**Group 6**: Identical treatment as Group 3.

The animals in Groups 1-3 were sacrificed at 26 weeks, while those in Groups 4-6 were sacrificed at 35 weeks after the first injection of 1,2-DMH or PS.

During the study, the animals were allowed to die or were killed with CO₂ when found to be in poor condition and complete necropsies were performed. All organs were examined macroscopically and were fixed in 10% buffered formalin. Histological examination was routine for the intestines (large and small) as well as any organs showing gross pathological changes. Sections from these tissues were stained with hematoxylin and eosin and studied by light microscopy.

**Results**

_Experiments in which the animals were sacrificed at 26 weeks after the first injection of 1,2-DMH or PS_. Table I summarizes the survival rates of the treated mice in five-week intervals. Of the animals treated with 1,2-DMH, 29 mice (96%) developed 438 tumors of the large intestine. Their average age at death was 35.0 weeks. The first tumor observed was at the 35th week of age, as was the last. Three mice had three adenocarcinomas of the cecum, 16 mice developed 39 adenocarcinomas of the colon, 11 mice had 21 adenocarcinomas of the rectum and one mouse developed a squamous cell carcinoma of the anus.

Among the PS-treated mice, only one (3%) developed a single adenocarcinoma of the cecum at the 35th week of age.

**Experiments in which the animals were sacrificed at 35 weeks after the first injection of 1,2-DMH or PS_.** Table III summarizes the survival rates of the treated animals in five-week intervals. Of the animals treated with 1,2-DMH, 30 mice (100%) developed 323 tumors of the large intestine. Their average age at death was 38.6 weeks. The first tumor observed was at the 27th week and the last at the 44th week of age. Of these, four mice had four adenocarcinomas of the cecum, 26 mice developed 217 adenocarcinomas of the colon, 24 mice had 95 adenocarcinomas of the rectum, one mouse had a polypoid adenoma and four adenocarcinomas of the rectum and two mice developed two squamous cell carcinomas of the anus.

Of the animals treated with C plus 1,2-DMH, 23 mice (76%, p<0.01) developed 134 tumors of the large intestine. Their average age at death was 43.3 weeks. The first tumor was observed at the 39th week and the last at the 44th week of age. Of these, one mouse developed an adenocarcinoma of the cecum, 20 mice had 78 adenocarcinomas of the colon, 20 mice developed 52 adenocarcinomas of the rectum, two mice had two squamous cell carcinomas of the anus and one mouse had a sebaceous gland adenoma of the anal gland.

None of the PS-treated mice developed tumors of the large intestine.

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Table I. Treatments and survival rates in 1,2-dimethylhydrazine dihydrochloride (1,2-DMH), celecoxib (C) and physiological saline (PS)-treated Swiss mice sacrificed at 26 weeks after the start of 1,2-DMH or PS treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Initial no. + sex of mice</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,2-DMH, 10 weekly s.c. injections at 20 ìg/g</td>
<td>30 ♀♂</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>29</td>
<td>29</td>
<td>27</td>
<td>21</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>C, 0.1% in diet + 1,2-DMH as in group 1</td>
<td>30 ♀♂</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>29</td>
<td>28</td>
<td>28</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>PS, 10 weekly s.c. injections at 0.01 ml/g</td>
<td>30 ♀♂</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>29</td>
<td>27</td>
<td>-</td>
</tr>
</tbody>
</table>

_Histological examination was routine for the intestines (large and small) as well as any organs showing gross pathological changes. Sections from these tissues were stained with hematoxylin and eosin and studied by light microscopy._
The location and distribution, gross appearance and histological descriptions of the large intestinal tumors were similar to those described in our previous publications (3). Table IV presents the number and percentage of animals with tumors and their ages at death.

Table IV.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. and sex of mice</th>
<th>Animals with tumors of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Large intestine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. % Latent period*</td>
<td>No. % Latent period*</td>
</tr>
<tr>
<td>1</td>
<td>1,2-DMH, 10 weekly s.c. injections at 20 µg/g</td>
<td>30 ♀</td>
<td>29 % 33 (28-35)</td>
</tr>
<tr>
<td>2</td>
<td>C, 0.1% in diet + 1,2-DMH as in group 1</td>
<td>30 ♀</td>
<td>18 % 35 (35-35)</td>
</tr>
<tr>
<td>3</td>
<td>PS, 10 weekly s.c. injections at 0.01 ml/g</td>
<td>30 ♀</td>
<td>1 % 35</td>
</tr>
</tbody>
</table>

*: Average and range in weeks.
**: Age at death given in weeks in parentheses.

Table III.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Initial no. + sex of mice</th>
<th>No. of survivors (age in weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td>1,2-DMH, 10 weekly s.c. injections at 20 µg/g</td>
<td>30 ♀</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>C, 0.1% in diet + 1,2-DMH as in group 1</td>
<td>30 ♀</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>PS, 10 weekly s.c. injections at 0.01 ml/g</td>
<td>30 ♀</td>
<td>30</td>
</tr>
</tbody>
</table>

Discussion

The aim of the current study was to further determine the possible anticarcinogenic effect of C in a serial sacrifice investigation. In an earlier study, the oral administration of C for life at a 0.1% dose level reduced the incidence of large intestinal cancer induced by 1,2-DMH at 20 µg/g body weight (1). In the present group of experiments, identical treatments were administered to Swiss mice, but they were sacrificed at 26 or 35 weeks after the first injection of the carcinogen or PS. As anticipated, the incidence of large intestinal cancer was substantially reduced in the mice sacrificed at 26 and 35 weeks after the first injection of 1,2-DMH. In both of our investigations, the administration of C reduced the induced large intestinal cancer incidence.
Our findings corroborate those of other investigators who used C to inhibit the development of cancers in the intestine, stomach, urinary bladder, skin, breast and prostate (5-15), while similar protective results were obtained in the duodenum, colon and rectum of humans (16-18).

After the anti-inflammatory painkiller rofecoxib (Vioxx) was withdrawn from the market, similar COX-2 inhibitors, including celecoxib, came under suspicion. The National Institutes of Health recently halted a large scale colorectal cancer prevention trial involving more than 2,000 people, since there was evidence of a 2.5-fold increased risk of major fatal and non-fatal cardiovascular events in those taking C versus the placebo patients (19). In addition, Pfizer, the manufacturer of the COX-2 inhibitor celecoxib, is involved in an international trial with approximately 20,000 patients, focusing on those with heart disease, including patients who had undergone bypass surgery and those at risk of cardiac problems (20). Even though the trial is still in progress, a number of criticisms have been leveled against the protocol in terms of the variables involved in the experimental set-up.

One advantage of the Swiss mouse model is that it develops few, if any, spontaneous large intestinal cancers. Whether the molecular genetics of chemically-induced rodent models are significantly different from those observed in human colorectal carcinogenesis remains to be seen. The Apc transgenic mouse strain, on the other hand, is certainly different since, in both humans and mice, over expression of the Apc gene is responsible for the appearance of intestinal tumors. The Apc transgenic murine model is characterized by the spontaneous development of small and large intestinal tumors, and our on-going studies are designed to clarify this field of interest.

Our preliminary study nevertheless indicated that the survival rate was substantially prolonged and the intestinal tumor incidence considerably reduced by the life-long administration of C to Apc mice.

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References


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