Induction of Cancer by 20-Methylcholanthrene in Different Regions of the Rat Stomach

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SUMMARY

We have developed a new method of application of carcinogen to the mucosa of any region of the stomach in rats. By means of an oval fastener and globular polyethylene ampul, a pocket from the stomach wall is formed in which blood circulation and innervation are kept uninterrupted. Ampuls with different numbers of apertures in walls and lipid vehicles with different melting points were investigated.

Cardiac, fundal, and pyloric regions of the stomach and the forestomach were subjected to the effect of 20-methylcholanthrene for 16 months. The forestomach is the most susceptible to the effect of carcinogen (17 tumors in 27 rats). The greatest susceptibility to carcinogenesis of the 3 regions of the glandular stomach is in the cardiac region (9 tumors in 30 rats), and the least is in the fundal region (2 tumors in 31 rats).

INTRODUCTION

An easy method of producing experimental stomach cancer in rodents in a laboratory would be of great importance for experimental oncology.

This can be done by introducing Plexiglas vessels containing a carcinogenic substance and a wick into the stomach wall. The carcinogen flows into the stomach cavity along the wick and produces tumors of the glandular part of the stomach in rats (2-4).

However, this method is rather complicated and the size of the wick and of the ampul prevents the whole rat stomach from being subjected to the carcinogen. Also, this method cannot be used in mice. Therefore we developed another simpler method, which makes it possible to apply carcinogens over a long period of time to any region of the stomach both in rats and in mice.

MATERIALS AND METHODS

The experimental animals were noninbred male rats weighing initially 100 to 200 g; they were fed a standard diet (ration of food value from natural foods).

The application of carcinogens was achieved by means of globular polyethylene ampuls. Polyethylene tubes 3 to 7 mm in diameter were stretched over the flame to a diameter of 2 mm. Then they were fused and a bubble 6.5 to 7.0 mm in diameter was blown. The bubble was separated from the tube, perforated by means of an injection needle 0.7 mm in diameter, and filled with carcinogen in a lipid vehicle.

The ampul (Chart 1A) was introduced surgically into the cavity of the stomach. Rats were subjected to laparotomy along the linea alba under sterile conditions, after ether anesthesia. The operation may be performed either after preliminary starving or when the stomach is filled. The stomach was taken out of the abdominal cavity. The wall of the forestomach was dissected with scissors, the ampul was pressed into the cavity of the stomach, and the wound (5 to 6 mm long) was sewn up. The ampul was then moved manually to the part of the stomach selected for the application of the carcinogen. Then a pocket was formed around the ampul by lifting and fastening the ampul to the wall of the stomach. An oval fastener (Chart 1, 2) was placed under the container from the serosal side of the stomach (cf. Chart 1, B and C). The fastener is made of a...
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single-core brass wire with a 1-mm section insulated with polyethylene or chlorvinyl. The fastener is closed carefully to avoid trauma to the stomach so that the ampul does not fall out of the pocket into the lumen. The stomach is then returned into the abdominal cavity, and the cavity wall is closed by a 2-layer suture.

After the operation the rats were maintained on their normal diet.

The oval form of the fastener (8 x 18 mm) was deliberately designed to allow peristalsis of the stomach only at 2 definite points, as shown in Chart 1A. The remainder of the stomach wall remains free, and the innervation and the vascularization of the pocket remain unchanged. The stomach pocket thus formed keeps its form, and the ampul remains unimpaired for up to 2.5 years of experiment.

For the greatest local effect, the carcinogenic agent should leave the ampul gradually during a long period of time (8 to 12 months). This depends on the number of perforations in the walls of the ampul and on the type of vehicle.

The ampul used by us holds 5 mg of carcinogen in 0.1 ml of a lipid vehicle. We investigated the use of three lipid vehicles, sunflower seed oil, Vaseline petrolatum jelly and mutton lard.

The animals were observed periodically during these experiments. Dead or sacrificed rats were subjected to necropsy, and tissues were obtained for microscopic examination. Histological preparations were stained with hematoxylin-eosin.

RESULTS

Experiment 1. Ampuls with 2 apertures at the poles were introduced into the fundus of the stomach. Sunflower seed oil (Group 1), Vaseline petrolatum jelly (Group 2), and mutton lard (Group 3) were used as vehicles for 20-methylcholanthrene at a 5% concentration.

Table 1

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Vehicle</th>
<th>No. of apertures</th>
<th>No. of rats</th>
<th>Discharge of contents from ampuls in rats killed at</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sunflower seed oil</td>
<td>15</td>
<td>4 mo.</td>
<td>++ +a</td>
</tr>
<tr>
<td>2</td>
<td>Vaseline petrolatum jelly</td>
<td>15</td>
<td>8 mo.</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>Mutton lard</td>
<td>15</td>
<td>12 mo.</td>
<td>+++</td>
</tr>
</tbody>
</table>

a+++, full discharge; -- --, no discharge.

Mutton lard (Group 3) remained in the ampuls during the whole experiment, and sunflower seed oil (Group 1) is discharged during the first months of the experiment. Therefore, contact of carcinogen with the mucosa of the stomach by means of these ampuls seems less desirable than with Vaseline petrolatum jelly.

Experiment 2. The increase of the number of apertures in the walls of the ampuls somehow increases the permeability of the ampuls and thus should improve the contact of its content with the mucosa of the stomach.

We investigated the carcinogenic effect of Vaseline petrolatum jelly containing 5% 20-methylcholanthrene on the mucosa of the glandular stomach, using ampuls with different numbers of apertures.

Four groups were used, 30 rats/group, implanted with 2, 3, or multiple (36 to 40) apertures.

The stomach with the ampuls was examined weekly by palpating the front abdominal wall.

During the 9th to 10th month after the beginning of the experiment, 3 rats died (1 rat in the 1st group and 2 rats in the 2nd) of pneumonia. There were no tumors in the stomach and other organs. The ampuls with 2 apertures were one-third empty; the ampuls with 1 aperture had discharged a little.

During the 11th month, solid tumors began to be palpable. At this time, 1 rat of Group 4 died of pneumonia; there was no tumor in the stomach and in other organs.

The experiment was terminated at 2 years, when the surviving rats were sacrificed. The results are given in Table 2.

Over 24 months, the content of the ampuls with 1 aperture (Group 1) remained almost fully undischarged, and the mucosa of the stomach wall showed no visible changes. The discharge from ampuls with many apertures was the most effective (Group 4).

As seen from Table 2, the number of apertures in the ampul has an influence upon the rate of discharge of their content and the induction of tumors in the stomach.

Experiment 3. In this experiment, we explored the responsiveness of functionally different parts of the stomach in rats to the carcinogenic effect of 20-methylcholanthrene, using 2-apertured ampuls and Vaseline petrolatum jelly as the vehicle.

Table 2

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of apertures</th>
<th>No. of rats</th>
<th>Time of palpable first tumors (mo.)</th>
<th>No. of tumors at 24th mo.</th>
<th>Morphology of tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>30</td>
<td>15</td>
<td>2</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>30</td>
<td>16</td>
<td>1</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>4</td>
<td>36-40</td>
<td>30</td>
<td>11</td>
<td>6a</td>
<td>Adenocarcinoma, one myosarcoma</td>
</tr>
</tbody>
</table>

aDifference in number of tumors between Groups 4 and 3 is statistically reliable (p < 0.05).
All 132 rats were divided into 4 groups. In rats of Group 1, the ampuls with carcinogen were fixed by means of the stomach pocket on the mucosa of the lateral wall of the forestomach; in rats in the 2nd, 3rd, and 4th group, ampuls were fixed in the cardiac, fundal, and pyloric parts, respectively.

During the first 10 months, 8 rats died from various causes. No tumors were found.

One rat of Group 1 died during the 11th month. The examination showed a thick layer of tumor enveloping the whole lateral wall of the forestomach. At the same time, 9 more rats had solid palpable tumors in the stomach. By 16 months, tumors were palpable in all groups. The rats were therefore killed and examined by autopsy.

The results are given in Table 3. The multilayered squamous epithelium of the forestomach was most sensitive to the effect of 20-methylcholanthrene. The tumors appeared earlier and were larger (about 2 x 3 mm), sometimes covering the whole forestomach and a considerable part of the glandular stomach, with extensions of the liver and the intestines. The 17 tumors originating from the squamous epithelium of the forestomach had many pearls, fields of cornified cells (Fig. 2) intermingled with columns, and fields of dedifferentiated epithelial cells with large, pale nuclei. In 3 of these 17 rats were found multiple metastases to the mesentery of the intestine and under the serosa of the small intestine, measuring 1 to 12 mm (Fig. 1). The histological appearance was identical with the primary, cornifying squamous cancer (Fig. 3).

Tumors of the glandular stomach in Experiments 2 and 3 were all adenocarcinomas. In spite of the fact that they were induced in different parts of the stomach, their structure was similar (Fig. 5). Many of these tumors were large (12 x 18 x 20 to 48 x 38 x 30 mm). They spread along the stomach wall and enveloped the entire stomach with a thick layer (Fig. 4). Necrosis was prominent, and the masses projected into the lumen of the stomach, forming deep cavities.

Necrotic areas in sections alternated with fields of dedifferentiated cells of the epithelium, with considerable mitotic activity. All layers of the stomach wall, serosa included, were destroyed and invaded; it was one of the main criteria of malignancy of stomach tumors (5, 6).

Almost all the tumors had extended to the liver, spleen, and loops of the intestine. No distant metastases were found.

**DISCUSSION**

By means of polyethylene ampuls implanted into the wall of the stomach and retained by metal fasteners, a constant, long-term exposure to a carcinogen was achieved. The ampuls require perforations through which the carcinogen can diffuse. The carcinogen used was 20-methylcholanthrene, as a 5% solution in Vaseline petrolatum jelly.

The new method of application of carcinogen to the mucosa of specific regions of the stomach in rats has been shown to produce malignant tumors within 16 months. We found the forestomach most susceptible to the effect of the carcinogen, producing cornifying squamous cell carcinomas in 17 of 27 rats.

The glandular portion of the stomach also showed differing sensitivity to the carcinogenic effect of 20-methylcholanthrene. The earliest adenocarcinomas appeared when the carcinogen was applied to the pyloric region, but by the end of the experiment, the greatest number of neoplasms was found in rats in which the carcinogen was applied to the cardiac area. The mucosa of the cardia, consisting mainly of tubular glands not secreting digestive juice, appears to be the most sensitive to carcinogenesis upon application of 20-methylcholanthrene. The fundal part of the stomach appears to be least sensitive to carcinogenesis.

Previous data from various sources (7-11) suggest that 20-methylcholanthrene is the most active polycyclic hydrocarbon for the induction of tumors of the glandular stomach.

The technique described is simple to perform surgically. The use of an inhaling apparatus for anesthesia (1) shortens the time under anesthesia and preserves the condition of the animals. We could complete the operation procedure at the rate of 25 to 30 rats over a period of 6 to 7 hr.

Further investigations with other carcinogens, other vehicles, and modifications of the ampuls should lead to improvements in the system. We have also been able to perform similar procedures in mice, using smaller fasteners and ampuls. The results in mice will be reported later.

**ACKNOWLEDGMENTS**

I extend my appreciation to Mr. A. I. Sobolev for the photographic work.

**Table 3**

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Site of application of carcinogen</th>
<th>Initial No. of rats</th>
<th>Duration of experiment (mo.)</th>
<th>No. of rats at the end of the experiment</th>
<th>No. of rats with tumors after 10 mo. (by palpation)</th>
<th>No. of rats with tumors after 16 mo. (by autopsy)</th>
<th>No. of rats with metastatic tumors</th>
<th>Morphology of tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Forestomach</td>
<td>33</td>
<td>16</td>
<td>27</td>
<td>9</td>
<td>17(^a)</td>
<td>3</td>
<td>Cornifying squamous cell cancers</td>
</tr>
<tr>
<td>2</td>
<td>Cardia</td>
<td>33</td>
<td>16</td>
<td>30</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>Adenocarcinomas</td>
</tr>
<tr>
<td>3</td>
<td>Fundus</td>
<td>33</td>
<td>16</td>
<td>31</td>
<td>0</td>
<td>2</td>
<td>6(^b)</td>
<td>Adenocarcinomas</td>
</tr>
<tr>
<td>4</td>
<td>Pylorus</td>
<td>33</td>
<td>16</td>
<td>29</td>
<td>2</td>
<td>6(^b)</td>
<td>0</td>
<td>Adenocarcinomas</td>
</tr>
</tbody>
</table>

\(^a\)Difference in number of tumors between Group 2 and 3 is statistically reliable (p < 0.05).

\(^b\)Difference in number of tumors in Group 4 and Groups 2 and 3 is not statistically reliable (p > 0.05).
REFERENCES


Fig. 1. Cornifying squamous cell cancer of the forestomach. The tumor has grown through the entire forestomach and partly through the glandular stomach. Multiple metastases in epiploon, mesentery, and the wall of the small intestine. H & E, X 20.
Fig. 2. Cornifying squamous cell cancer of the forestomach. Wide cornifying fields and pearls intermingle with columns of epithelium cells. H & E, X 20.
Fig. 3. Metastasis of cornifying squamous cell cancer of the forestomach. H & E, X 20.
Fig. 4. Adenocarcinoma of a rat stomach. The tumor has grown through the walls of both regions of the stomach. The appearance of the stomach is that of a thick-walled bag. The wall is 10 to 30 mm thick. H & E, X 180.
Fig. 5. Adenocarcinoma of the stomach. Dedifferentiated glandular epithelium. Cancer cells are atypical and polymorphic. H & E, X 180.
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