Aroclor 1254-induced Intestinal Metaplasia and Adenocarcinoma in the Glandular Stomach of F344 Rats

Robin W. Morgan, Jerrold M. Ward, and Philip E. Hartman

Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218 [R. W. M., P. E. H.,] and Tumor Pathology and Pathogenesis Section, Laboratory of Comparative Carcinogenesis, Division of Cancer Cause and Prevention, National Cancer Institute, Frederick Cancer Research Center, NIH, Frederick, Maryland 21701 [J. M. W.]

ABSTRACT

Ingestion of diets containing Aroclor 1254, a mixture of polychlorinated biphenyls, for 2 years led to a dose-related increase in the incidence of focal lesions in the glandular stomachs of male and female F344 rats. The incidence of stomach lesions was 6% in control specimens and in specimens from rats fed a diet containing 25, 50, or 100 ppm Aroclor 1254, the incidences of stomach lesions were 10, 17, and 35%, respectively. The majority of gastric lesions in treated rats were histologically identified as intestinal metaplasia characterized by an architecture resembling that of intestinal crypts and particularly by goblet cells, which stained with Alcian blue and periodic acid-Schiff reagent. Adenocarcinomas were found in six specimens. Most (88%) of the lesions were located in the pyloric region of the glandular stomach. No multiple lesions were observed among 47 control specimens examined; however, nine cases of multiple lesions were observed in 30 lesion-containing specimens from Aroclor 1254-treated rats. Although the exact relationship between gastric intestinal metaplasia and adenocarcinoma remains to be established, they commonly coexist and may share initiating mechanisms.

INTRODUCTION

PCBs are remarkably inert organochlorine compounds used from 1929 until the late 1970's in electrical systems, investment casting, and numerous commercial products including carbonless copy paper and microscope immersion oil. It has been estimated that only 4.4% of the PCBs purchased by United States industry have been incinerated or degraded in the environment; the majority of PCBs are either still in use (60%), in land fills or dumps (23.5%), or free in the environment (11). Due to the resistance of PCBs to degradation, their widespread use has led to global contamination of the environment; the majority of PCBs are still in use (60%), in land fills or dumps (23.5%), or free in the environment (11). The test chemical was analyzed at Stanford Research Institute and was found to contain 54.67% chlorine and to be a mixture of at least 18 isomers ranging from 4 to 7 chlorine atoms per molecule. Identification or quantitation of impurities was not done. Aroclor 1254 (24, 29, 43). Hepatocellular carcinomas have been reported in female Sherman rats fed Aroclor 1260 (30), and hepatomas have been induced in mice fed PCBs (23, 28). Gastric lesions, such as epithelial hypertrophy, hyperplasia, and ulceration, have been described in PCB-exposed monkeys (1, 2, 4, 34) and swine (20). Additionally, mucus-filled cysts that penetrate the muscularis mucosa have been reported in rhesus monkeys exposed to PCBs (3, 6).

MATERIALS AND METHODS

Bioassay. Male and female F344 rats (Simonsen Laboratory, Gilroy, Calif.) were fed a diet consisting of low-fat laboratory chow (Ralston Purina Co., St. Louis, Mo.) that contained 0, 25, 50, or 100 ppm Aroclor 1254 (CAS No. 27323-18-8; Lot KBO1-604; Monsanto Chemical Company, St. Louis, Mo.) from July 1972 until September 1974. The test chemical was analyzed at Stanford Research Institute and was found to contain 54.67% chlorine and to be a mixture of at least 18 isomers ranging from 4 to 7 chlorine atoms per molecule. Identification or quantitation of impurities was not done. Aroclor 1254 was dissolved in corn oil (Staley Manufacturing Co., Orange, Calif.) prior to its addition to the laboratory chow. Lungs, bronchi, spleen, liver, testes, pituitary gland, kidneys, brain, and any grossly visible lesions were prepared for microscopy. In the initial studies performed at necropsy (43), stomachs from 42 control animals were examined histologically. Stomachs from PCB-treated animals were examined histologically only in 18 cases in which gross lesions were noted at necropsy. All tissues were preserved in 10% buffered formalin, sealed in plastic bags, and stored at room temperature for 6 years at Stanford Research Institute and the NCI Tissue Repository in Rockville, Md. Detailed information on methods, results, and conclusions of the bioassay are available (43).

Detection of AP Activity in Formalin-fixed Specimens. Whole-tissue specimens of stomachs, which had been preserved in formalin for 6 years, were washed overnight in deionized water and stained for AP activity, as described previously (35). Tissues were incubated at room temperature for 3 hr in a solution of β-naphthyl acid phosphate.
ent, intestinal metaplasia was associated both with focal and with sites at which intestinal metaplasia or other pathological lesions were detected by microscopic examination. When present, intestinal metaplasia was focal, while in others AP activity was diffuse and less intense. The percentage of total specimens with focal AP was 11% in the control group, 13% in the group exposed to the low dose, 46% of the group exposed to the high dose survived until the termination of the experiment (43). Specimens were stained for AP activity.

RESULTS

Growth, General Health, and Survival. With the exception of males exposed to low doses of Aroclor 1254, the mean body weights of male and female rats fed Aroclor 1254 were lower than those of controls fed corn oil. Males and females fed the high dose had mean body weights that were roughly three-fourths and two-thirds those of controls, respectively. Beginning at Week 7 for rats fed the high dose and Week 104 for rats fed the medium dose, symptoms of Aroclor 1254 exposure, including alopecia, amber-colored urine, facial edema, exophthalmos, and cyanosis, were evident (43). For males, 92% of the control group, 83% of the group exposed to the low dose, 58% of the group exposed to the medium dose, and 46% of the group exposed to the high dose survived until the termination of the study. For females, 67% of the control group, 79% of the group exposed to the low dose, 83% of the group exposed to the medium dose, and 71% of the group exposed to the high dose survived until the termination of the experiment (43).

Gross Morphology of the Stomachs. We first verified that stomachs from the NCI Tissue Repository from both control and Aroclor 1254-treated rats had a normal gross morphology, except those from 18 Aroclor 1254-treated rats noted in the original NCI report to have stomach tumors or other stomach lesions at necropsy (43). Specimens were then stained for AP to pinpoint areas of possible pathological significance for histological examination. In some specimens gross AP activity was focal, while in others AP activity was diffuse and less intense. The percentage of total specimens with focal AP was 11% in the control group, 13% in the group exposed to the low dose, 27% in the group exposed to the medium dose, and 38% in the group exposed to the high dose of Aroclor 1254. Diffuse AP was present in 47% of the specimens from the control group and greater than 75% of the specimens from each of the Aroclor 1254-treated groups.

Sites of detectable AP activity overlapped only incompletely with sites at which intestinal metaplasia or other pathological lesions were detected by microscopic examination. When present, intestinal metaplasia was associated both with focal and diffuse sites of AP activity. For specimens from the group fed the high dose of Aroclor 1254, 10 of 16 (63%) pathological lesions were at sites of focal AP activity, 4 of 16 (25%) were at regions of diffuse AP activity, and 2 of 16 (13%) were from specimens which had no detectable AP. One lesion scored from an NCI tissue block was not included, since the portion of the stomach containing the lesion was not available for staining for AP activity.

Histological Observations. All 191 stomachs available were sectioned through the junction of the pyloric region of the stomach and the duodenum, a known "hot spot" for gastric neoplastic lesions in humans (36) and rats (33), as well as at any AP-positive sites. The incidence of microscopically confirmed lesions in the glandular stomachs of F344 rats is presented in Table 1. The incidence of lesions in the stomachs increased as a function of the concentration of Aroclor 1254 in the diet, and at the highest concentration tested one-third of the animals had gastric lesions. No significant differences were apparent between males and females (Table 1). Multiple lesions were found only in specimens from Aroclor 1254-treated rats (Table 1).

A histological classification of the types of gastric lesions induced by Aroclor 1254 is presented in Table 2, and examples of the lesions are shown in Figs. 1 to 12. Metaplastic lesions in 3 controls were generally associated with aggregates of lymphocytes, and included AP-positive areas. Specimens were also trimmed so that samples spanned the duodenum-glandular stomach junction and included AP-positive areas. Specimens were sectioned to thicknesses of 5 to 15 μm. Duplicate slides were stained with H & E and with Alcian blue-periodic acid Schiff reagent. Selected slides were stained with phosphotungstic acid-hematoxylin or eosin B-aniline blue (9), both of which stain Paneth cell granules. Slides that were stored at the NCI Tissue Repository since the bioassay was performed 6 years ago were also reexamined.

<table>
<thead>
<tr>
<th>Concentration of Aroclor 1254 (ppm)</th>
<th>No. of males with stomach lesions</th>
<th>Rats with multiple stomach lesions</th>
<th>Total rats with stomach lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2/24 (8.3)</td>
<td>0/47</td>
<td>3/47 (6.4)</td>
</tr>
<tr>
<td>25</td>
<td>2/24 (8.3)</td>
<td>3/24 (12.5)</td>
<td>5/24 (10.4)</td>
</tr>
<tr>
<td>50</td>
<td>4/24 (16.7)</td>
<td>1/48 (2.1)</td>
<td>8/48 (16.7)</td>
</tr>
<tr>
<td>100</td>
<td>7/24 (29.2)</td>
<td>6/48 (12.5)</td>
<td>17/48 (35.4)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentages.

Table 2

Histological analysis of stomach lesions* in male and female F344 rats exposed to Aroclor 1254 in their diets

<table>
<thead>
<tr>
<th>Concentration of Aroclor 1254 (ppm)</th>
<th>Cryptotype, over lymphoid cluster</th>
<th>Diffuse in epithelium</th>
<th>Adenocarcinoma</th>
<th>Total rats with stomach lesions</th>
<th>Total rats examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>ND</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>47</td>
</tr>
<tr>
<td>25</td>
<td>3</td>
<td>1</td>
<td>4</td>
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<td>13</td>
<td>2</td>
<td>15</td>
<td>2</td>
<td>48</td>
</tr>
</tbody>
</table>

* Rats containing multiple lesions were classified by the most severe lesion (see text).  
† ND, none detected.  
| Regions of severe dysplasia were identified in 2 additional foci of intestinal metaplasia, but sections available did not allow confirmation of the presence of adenocarcinoma.  
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| ND, none detected.
phocytes and/or inflammation (Fig. 1). Metaplastic cells were few (Fig. 2). The histology of experimentally induced adenocarcinoma of the rat glandular stomach has been described in detail (42). Most of the lesions were focal intestinal metaplasia characterized by mucin-containing goblet cells that stained with Alcian blue-periodic acid Schiff reagent (Figs. 3 to 6). Tall columnar cells were commonly seen, and crypts of intestinal metaplasia sometimes had atypical cells and mitotic figures (Fig. 4). Twenty-one of 33 lesions (64%) were adjacent to foci of lymphocytes (Fig. 1), and some eosinophils and other inflammatory cells were noted. No Paneth cells were seen in slides stained with H & E or in any of 14 lesions stained with phosphotungstic acid-hematoxylin or eosin B-aniline blue, which specifically stained Paneth cell granules in specimens from the rat ileum.

Nineteen specimens contained intestinal metaplasia organized into cysts (Figs. 3, 5, 7 to 10). Twelve cystic lesions involved the muscularis mucosa, and 7 of the cysts distorted the muscularis mucosa but did not actually appear to penetrate it. Three of the lesions in control rats, one in a rat exposed to the medium dose of Aroclor 1254 and 2 from rats exposed to the high dose of Aroclor 1254, were crypts of intestinal metaplasia located above large aggregates of lymphocytes (Figs. 1 and 2). One lesion from a rat exposed to the low dose of Aroclor 1254 and one from a rat exposed to the medium dose consisted of goblet cells scattered in the gastric epithelium but not organized into intestinal crypts.

Adenocarcinomas were found in one specimen from the group exposed to the low dose of Aroclor 1254, in 3 specimens from rats exposed to the medium dose of Aroclor 1254, and in 2 specimens from the group exposed to the high dose of Aroclor 1254 (Figs. 7 to 11). The carcinomas invaded the muscularis mucosa (1 case), the submucosa (4 cases), or the serosa (1 case) and contained areas resembling those of intestinal metaplasia (Fig. 12) in addition to more undifferentiated and scirrhous areas (Fig. 11). Twenty-nine of the 33 (88%) lesions scored were located in the pyloric region of the stomach. Two of the lesions were in the cardiac portion of the stomach, one of which was adjacent to the glandular stomach-foregut junction. For 2 of the lesions scored from slides stored at the NCI Tissue Repository, the locations were unknown.

Intestinal metaplasia was not seen in any of 8 animals that died before the 73rd week of the experiment. Because the number of animals that died during the first year of the experiment was so small, no conclusions can be drawn as to when intestinal metaplasia first appeared. No correlation was found between animals dying before the experiment ended at 105 weeks and those having gastric lesions.

**DISCUSSION**

AP activity normally is not detectable in the rat glandular stomach (40) but is present at high levels in the brush borders of the small intestine and persists after fixation of the tissues in formalin at room temperature (17, 19, 41). Since intestinal metaplasia and some adenocarcinomas contain AP, histochemical staining for AP can be used to macroscopically pinpoint sites of possible pathological significance in stomachs which have been preserved for extended periods of time (35). A dose-dependent increase was seen in the incidence of focal gastric AP in Aroclor 1254-treated animals. Although the incidence of lesions was not 100% in animals exposed to the high dose, the high dose appeared to represent a maximally tolerated one. Diffuse AP activity was elevated in Aroclor 1254-treated animals, but the increase was not dose dependent. Diffuse AP activity was not seen in tissues from a similar bioassay in which rats were exposed to N-methyl-N'-nitro-N-nitrosoguanidine (35). Some areas of diffuse AP activity may stem from inflammation of the tissue or may reflect widespread alteration of the epithelial cells of the gastric mucosa. Most of the stomachs with AP activity were histologically normal; however, 26 AP-positive specimens contained lesions of pathological significance. Seven specimens which contained gastric lesions did not stain for AP, including 2 of the adenocarcinomas, 3 crypt-type lesions over lymphoid follicles, and 2 cystic lesions (Table 2). Such lesions may represent a biochemically distinct category in that they lack AP. It has been reported that human intestinal metaplasia of the incomplete type lacks AP activity (26). An alternative explanation is that AP-negative gastric lesions contain AP at a level below the limit of detection by macroscopic staining.

Intestinal metaplasia and total lesions in the glandular stomachs of F344 rats increased as a function of Aroclor 1254 concentration in the diet, indicating that the lesions were either induced or enhanced by exposure to the PCBs (Table 2). In some cases, intestinal metaplasia disrupted the muscularis mucosa, had features of adenocarcinoma, and resembled areas of invasive carcinomas. A definite sequence of cancer development from these metaplastic areas, however, could not be demonstrated.

Stomach adenocarcinomas are rarely found in aging F344 rats. In untreated F344 rats used as controls in carcinogenesis tests performed as part of the NCI’s Carcinogenesis Testing Program, 1 of 1754 (0.05%) stomach adenocarcinomas were observed in females and 0 of 1794 (<0.05%) were observed in males (18). The initial pathological analysis performed at Stanford Research Institute detected one adenocarcinoma in an animal from the low-dose groups and 2 in animals from the medium-dose groups. Our study has detected 3 additional early adenocarcinomas at sites of AP-positive intestinal metaplasia (Table 2) as well as 2 foci of severe intestinal metaplasia in which adenocarcinoma could not be identified with certainty (Table 2, Footnote c). Using simultaneous controls in which the frequency of stomach adenocarcinomas is less than 2% (<1 of 47) and assuming that the occurrence of adenocarcinoma is random, the likelihood of observing 6 adenocarcinomas in 144 rats is less than 0.05 (significant at p < 0.05). With the use of historical controls in which the incidence of adenocarcinomas of the stomach is less than 0.05% (1 of 3548) and assuming that the occurrence of adenocarcinoma is random, the chance of observing 6 adenocarcinomas in 144 rats is less than 1.3 × 10⁻¹⁰ (significant at p < 0.001).

Over one-half of the gastric lesions were adjacent to aggregations of lymphocytes. Similar clusters were found in areas lacking AP staining or proliferative lesions in the stomachs of treated animals. The lymphoid aggregates may be follicles normally present in the stomach, although we detected no such striking clusters in control animals in this or an earlier experiment (35). It is also possible that the lymphoid aggregates stem from an immune response to inflammation of the gastric mucosa or to altered cellular antigens. Although striking when
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Fig. 1. Glandular stomach of control female F344 rat showing a focus of hyperplastic glands within lymphoid aggregate. Note disruption of muscularis mucosa. H & E, X 54.

Fig. 2. Alcian blue-periodic acid-Schiff staining of lesion in Fig. 1. A few mucus cells are present. X 130.

Fig. 3. Focal intestinal metaplasia in glandular stomach of rat receiving Aroclor 1254. Note fibrosis and inflammation adjacent to the lesion. H & E, X 80.

Fig. 4. Portion of gastric intestinal metaplasia in Fig. 3 showing mitotic figures, hyperplasia, goblet cells, and tall columnar cells. Note inflammatory cells in lamina propria. H & E, X 220.
Fig. 5. Alcian blue-periodic acid-Schiff staining of lesion in Fig. 3 showing goblet cells and mucus in lesion but not in adjacent normal epithelium. X 68.

Fig. 6. Alcian blue-periodic acid-Schiff staining of lesions in Figs. 3 and 5 showing Alcian blue-positive goblet cells and periodic acid-Schiff-positive brush border. X 220.

Fig. 7. Portion of hyperbasophilic focus of poorly differentiated intestinal metaplasia. Note similarity to invasive adenocarcinoma. H & E, X 54.

Fig. 8. Gastric adenocarcinoma invading the tunica muscularis. Note ulceration and inflammation. H & E, X 54.
Fig. 9. Portion of large metaplastic lesion showing apparent early invasion into submucosa. H & E, × 54.

Fig. 10. Portion of large cystic gastric submucosal lesion with similarities to intestinal metaplasia and adenocarcinoma. H & E, × 55.

Fig. 11. Scirrhouos gastric adenocarcinoma which invaded the gastric serosa. H & E, × 80.

Fig. 12. Portions of gastric adenocarcinoma which invaded the gastric serosa showing areas of well-differentiated epithelial cells resembling those in intestinal metaplasia. H & E, × 220.
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