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

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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 (5, 13, 14, 15). A 1972 report indicated that 32.5% of randomly collected samples of adipose tissue from the United States population contained 1.0 or greater ppm PCBs, 33.3% contained less than 1.0 ppm PCBs, and 34.2% contained no detectable PCBs (46).

Biological effects of PCB exposure were suspected in 1968 when Yusho, also known as “oil disease,” reached epidemic proportions in Japan due to widespread ingestion of rice oil contaminated with PCBs (31). Symptoms of Yusho include eye discharge, swelling of eyelids, acne-like skin eruptions, skin pigmentation, and weakness (31).

In experimental animals, some of the reported effects of PCB-toxicity are liver alterations (chicken, mouse, guinea pig, and rabbit) and skin lesions (guinea pig, rabbit, mouse, and monkey) (1, 2, 4, 34). PCBs have been reported to induce hepatic smooth endoplasmic reticulum (38), hepatic enzymes (8, 32, 38), and clusters of pancreatic tissue in the livers (27) of exposed rats. Proliferative lesions in the liver, specifically hepatocellular neoplastic nodules, have been reported in rats fed 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). In this report, we present evidence that Aroclor 1254 induces intestinal metaplasia in the glandular stomachs of F344 rats and that such lesions can involve the muscularis mucosa and the submucosa and may lead to adenocarcinoma.

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 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 lymphoid clusters and intestinal metaplasia, in the glandular stomachs of F344 rats exposed to Aroclor 1254.

### 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>No. of males with stomach lesions</th>
<th>No. of females 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>1/23 (4.3)%</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>2/48 (4.2)%</td>
<td>5/48 (10.4)%</td>
</tr>
<tr>
<td>50</td>
<td>4/24 (16.7)%</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>10/24 (41.7)%</td>
<td>6/48 (12.5)%</td>
<td>17/48 (35.4)%</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentages.

### Table 1
Incidence of stomach lesions, namely intestinal metaplasia and adenocarcinoma, in the glandular stomachs of F344 rats exposed to Aroclor 1254

<table>
<thead>
<tr>
<th>Concentration of Aroclor 1254 (ppm)</th>
<th>Incidence of lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3/24 (12.5%)</td>
</tr>
<tr>
<td>25</td>
<td>4/24 (16.7%)</td>
</tr>
<tr>
<td>50</td>
<td>7/24 (29.2%)</td>
</tr>
<tr>
<td>100</td>
<td>10/24 (41.7%)</td>
</tr>
</tbody>
</table>

* The bioassay was performed at Stanford Research Institute, Menlo Park, Calif, under the direction of Dr. David C. L. Jones. Necropsies were supervised by Dr. Daniel Sasmore of Stanford Research Institute.
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 aggregates 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
present, lymphoid clusters are not obligatory in the development of intestinal metaplasia since some sites of intestinal metaplasia lacked discernible lymphoid aggregates.

Individual animal pathology data were examined in an attempt to uncover any correlation between rats having stomach lesions and those with liver lesions, namely, hepatocellular carcinomas and hepatocellular neoplastic nodules. Hepatocellular neoplastic nodules can be induced by chemical carcinogens and increasingly are recognized as "precancerous" lesions (16, 39, 45). No correlation was found between animals having gastric lesions and those with liver lesions; therefore, the presence of intestinal metaplasia does not appear to predispose an animal to "precancerous" lesions in the liver, or vice versa.

The presence of intestinal enzymes and cell types in gastric foci may be significant in stomach cancer progression (21, 26). Although it remains to be established that intestinal metaplasia is "precancerous," intestinal metaplasia accompanies stomach cancer (25, 36), and both carcinoma and intestinal metaplasia are found in the pyloric region of the stomach (37). The incidences of intestinal metaplasia and carcinoma increase with age, and populations with high frequencies of gastric cancer have increased incidences of intestinal metaplasia compared to those with low frequencies of gastric cancer (10, 22). Furthermore, in this and in a previous study (35), we have found early carcinomas preferentially located at sites of intestinal metaplasia. Thus, adenocarcinoma and intestinal metaplasia appear to be related and may share initiating mechanisms. Our results indicate that evaluation of data on intestinal metaplasia can increase the sensitivity of an animal bioassay for detecting gastric carcinogens and/or promoters.

The conclusion from the NCI bioassay was that the combined incidence of hepatocellular neoplastic nodules and hepatocellular carcinomas in animals treated with Aroclor 1254 was significantly higher than that in controls. We have extended the results from this bioassay and conclude that Aroclor 1254 certainly leads to induction of intestinal metaplasia and probably leads to induction of adenocarcinoma in the glandular stomachs of F344 rats.

REFERENCES


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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. × 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. × 220.

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

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

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

Fig. 11. Scirrhous gastric adenocarcinoma which invaded to the gastric serosa. H & E, x 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, x 220.
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