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Opposite Effects of Lead on Chemical Carcinogenesis in Kidney and Liver of Rats

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The vast number of animal experiments designed to determine the tumorigenic effects of carcinogenic substances relate to a single compound and its effect upon one or more target tissues. Less commonly pursued are the interactions and results which may arise from the combination of two or more potential carcinogens in an experimental situation. One potentially important factor in the metabolism and ultimate fate of carcinogenic compounds in animal systems could be the degree of exposure of the animals to environmental pollutants, for example heavy metals. Recent evidence suggests that microsomal function may be inhibited or enhanced by specific heavy metal compounds (ALVAREZ et al. 1972, ALVAREZ et al. 1976, BECKING 1976).

The heavy metal lead (Pb^{++}) is an ubiquitous environmental pollutant present in particulates of air following fossil fuel combustion (GOYER 1971), and can be found as a contaminant in foods and water (CANNON and BOWLES 1962, BERG and BURBANK 1972). In the rodent kidney, acute exposure to lead results in a wave of mitotic activity within the epithelium of proximal tubules (CHOIE and RICHTER, 1974), while prolonged exposure results in adenocarcinoma (VAN ESCH et al. 1962, BOYLAND et al. 1962). Lead has also been shown to be cocarcinogenic with benzo(a)pyrene in lung tumorigenesis in the syrian hamster (KOBAYASHI and OKAMOTO 1974).

Previous work in this laboratory (DEES et al., 1976; HEATFIELD et al., 1976) has resulted in the development of an animal model for human renal adenocarcinoma utilizing the chemical carcinogen N-(4'-fluoro-4-biphenyl) acetamide (FBPA). When rats are fed this compound for a period of 9 months to 1 year, grossly observable renal tumors occur in approximately 70% of the animals (DEES et al., 1976). In addition, the development of these tumors goes through an easily recognized series of histogenetic stages (HEATFIELD et al. 1976). These include: Stage 1, cytomegaly of proximal tubule lining epithelium with large, bizarre nuclei; stage 2, tubular hyperplasia; stage 3, microscopic nodules resulting from enlargement of stage 2 lesions and stage 4, gross nodules. Transplant studies into syngeneic animals have been successful on tumor masses as small as 1 mm in diameter, indicating the malignant nature of these neoplasms. FBPA, but not Pb^{++}, has been shown to cause a high incidence of liver neoplasms (MORRIS et al. 1957). We report, here, a co-carcinogenic interaction between lead and FBPA in relation to the development of renal adenocarcinoma and a delay of onset and retardation of later development of hepatocellular carcinoma.

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MATERIALS AND METHODS

Male rats of the highly inbred Fisher-344 strain, (Microbiological Associates, Rockville, Maryland) weighing 125-175 grams, were used. Three to four animals were housed per stainless steel cage in temperature and humidity controlled rooms on a 12 hour light-dark cycle. Food and water were available ad libitum. Animals were divided into 4 groups of 150 rats each: Group I - (controls) were fed a semi-synthetic diet developed by MORRIS et al. (1957). Group II (were fed the above diet to which 0.04% (W/W) FBPA was added. Group III were fed the semisynthetic diet plus 1% (W/W) Pb^{++} as lead acetate. Group IV animals were fed the control diet to which 0.04% FBPA and 1% lead acetate were added. Following exposure of 3, 7, and 14 days and 4, 8, 16, 24, 36, and 52 weeks, 10-20 rats per group were sacrificed and their livers and kidneys were removed, weighed and processed for subsequent examination.

Tissues for histologic examination were fixed in 4% formaldehyde, 1% glutaraldehyde in 200 milliosmolar phosphate buffer, pH 7.4 (MCDOWELL and TRUMP 1976) and processed by routine methods. In order to estimate the capacity for mixed function oxidase activity, chtochrome P-450 of liver microsomal fraction was monitored throughout the experiment according the the method of OMURA and SATO (1964).

RESULTS

Table 1 summarizes the data on kidney tumors in each of the groups through 52 weeks. The first indication (via gross inspection) of kidney tumors was in group IV at 24 weeks. After 36 weeks of feeding, tumors were seen in kidneys of group II animals as well. Throughout the remaining period of study, a higher total number of gross kidney tumors was found in Group IV animals. In addition, the highest % of tumor-bearing rats was in this group.

TABLE 1

Number of gross renal adenocarcinomas per group with time.

		Weeks of Exposure			
Group		16	24	36	52
Group I	Control	0	0	0	0
Group II	FBPA	0	0	37	65
% Tumor	bearing rats			73%	90%
Group III	Pb	0	0	0	1
% Tumor	bearing rats				6.5%
Group IV	FBPA and Pb	0	2	61	88
% Tumor	bearing rats		13%	87%	100%

Each value represents analysis of both kidneys of 15 animals.

Upon histologic examination, the tumors were shown to be renal adenocarcinomas, identical to those previously described (DEES et al. 1976, HEATFIELD et al. 1976). The microscopic appearance was identical for tumors produced by FBPA alone and by FBPA + Pb^{++}. Measurements of the gross nodules revealed a larger average size for tumors in Group IV animals (fig. 1). Prior to 24 weeks, Group IV animals displayed an increased number and more rapid development of microscopic lesions which were previously determined as part of the histogenetic development of the gross tumors. The greatest number of microscopic tumor stages was seen in Group IV, although an appreciable number was also encountered in Group II animals. In general, the animals that received Pb^{++} alone showed only a few formative lesions.

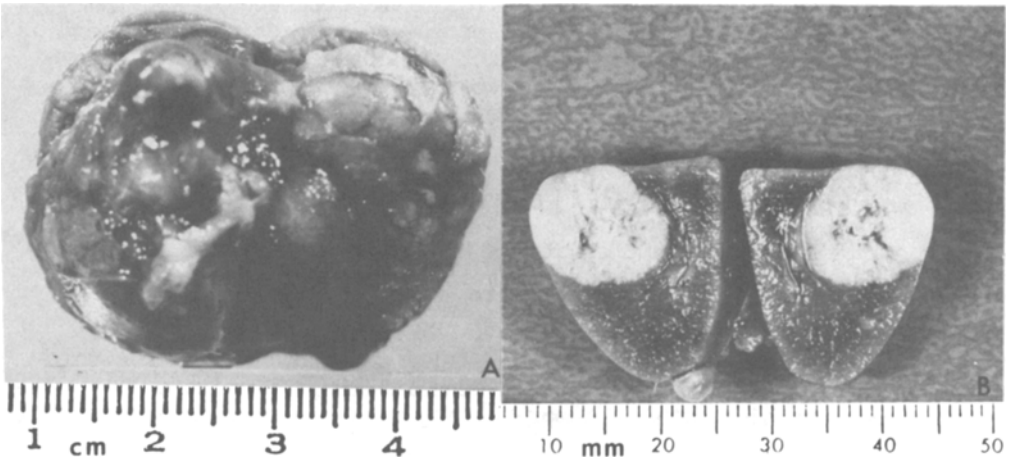

Figure 1

A. Maximum tumor involvement in the kidney of a rat fed FBPA and Pb.

B. Typical renal adenocarcinoma following exposure to FBPA alone.

In the liver, no lesions were seen before 24 weeks in any group. However, liver to body weight ratios of Group II and IV animals increased with respect to controls (TABLE 2). The greatest increase in liver weight was seen in Group II animals followed by Group IV. Group III liver weights were not significantly different from controls.

TABLE 2

Effect of dietary exposure on liver to body weight ratios

Group	Weeks of Exposure 16	24	36	52
Group I Control	.033±.004	.025±.009	.020±.007	.027±.003
Group II FBPA	.043±.005	.062±.007[2]	.132±.023[2]	.219±.060[2]
Group II Pb^{++}	.032±.005	.028±.003	.031±002	.036±.004[1]
Group IV FBPA and Pb^{++}	.040±.004	.043±.003[1]	.066±.006[2]	.098±.038[2]

All values represent means of 10 animals ± one standard deviation.
[1] $P < 0.05$; [2] $P < 0.01$ with respect to controls.

After 24 weeks of exposure, the livers of Group II rats revealed numerous pale nodules, 1-2 mm in diameter. Histologic analysis showed these nodules to be identical to the neoplastic nodules as described in the recent National Cancer Institute classification (SQUIRE and LEVITT 1975) of rat liver neoplasms. Due to elevation above the surface of the liver, nodules were easily recognized. At this time the livers of Group IV animals were granular in appearance but no nodules were encountered. By 36 weeks, histological examination of the livers of the Group II animals revealed numerous examples of hepatocellular carcinoma. In addition to neoplastic changes, extensive areas of necrosis, fibrosis and degeneration were seen. Following 36 weeks of exposure, livers of Group IV animals showed small 1-2 mm nodules. These were identical grossly and microscopically to those which were seen 12 weeks earlier in Group II. We have interpreted this finding as representative of a possible retarding effect of Pb upon the development of FBPA-induced hepatocellular carcinomas. Also, with respect to liver weights at 52 weeks, an indication of a protective or retarding effect of lead upon FBPA-induced liver alteration was seen. After 52 weeks of feeding, the histologic pattern of hepatocellular carcinoma was identical in Group II and IV animals. However, on the basis of liver weight and liver to body weight ratio, the extent of alteration in Group II animals was three times greater than in Group IV (Table 2). No evidence of hepatocellular carcinoma or of neoplastic change was observed in Group III rats.

In the early time periods (i.e. 3, 7, 14 days), a pattern of enzyme induction as evidenced by increased cytochrome P-450 was

seen in livers of Group II rats (Table 3). Group III animals showed a depression of cytochrome P-450 below control values. Group IV animals showed cytochrome P-450 values similar to controls (i.e. > Group III but < Group II). Thus, the preliminary microsomal data suggest an inhibition of cytochrome P-450 by Pb^{++}, as has been noted by others (ALVAREZ et al. 1972, ALVAREZ et al. 1976, BECKING 1976) and an induction by FBPA.

TABLE 3

Effect of diets on liver Cytochrome P-450

		Exposure Period		
Group		7 day	14 day	16 weeks
Group I	Control	.347±.101	.351±.047	.545±.065
Group II	FBPA	.475±.109[1]	.458±.069[2]	.703±.100[3]
Group III	Pb^{++}	.333±.180	.251±.105	.453±.154
Group IV	FBPA and Pb^{++}	.293±.131	.419±.032	.590±.084

All values represent means of 4-5 animals ± one standard deviation.
[1] $P < 0.05$; [2] $P < 2.5$; and [3] $P < 0.5$ with respect to controls.

DISCUSSION

Our data show that Pb^{++} enhances the formation of FBPA-induced renal adenocarcinoma by decreasing the latency period, increasing the tumor yield and increasing the percentage of tumor bearing rats from 70% to 100%. Furthermore, early protection from onset and retardation of later development of hepatocellular carcinoma was provided by addition of 1% Pb^{++} acetate to the FBPA diet. The addition of Pb^{++} to the FBPA diet did not result in any alterations in tumor morphology in either the kidney or liver. The primary effect, therefore, was on the time of gross development (as measured by tumor size and liver to body weight ratios).

The metabolism of FBPA has not been studied. It is similar in structure, however, to acetylaminofluorene (AAF). We can postulate that like AAF, FBPA must undergo oxidative metabolism to exert its carcinogenic potential. Acute and subacute Pb^{++} administration resulted in decreases in mixed-function oxidase activity (1,2,3). In this study, FBPA caused induction of cytochrome P-450, while the coadministration of FBPA and lead did not result in an increase of

cytochrome P-450. Indirect evidence, therefore, indicates that the mixed-function oxidase system mediates FBPA carcinogenicity. Further work is needed to determine if this is responsible for the differences in target organ response. Thus by synergism and/or antagonism ubiquitous environmental pollutants, such as heavy metal, may alter target organ response in chemical carcinogenesis.

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