Effect of 3-Methylcholanthrene on Hyperplastic and Early Neoplastic Hepatic Lesions Induced in Rats by Carbon Tetrachloride

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SUMMARY—Buffalo male and female rats 5, 8, 12, 24, 52, and 76 weeks old, with hepatic lesions induced by carbon tetrachloride, were fed 3-methylcholanthrene to study the effect on hepatic lesions induced by CCl₄. This chemical increased the incidence of hyperplastic nodules and small hepatocellular carcinomas in animals of all ages. The difference was greater in animals 12 weeks of age and older. Females were more susceptible to the development of hyperplastic nodules and carcinomas than were males. Multiple nodules and carcinomas were observed in the livers of animals given both chemicals, whereas animals receiving CCl₄ had fewer lesions per liver. Cirrhosis of the liver was more advanced in the animals given methylcholanthrene and carbon tetrachloride simultaneously.—J Nat Cancer Inst 45: 1237-1242, 1970.

THE DEVELOPMENT of hyperplastic hepatic nodules and small hepatocellular carcinomas in Buffalo strain rats given subcutaneous injections of carbon tetrachloride (CCl₄) is related to the age and sex of the animals (1, 2). The 24- and 52-week-old males and females had more hyperplastic nodules, as well as an occasional small hepatocellular carcinoma, than did animals 4, 12, and 76 weeks of age.

The incidence of hyperplastic nodules increased in male rats 12 weeks old when 3-methylcholanthrene (MCA) was given simultaneously with CCl₄ (3). The present study was done to determine if MCA would increase the hyperplastic and early neoplastic lesions of the liver in male rats of other ages and in females of varying ages.

MATERIALS AND METHODS

Inbred Buffalo strain male and female rats 5, 8, 12, 24, 52, and 76 weeks old were used. Groups of animals of each age and sex were treated with: 1) only CCl₄ [Mallinckrodt (low sulfur) CCl₄ analytical reagent, Mallinckrodt Chemical Works, New York, N.Y.], 2) only MCA (Eastman Kodak Co., Rochester, N.Y.), or 3) CCl₄ and MCA simultaneously. There were 10–17 rats in each group (text-figs. 1, 2). In addition to the experimental animals,

1 Received July 7, 1970; accepted September 14, 1970.
2 This research was started in the Laboratory of Biology, General Laboratories and Clinics, and completed in the Experimental Pathology Branch, Carcinogenesis, Etiology, National Cancer Institute.
control groups of 6 rats of each age and sex were fed only the basal diet of laboratory meal.

The laboratory meal was ingested ad libitum. At a dosage of 1.3 ml/kg body weight, carbon tetrachloride was injected subcutaneously twice weekly; 0.033% MCA was added to the meal.

Animals given CCl₄ and MCA simultaneously were killed when moribund or at the end of 12 weeks. Animals given CCl₄ only were killed between 6 and 12 weeks to correspond to the time of death for those receiving CCl₄ and MCA. Rats receiving MCA only, as well as control rats, were killed at the end of 12 weeks. Complete autopsies were done and tissues were fixed in 4% formaldehyde solution. Paraplast sections were stained with hematoxylin and eosin. Sections of liver were also stained with periodic acid-Schiff for glycogen and mucin, Masson trichrome for connective tissue, acid-fast and oil red O for ceroid, phosphotungstic acid-hematoxylin for canaliculi and fibrin, Perls' for hemosiderin, Hall's for bilirubin, and Wilder's for reticulum. Oil red O staining for lipid was done on frozen sections.

For evaluation of the findings in the livers of each experimental animal, the lesions were classified and tabulated as follows: 1) no hyperplasia, 2) hyperplasia, 3) hyperplastic nodules, 4) small hepatocellular carcinomas (4). Well-developed hepatocellular carcinomas like those seen in animals given other chemical carcinogens were not observed. These lesions were detailed and illustrated by photomicrographs in (1, 4).

RESULTS

The number of experimental animals with each type of lesion is shown in text-figures 1 and 2. Hepatic lesions or lesions in other organs were not observed in the control animals.

The animals given both CCl₄ and MCA lost weight, whereas those given only CCl₄ or MCA gained weight. Terminally, the male rats receiving both chemicals weighed 45–100 g less than those given CCl₄; the females, 20–55 g less. The controls and the animals ingesting MCA gained more weight than those on CCl₄.

The male rats of all ages treated with CCl₄ and MCA survived an average of 9.2 weeks (range, 6.2–12), and the females, an average of 11.0 weeks.

Male Animals

Hyperplastic nodules were observed in rats 8, 24, and 52 weeks of age given CCl₄ only. There was one small hepatocellular carcinoma in an 8-week-old animal. Hyperplastic nodules were induced by MCA and CCl₄ in animals of all ages. Carcinomas were present in rats 12 weeks of age and older. The incidence of nodules and carcinomas increased with increasing age of the rats, with one exception. Curiously, the incidence of nodules and carcinomas was lower in the 8-week-old male rats given both chemicals than in those treated with CCl₄ alone. In animals of all other ages receiving both chemicals, there were more nodules and carcinomas per liver. This number increased with the age of the animals.

Female Animals

The incidence of hyperplastic nodules in female animals 12–52 weeks old that received CCl₄ only was greater than in male rats. Females 24 and 52 weeks of age developed the highest incidence of nodules. In comparison to the 5-, 8-, and 12-week-old rats, 76-week-old females were less prone to develop nodules. Males 8 weeks old were more susceptible to the growth of nodules and carcinomas than were females of the same age. One 24-week-old and another 52-week-old animal had small carcinomas.

Nodules and small hepatocellular carcinomas increased notably in females 24, 52 and 76 weeks of age that were treated with CCl₄ and MCA simultaneously. Almost all rats 52 and 76 weeks old had nodules or carcinomas of the liver. The increase in number of nodules and carcinomas per liver was even more striking in the females than in the males.

Female rats of all ages were more susceptible to the development of hyperplastic nodules and small carcinomas than were male rats. This was evident for animals given CCl₄ only (except for 8-week-old males), or CCl₄ and MCA, though the findings were more notable in the latter groups of animals.
Other Hepatic Lesions

Animals given injections of CCl₄ only had mild or moderate cirrhosis. Those fed MCA in the diet simultaneously with injections of CCl₄ developed severe cirrhosis (5, 6). The incidence of hepatic vein thrombosis was markedly increased in the rats given both chemicals, except for both males and females 8 weeks of age (6, 7). Complete occlusion with infarcts of the liver was observed in three animals: one 5-week-old male and one 24-week-old and one 52-week-old female. Cholangiofibrosis was increased in 5-week-old males and 24-week-old females treated with both chemicals.

Lesions in Organs Other Than Liver

The spleen was considerably enlarged in rats given both CCl₄ and MCA, particularly in 5- and 12-week-old animals of both sexes. There was focal or mild, diffuse fibrosis.

Testes, seminal vesicles, and prostate glands were more atrophied in animals with severe cirrhosis, i.e., animals receiving both chemicals. The pancreas was edematous in rats with ascites.

Fibrin thrombi were present in the left ventricle in one 5-week-old and two 12-week-old females and in the splenic artery in one 5-week-old female and one 24-week-old male. There were focal renal

Text-Figure 1.—The most advanced lesion in a specified animal is shown for each age at the beginning of the experiment. Number above each black bar is the total number of animals developing such lesions. The number of animals per group is given above each white bar under cirrhosis. Cross-hatching indicates number of rats with cirrhosis.
infarcts in one 12-week-old female and infarction of the entire kidney in two 12-week-old females.

Thyroiditis in rats ingesting MCA in the diet or receiving CCl₄ was detailed in (8, 9). The incidence of thyroiditis in animals given both chemicals was similar to that of animals fed MCA only in the diet.

There was a transitional cell carcinoma of the urinary bladder in one 8-week-old female rat receiving both chemicals (5).

**DISCUSSION**

MCA decreases the incidence of carcinomas of the liver in rats given 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) (10). The chemical protects against hepatic carcinogenesis in rats ingesting N-2-fluorenylacacetamide (2-FAA) or N-2-fluorenyldiacetamide (2-FdiAA) (6, 11). The incidence of carcinomas of the liver is markedly reduced in rats given MCA and diethylnitrosamine simultaneously (6); however, it is only slightly decreased in rats receiving MCA and dimethylnitrosamine (12).

Contrary to these previous reports, MCA, in this experiment, increased the incidence of hyperplastic nodules and small carcinomas of the liver in rats given CCl₄.

The hyperplastic hepatic nodules are generally

![Text-figure 2. Lesions in each animal for each age at the beginning of the experiment. Explanation of bars same as in text-figure 1.](https://academic.oup.com/jnci/article-abstract/45/6/1237/907349)
accepted as being preneoplastic (4). The nodules, as well as the cirrhosis, developed rapidly and the animals died as a result of the cirrhosis. It was shown previously that animals given CCl₄ must survive for a longer period for the hyperplastic nodules and small carcinomas to become larger, well-developed hepatocellular carcinomas (13). The severity of the cirrhosis is related to the dosage of MCA and CCl₄. Smaller dosages are being administered so that the animals will live longer. Also, the chemicals are being discontinued, and the animals allowed to live longer.

Earlier studies, with one exception, concerned with hepatic carcinogenesis and MCA did not evaluate the importance of cirrhosis (10–12). MCA not only protected against hepatic carcinogenesis, but also against the development of cirrhosis of the liver in rats ingesting 2-FdlAA (6). In the present work, not only was the incidence of hyperplastic and early neoplastic lesions of the liver increased, but the severity of the cirrhosis also was enhanced. The significance of cirrhosis in the development of hepatocellular carcinomas in experimental animals has not been clarified.

The mechanism by which MCA protects against hepatic carcinogenesis induced by 3'-Me-DAB or 2-FAA has been studied. MCA increases the activity of the enzymes concerned with the hydroxylation of aromatic amines and the reduction of azo bond linkage and the N-demethylation of amino azo dyes (14, 15).

MCA and CCl₄ both affect the liver. CCl₄ causes intracellular accumulation of lipid (16–20), disturbances in protein synthesis and other enzymes of the endoplasmic reticulum (19, 21, 22), failure of energy synthesis by mitochondria (17, 23), and necrosis of parenchymal cells (16). MCA increases amino acid incorporating activity, protein synthesis, and total protein and acts as an effective inducer of microsomal enzymes (24–29).

The microsomal enzymes are involved in metabolic detoxication. The metabolism of 3'-Me-DAB or 2-FAA by microsomal enzymes leads to inactive products. Metabolism of CCl₄ is the activation step that leads to its toxicity.

McLean et al. (30) reported that phenobarbitone in the drinking water increased the severity of CCl₄-induced cirrhosis of the liver. Marshall and McLean (31, 32) showed that phenobarbitone increased the amount of cytochrome P-450 in rat liver. The higher level of cytochrome P-450 increased the metabolism of CCl₄ and caused severe cirrhosis in animals given both chemicals. CCl₄ also decreased markedly the amount of this pigment in the hepatic microsomes (33).

With most potent hepatic carcinogens, MCA is protective; however, it increases the action of the weaker hepatic carcinogen, CCl₄, and also adds to the severity of the cirrhosis of the liver. It is not clear whether the effects of MCA on CCl₄ carcinogenesis are related to the increased severity of the cirrhosis and the increased development of hyperplastic nodules, or whether both are important.

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