

LUNG TUMORS IN MICE SUCKLED BY 3-METHYLCHOLANTHRENE-TREATED MOTHERS

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INTRODUCTION

Several environmental carcinogens have been reported to be transferred to sucklings via mother's milk¹⁾. Cycasin²⁾, pyrrolidine alkaloids³⁾, Petridium aquilinum toxin⁴⁾, aflatoxinB₁ and G⁵⁾ are examples of the carcinogens which have been found in the milk of lactating mothers. Experimental chemical carcinogens including urethane⁶⁾, 3-methylcholanthrene (3-MC)^{7,8)}, diethylnitrosamine⁹⁾, dimethylbenz (a) anthracene¹⁰⁾, ethyl- or methyl-nitrosourea^{11,12)}, methyl-nitrosamine¹³⁾, benzo (a) pyrene¹⁴⁾, 4-nitroquinoline 1-oxide and methylnitrosourethan¹⁵⁾, are also excreted in the milk and transferred to sucklings which later develop carcinoma in the lung.

In our previous study¹⁶⁾ transplacental transfer of 3-MC to fetal mice was confirmed by radiometric and macroautoradiographic examinations. The present paper reports a high incidence of lung tumor in ICR mice suckled by mothers receiving 3-MC by stomach tube only during the lactation period. Milk removed from the sucklings' stomachs was studied for its content of 3-MC and its metabolites, and their ethanol-soluble fractions were analyzed by a gas chromatograph-mass spectrometer (GC-MS) system for identification of carcinogenic metabolites.

MATERIALS AND METHODS

Chemicals: 3-MC and ³H-labeled 3-MC (13 Ci/mmmole) were purchased from Sigma Chem. Co. and New England Nuclear Co., respectively. Dehydro 3-MC, 1-hydroxy 3-MC, 2-hydroxy 3-MC and trans 1,2-dihydroxy 3-MC were prepared in our laboratory according to the methods of Smis¹⁷⁾, as described previously¹⁸⁾.

Animals: Pregnant ICR mice were obtained commercially. They were housed separately until parturition.

Incidence of tumor: Five mg of cold 3-MC in 0.5 ml of sesame oil was administered to lactating mice by stomach tube on the second and fourth days after delivery. The suckling mice were nursed by the 3-MC-treated mothers for one month and then were fed a laboratory chow and tap water *ad libitum*. They were sacrificed at six months of age. Organs were checked macroscopically at autopsy, and tumors in the lung, if any, were counted and examined histolo-

gically.

Time course of excretion: ^3H -labeled 3-MC (5 mg and 53.6 μCi) was administered to lactating mice one day after delivery in the manner described above. The lung, liver and caseated milk in the stomach were removed from the sucklings one, two, three, and seven days after administration. Each organ or milk was weighed, mixed with 0.5 ml of NCS tissue solubilizer, incubated at 65°C for five hours and its radioactivity was measured in a Nuclear Chicago liquid scintillation spectrometer, as described previously¹⁶.

Analysis of carcinogens in the milk: ^3H -labeled 3-MC (5 mg and 53.6 μCi) was administered to lactating mice three days after delivery, and their sucklings were sacrificed 24 hours later. Caseated milk from the suckling stomach was extracted with ethanol. The extracts obtained were applied to a Sephadex LH-20 column in ethanol. This resulted in separation and purification of intact 3-MC and its hydroxy metabolites, as described previously^{18,19}. The chromatographic fraction containing 3-MC or its metabolites was analyzed by GC/MS, according to the method of Takahashi²⁰. The fraction was purified further by silica gel column chromatography in a benzene:ethanol (9:1) solution as described previously²¹ and analyzed by GC/MS also.

GC/MS system: A combined system of a JEOL JGC-20K gas chromatograph, a JEOL JMS D-300 mass spectrometer and a mass data analysis system JEOL JMA-2000 was used at an ionization current of 300 μA , an ionization voltage of 25 eV and a mass range from 200 to 500. The samples were silylated at 65°C for 15 minutes with pyridine: N,O-bis(trimethylsilyl)acetamide: trimethylchlorosilane (2:2:1) in N_2 gas-sealed glass tubes. They were applied to gas chromatograph columns of OV-1 (2%) and Dexsil-300 (6%) which were packed in glass tubes (50 cm \times 2 mm) together with Chromosorb W. The gas chromatograph was operated at a constant temperature of 260°C on OV-1 and 290°C on Dexsil-300. The carrier gas was helium and the flow rate was about 20 ml/min after injection of the sample and continued for 10 minutes at 4-second scanning intervals.

RESULTS

The incidence of lung tumor is summarized in Table 1. Tumors were found in about 60% of the male and 30% of the female progeny. No tumors were observed in the progeny of control mothers. All lung tumors were confirmed to be adenoma or adenocarcinoma histologically. No tumors occurred in any other organs, except for some cases with lymphoid cell proliferation in the lung, thymus and some other organs.

The time course of the radioactivity in the milk is shown in Table 2. The activity was most intense one day after administration and then decreased rapidly. It was still present on the seventh day, but only slightly. In the liver and lungs, the activity was most intense two days after administration and it remained through the seventh day.

About 40% of the radioactive substances in the milk were ethanol-soluble. Mass fragmentogram results of this ethanol-soluble fraction are shown in Fig. 2. Many peaks appeared by plotting total ions from 200 to 500. They might originate in components derived from both tissue and the carcinogen. Intact 3-MC can be plotted by the molecular ion at m/e 268. Therefore,

Table. 1 Incidence of lung tumor

Treatment of mother	Sex of suckling	Effective ^{c)} number	Tumor-bearing	
			Number	%
3-MC ^{a)}	male	46	28	61
//	female	36	10	28
— ^{b)}	male	15	0	0
—	female	14	0	0

^{a)} Lactating mice given two gastric intubations of 5 mg of 3-MC in sesame oil soon after delivery.

^{b)} Mice given sesame oil alone.

^{c)} Surviving mice at 6 months of age.

Table. 2 Distribution of 3-MC and its metabolites in suckling mice (dpm/mg wet tissue)

Tissue	Days after administration of 3-MC to mother			
	1	2	3	7
Lung	7.6± 0.8 ^{a)} (3.2± 0.4) ^{b)}	13.5±1.3 (5.7±0.6)	4.4±0.2 (1.9±0.8)	6.3±1.3 (2.7±0.5)
Liver	9.9± 2.6 (4.2± 1.1)	13.7±1.5 (5.8±0.6)	5.9±0.7 (2.5±0.3)	6.2±1.6 (2.6±0.7)
Milk	55.9±16.5 (23.5± 6.9)	28.0±4.6 (11.8±1.9)	9.2±1.3 (3.9±0.6)	2.8±0.4 (1.2±0.2)

^{a)} Mean and standard deviation in 6 animals.

^{b)} ng of hydrocarbons/mg of wet tissue. Calculated by conversion to 3-MC.

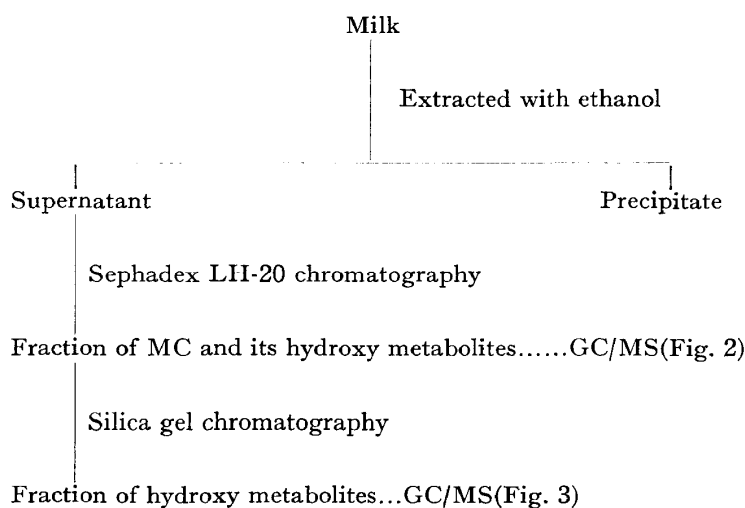


Fig. 1 Scheme for anzlysis of carcinogen in milk.

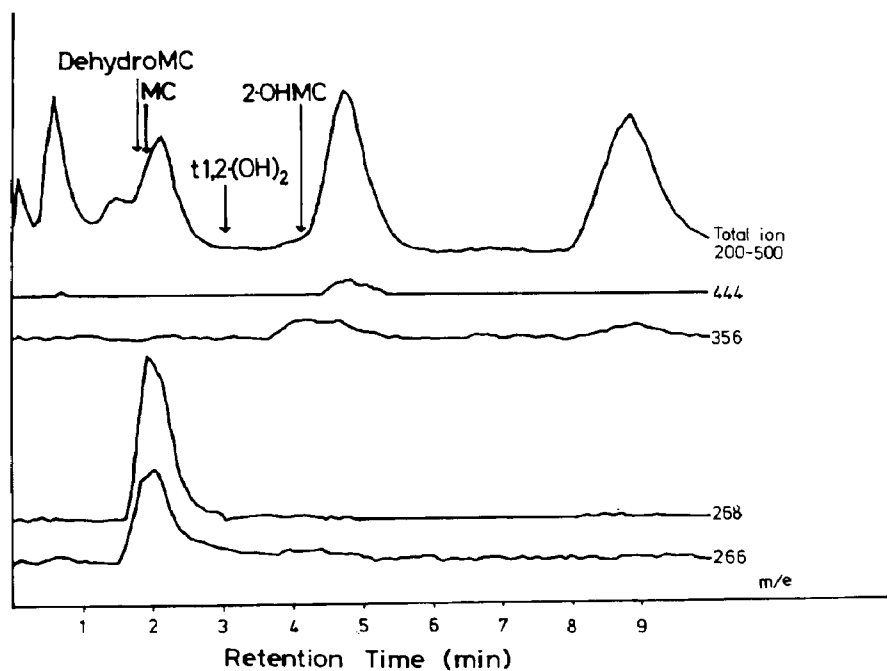


Fig. 2 Mass fragmentgram of 3-MC and its metabolites in milk. Fractions of 3-MC and its hydroxy metabolites purified by Sephadex LH-20 were applied to OV-1 column. Fragment ions at m/e 266, 268, 356 and 444 are for detection of dehydro 3-MC, intact 3-MC, 2-OH 3-MC and *t* 1,2-(OH)₂ 3-MC, respectively.

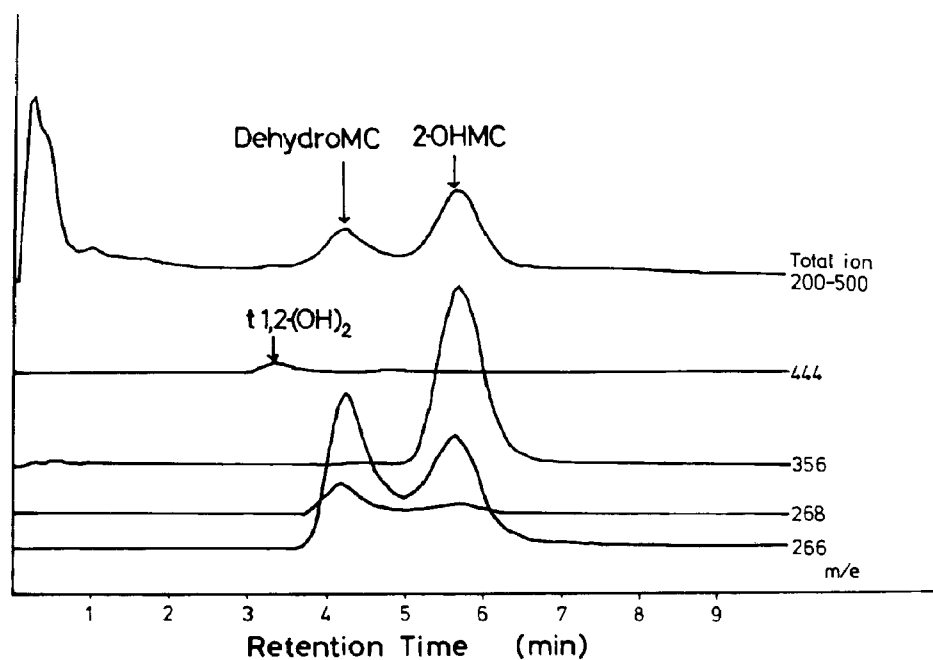


Fig. 3 Mass fragmentogram of hydroxy metabolites of 3-MC in milk. The fraction of hydroxy metabolites was obtained from the sample in Fig. 2 by further purification on silica gel column chromatography and applied to Dexsil-300 column.

the highest peak at m/e 268 of this fraction was identified as intact 3-MC. Another peak at m/e 266 was also high. Dehydro 3-MC has a molecular ion at m/e 266 and its retention time on gas chromatography is almost the same as that of intact 3-MC, as described previously.¹⁶⁾ Therefore, this peak was identified as dehydro 3-MC. This compound is known to be a break-down product of intact 3-MC and 1- or 2-hydroxy 3-MC. Mono- or di-hydroxy 3-MC was never identified in this fraction, as no peaks were observed definitely by plotting the molecular ion at m/e 356 or 444.

Fig. 3 shows a mass fragmentogram of the hydroxy metabolite fraction obtained from silica gel column chromatography. In this fraction the number of peaks plotted by the total ions was reduced markedly because of successful purification. Dehydro 3-MC, 2-hydroxy 3-MC and trans 1,2-dihydroxy 3-MC were identified in this fraction.

DISCUSSION

In the embryo, transplacental transmission is the main route of environmental carcinogenesis and at least 30 chemicals have been found to be carcinogenic transplacentally. After delivery, mother's milk plays the main role in the transfer of carcinogens to their sucklings.

Tomatis et al.²²⁾ noted no difference in incidence of lung tumor between offspring of untreated pregnant mice suckled by 3-MC-treated mothers and those suckled by untreated mothers. In the present experiment a high incidence of lung tumor was shown in mice suckled by 3-MC-treated mothers, while no tumors developed in controls. The discrepancy between the data from these two experiments may be due to the different methods of carcinogen application.

The present experiment revealed that radioactivity in the suckling lungs was less than 13.5 dpm/mg wet weight of tissue which corresponds to 5.7 ng of the carcinogen. Our previous publication¹⁶⁾ reported that 0.26% of the 3-MC given to a pregnant mouse was transferred to the fetuses and that 2 μ g of the carcinogen/mg of wet tissue was found in the fetal lung when 5 mg was given to a pregnant mouse near term. This amount was thought to be sufficient to initiate lung tumors. The present experiments show that the same level of carcinogen in tissue is necessary for blastomogenesis in the lung.

Radiometric analysis of 3-MC-related compounds in the milk showed that half of the radioactive chemicals were ethanol-insoluble and consisted of binding forms of 3-MC metabolites to tissue macromolecules. The other half were ethanol-soluble and consisted of intact 3-MC and 2-hydroxy 3-MC, both of which are known to be carcinogenic in mice^{23,24)}. Dehydro 3-MC, which is a break-down product of 1- and 2-hydroxy 3-MC, was also detected in this ethanol-soluble fraction. 1-Hydroxy 3-MC was not detected in the milk; however, further studies are required to confirm its absence, since 1-hydroxy 3-MC is less stable in this analytical system than 2-hydroxy 3-MC.

SUMMARY

A potent chemical carcinogen, 3-methylcholanthrene, given to lactating mice, is transferred via the milk to their suckling offspring, which develop lung tumors in their adult ages. The incidence of tumor-bearing animals was about 60% in male and 30% in female offspring, while

no tumors developed in mice suckled by untreated mothers. The carcinogen in the milk and tissues was measured radiometrically to confirm its transfer via milk to the suckling mice. The amount of carcinogen in the lungs of the sucklings was less than 13.5 ng/mg of wet tissue when 5 mg was given to lactating mothers by stomach tube. This agrees well with the data obtained previously in fetal lungs when mother animals near term were treated with the carcinogen in the same manner. By means of gas chromatography-mass spectrometry, ethanol-soluble fractions of milk from the stomachs of suckling mice were analyzed and the parent carcinogen, its 2-hydroxy, dehydro, and 1,2-dihydroxy derivatives were detected. These chemicals are all carcinogenic in mice and reported to be more potent in this order²³).

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