MACROAUTORADIOGRAPHIC ASSAYS OF ^{14}C-LABELED 4-NITROQUINOLINE 1-OXIDE IN ADULT AND FETAL MICE

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INTRODUCTION

Among the chemical carcinogens, at least 30 have been proved to be carcinogenic in the offspring when given to the mother in late pregnancy[1]. It is likely that such chemicals or their metabolites pass across the placenta and have carcinogenic effects on fetuses. Takahashi et al. demonstrated by macroautoradiography that cetain carcinogens and/or their metabolites passed quickly across the placentas and were transferred to the fetus[2,3,4,5,6].

Since first reported by Nakahara et al.[7], 4-nitroquinoline 1-oxide (4-NQO) has been known as a potent carcinogen in experimental animals. Recently Nomura et al.[8] reported that this chemical is also carcinogenic to mouse fetuses or sucklings when administered to their mothers during pregnancy or lactation.

The present paper deals with the distribution of 4-NQO and its metabolites demonstrated by macroautoradiography an adult, pregnant and fetal mice. The relationship is discussed between the distribution of the carcinogen in the tissues and the susceptibility of the tissues to it.

MATERIALS AND METHODS

Chemicals: 4-NQO 5, 6, 7, 8, 9, 10-^{14}C (32.6 μCi/mg) was purchased from Daiichi Pure Chemical Co. This chemical was 96% pure and was used one month after the purity analysis. Macroautoradiography: Sixty μCi of ^{14}C-labeled 4-NQO dissolved in 0.6 ml of acetone was mixed with 1.2 ml of 1% gelatine and the acetone was evaporated in vacuum. The final volume of the solution was 0.8 ml. One tenth ml of the 4-NQO solution (7.5 μCi and 230 μg) was injected intravenously in each pregnant mouse on the 19th or 16th day of gestation. The same dose of ^{14}C-labeled 4-NQO was injected in young adult mice intravenously or subcutaneously. The treated mice were killed under ether anesthesia 1, 3, 5 or 24 hours later. Fetuses in the left horn were separated from their mother and were frozen in an acetone-dry ice mixture. Mothers with fetuses in the right horn were frozen whole. Macroautoradiography was carried out according to the method described previously[2].

RESULTS

Adult mice: As shown in Fig. 1, radioactive substances derived from ^{14}C-labeled 4-NQO were accumulated intensely in the gallbladder and small intestinal tract and reached the cecum one hour after intravenous administration. They were also accumulated intensely in the lungs, kidneys and urinary bladder, moderately in the liver, heart, muscle, salivary glands and blood and slightly in the brain. The patterns of distribution were almost the same 5 hours later. However, radioactive substances appeared in the large intestinal tract and were eliminated gradually from the organs described above.

Large amounts of radioactive substances were deposited at the injection site even 24 hours after subcutaneous administration (Fig. 2). They were distributed intensely in the gallbladder, intestinal tract, urinary bladder and kidneys, moderately in the liver, lungs, blood and muscles and slightly in the brain.

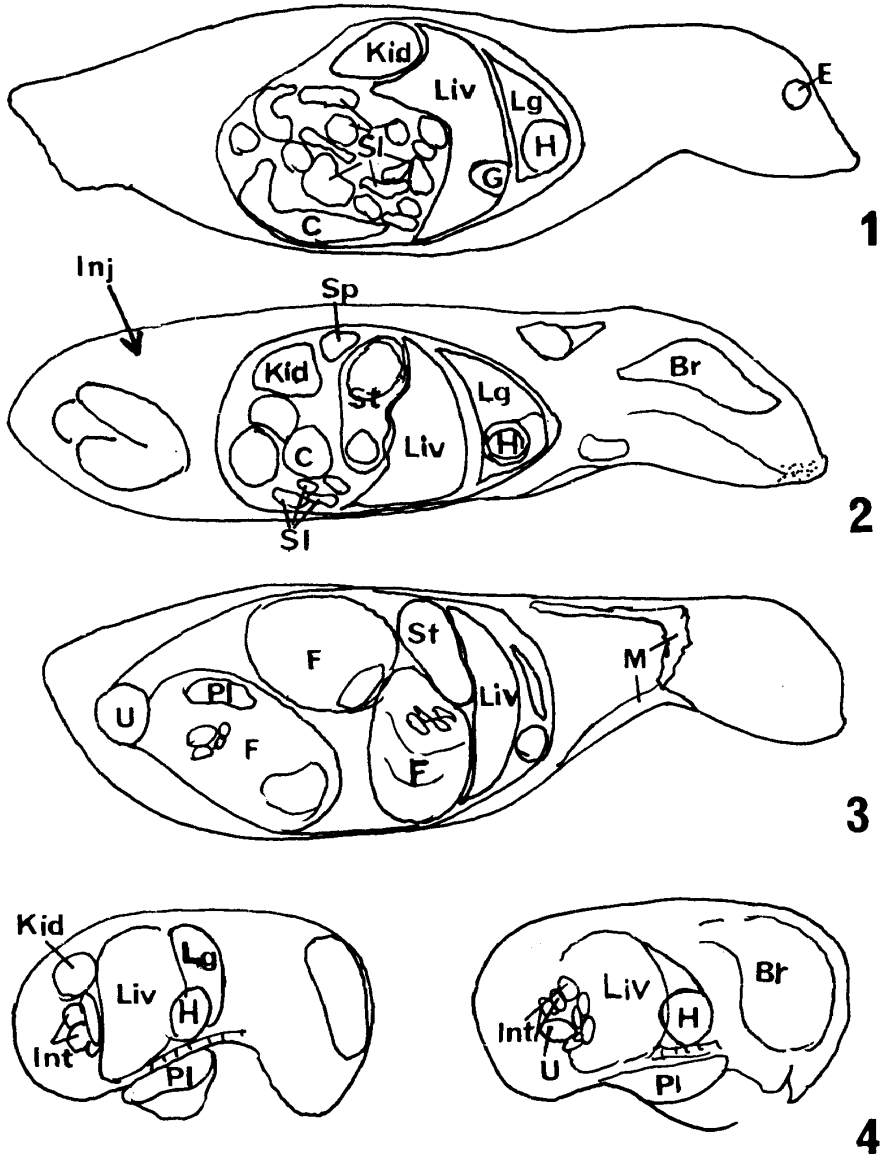
Pregnant and fetal mice: As shown in Fig. 3, radioactive substances appeared in the placentas and fetuses. Their concentrations in fetal tissues and placetas were nearly the same as in the blood of the mother one hour after intravenous administration. Mammary glands were labeled rather intensely at that time.

Among fetal organs on the 19th day of gestation, the kidneys, urinary bladder and intestinal tract were labeled intensely (Fig. 4). The brain was labeled slightly, and the other organs were labeled moderately. On the other hand, radioactivity was detected uniformly in all organs of fetuses on the 16th day of gestation.

DISCUSSION

Since Takahashi et al.[2] first observed the distribution of ^{14}C-labeled 3-methylcholanthrene (3-MC) in adult and fetal mice by macroautoradiography, certain carcinogens such as acetylaminofluorene (AAF)[3], dimethylaminoazobenzene (DAB)[3], benzo(a)pyrene (BP)[4], N-methyl-N′-nitro-N-nitosoguanidine (MNNG)[5] and dimethylnitrosamine (DMN)[6] have been examined. Among them, radioactivity derived from 3-MC and BP was distributed selectively in the excretory organs of adult mice and fetuses near term. On the other hand, radioactivity derived from DMN and MNNG was distributed rather uniformly in all organs. The distribution patterns of AAF and DAB appeared intermediate. These differences may be attributable to their solubilities in water. The distribution of ^{14}C-labeled 4-NQO observed herein was similar to that of 3-MC and BP.

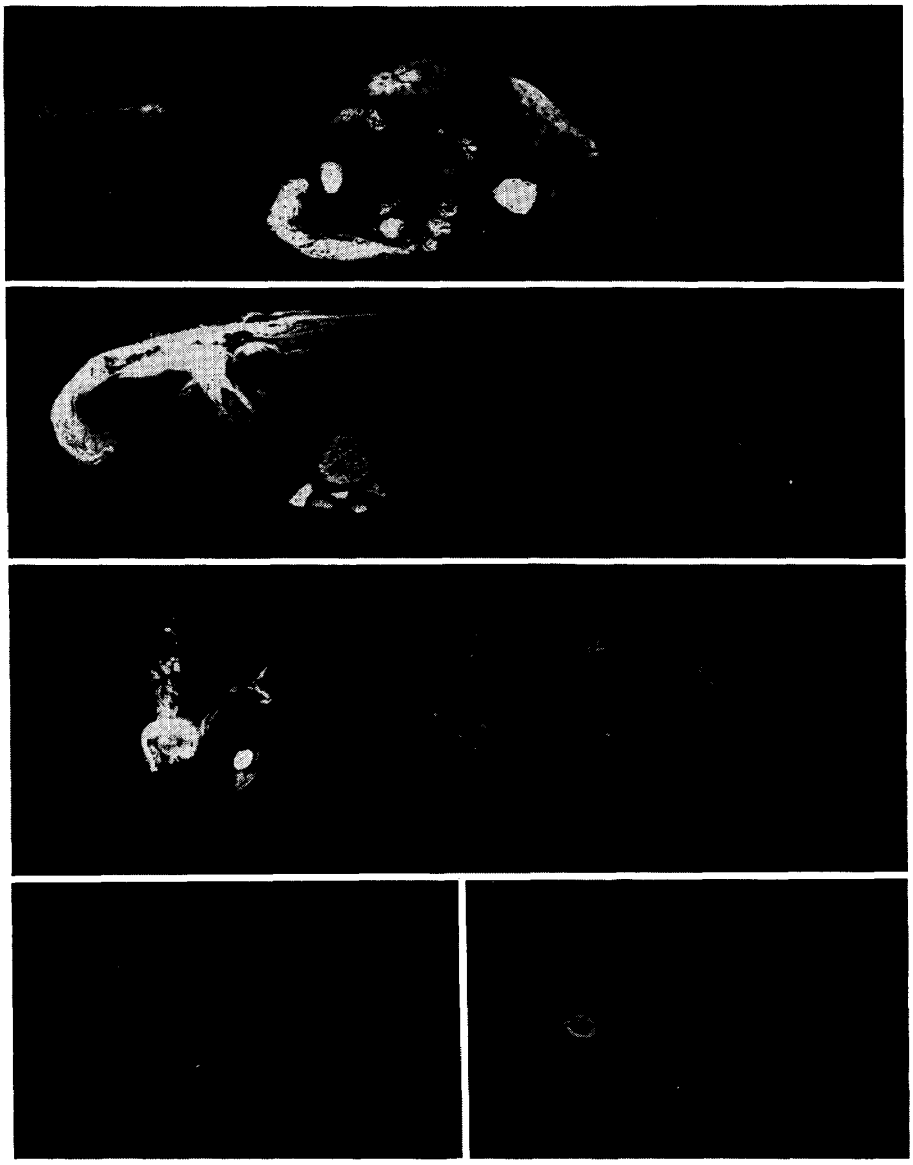
When 4-NQO was administered transplacentally to fetuses, tumors were observed in the lungs, liver and some other organs[8]. But these organs were not labeled intensely on the macroautoradiograms. So a direct relationship was not observed between the concentration of radioactivity and the susceptibility to the carcinogen. It may be noteworthy that we can get information on the total radioactivity of intact 4-NQO and its metabolites, but that information about its metabolites or binding forms to macromolucules cannot be obtained by macroautoradiography. 4-NQO is considered to be metabolized to an active form, which is 4-hydroxyamino-



Macroautoradiograms of sagittal sections of mice given ^{14}C-labeled 4-NQO.
Fig. 1, One hour after intravenous administration.
Fig. 2, Twenty four hours after subcutaneous administration.
Fig. 3, One hour after intravenous administration in a pregnant mouse on the 19th day of gestation.
Fig. 4, Fetal mouse removed from the mother shown in Fig. 3. Two different dimensions of the same fetus.

quinoline 1-oxide, to interact with cell components[9]. So further studies are required on individual metabolites in fetal organs to establish a direct relationship.

In any case, 4-NQO or its metabolites pass across the placenta rather easily. Thus one may consider that active forms of 4-NQO pass across the placenta or that fetuses have an ability to convert 4-NQO to its active forms. As shown in a previous paper[10], the fetus near term has an ability to metabolize 3-MC. It was shown herein that the radioactive substances derived from 4-NQO were eliminated from the excretory organs in the fetuses, following the mode of metabolism in adult mice. We therefore assume that 4-NQO is changed to an active form in fetal as well as in adult mouse tissues.



Abbreviations: Br, brain; C, cecum; E, eye ball; F, fetus; G, gallbladder; H, heart; Inj, injection site; Kid, kidney; Lg, lung; Liv, liver; M, mammary glands; Pl, placenta; SI, small intestine; Sp, spleen; St, stomach; U, urinary bladder.

SUMMARY

The distribution of radioactivity from ^{14}C-labeled 4-nitroquinoline 1-oxide (4-NQO), which is a known transplacental carcinogen, was observed by macroautoradiography in adult and pregnant mice. Radioactive substances derived from the labeled carcinogen passed across the placenta and were distributed intensely in the kidneys, urinary bladder and intestinal tract of the fetus near term and uniformly in all organs of the fetus on the 16th day of gestation. This indicates that fetus near term has an ability to metabolize this carcinogen. No significant differences in specific radioactivity was noted between tissues that were susceptible or resistant to this carcinogen.

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