DISTRIBUTION AND METABOLISM OF BENZO (a) PYRENE IN FETAL MOUSE

Gonya TAKAHASHI

Department of Pathology, Chest Disease Research Institute, Kyoto University

(Received for publication on January 31, 1974)

INTRODUCTION

It is well known that many carcinogens produce tumors in offsprings of animals so treated during pregnancy.[1-3] Benzo(a)pyrene (BP) is one of the most potent carcinogens and has been detected in cigarette smoke[4], broiled foods[5,6] and exhaust.[7] Thus this compound can be classified as one of the most significant agents regarding environmental carcinogenesis.

Bulay *et al*[8] demonstrated that i.p. or s.c. administration of BP to mice in the last half period of pregnancy resulted in an increased incidence of pulmonary adenoma in the offsprings. Rigdon *et al.*[9] reported teratogenic effects of BP in the rat.

In the present study, radioactive BP was given to pregnant mice and the radioactivity both in the mother and fetus was examined by macroautoradiography. BP and its metabolites were removed from organs or excreta of the mother as well as of its fetuses and chromatographic studies were done in order to determine the distribution and metabolism of this carcinogen especially in the fetal mouse.

MATERIALS AND METHODS

Chemicals:

BP was obtained from Nakarai Chemical Co. (Kyoto, Japan) and BP-^{3}H (25 Ci/mM) and BP-^{14}C (19 mCi/mM) were purchased from the Radiochemical Center (Amersham Buckingamshire, England).

Animals:

DDD strain mice used in this experiment were propagated at the Animal Center, Kyoto University. Three females were housed with a male overnight and the fetuses were labeled to be on the 0 day of pregnancy when a vaginal plug was found the next morning.

Macroautoradiography:

Five μ Ci of BP-^{14}C suspended in 0.15 ml of bovine serum was injected i.v. into a mouse on the 18th day of pregnancy. The treated mouse was sacrificed 5 hr after the administration and was immediately frozen in an acetone-dry ice mixture. Whole body saggital sections of the

frozen mouse were prepared at −12°C according to the methods of Ullberg.[10] Each serial section was 100 μm thick. The fetal mouse was separated from its mother, frozen, and prepared into sections of 50 μm thick. The sections were exposed to Sakura industrial type N X-ray film for 2-3 weeks.

Radiochemical analysis:

Twenty μ Ci (0.2 μg) of BP was administered i.v. to pregnant mice on the 12th to 18th day of gestation. At various intervals, organs were removed from both the mother and the fetus. These tissues were homogenized and extracted with ethanol and the extracts were evaporated in vacuum to dryness. The residue from the evaporation was extracted again in an adequate volume of ethanol and the solution obtained was subjected to chromatographic analysis.

Column chromatography:

Sephadex LH-20 was allowed to swell in ethanol and was packed in a column (gel bed volume, 8 ml). A sample was applied to the column and eluted with ethanol. The effluent was collected in 1 ml fractions with automatic fraction collector. Each fraction was counted with a Nuclear-Chicago liquid scintillation counter in toluene-ppo-popop scintillator.

Thin layer chromatography:

Thin layer chromatography was prepared by coating glass plates (20×5 cm width and 0.25 mm depth) with Merck Silica Gel HF254. Some of the test materials dissolved in ether were applied to the plates and were developed using benzene. The material on Rf 0.95 was identified as intact BP according to the method of Sims.[11]

Hydrolysis by β-glucuronidase and aryl-sulfatase:

The BP metabolites from FIII were dissolved in 2 ml of 0.1 M acetate buffer (pH 4.5) and 0.02 ml of β-glucuronidase and sulfatase from Helix Pomatia (Industrie Biologique Francaise). These were incubated at 37°C for 3 hr after which 3 ml of acetone was immediately added. The products were evaporated and prepared for chromatographic analysis.

RESULTS

Autoradiographic findings:

BP was distributed very distinctly in the lungs and liver immediately after i.v. administration and as illustrated in Fig. 1, was most intensive in the lungs, liver, kidneys and contents of the intestine 5 hr after administration. The urinary bladder and gall bladder appeared to be strongly labeled, indicating the excretion of this chemical into urine and bile. Radioactivity was observed also in adipose tissues and mammary glands but rarely in the muscle, brain and placenta. Amniotic sacs were labeled very clearly. In addition, this chemical was found distributed in the fetus, and was concentrated mostly in the intestine, kidneys and liver respectively. The lungs, brain and other tissues of the fetus were rarely labeled. These findings indicate that the fetal mouse in utero does metabolize and excrete BP.

Chromatographic findings:

Four peaks (pF, FI to FIII) appeared in chromatograms of ethanol extracts of the liver separated from an adult mouse 15 min and 1 hr after i.v. administration (Fig. 2-A). Intact BP at the FI peak was confirmed on thin layer chromatography and appeared at the FI peak

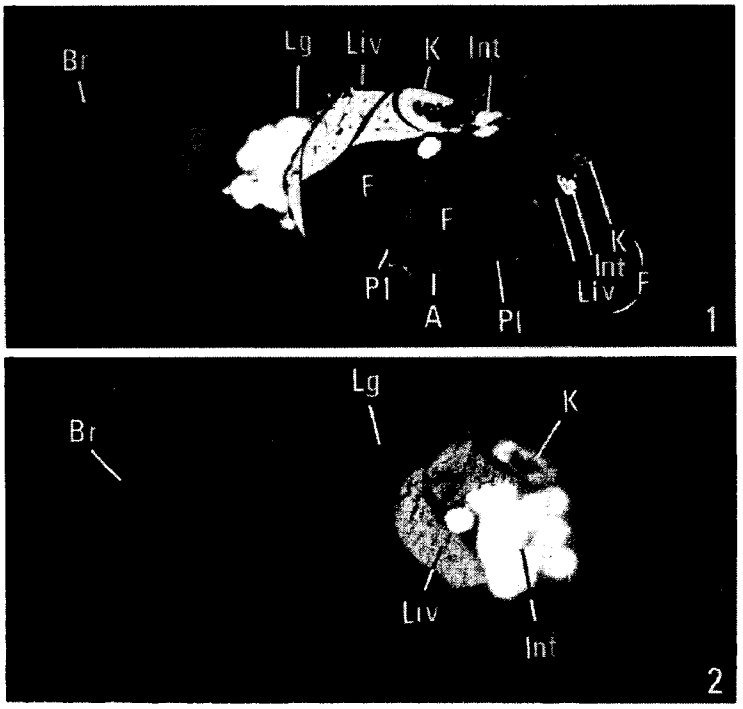

Fig. 1 Macroautoradiography of pregnant mouse and fetus. The mouse was sacrificed on the 18th day of gestation 5 hr after i.v. administration of BP-^{14}C. (1) the mother, (2) the fetus.
Abbreviations: A; amniotic sac, Br; brain, F; fetus; intestine, K; kidney, Lg; lung, Liv; liver, P; placenta

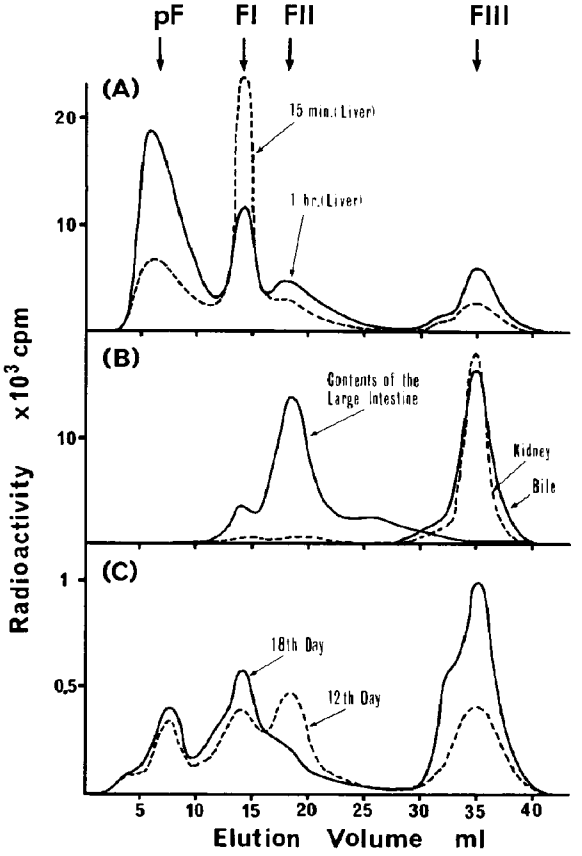

Fig. 2 Chromatograms of ethanol extracts from tissues of both adult and fetus. The mother had been given BP-^{3}H i.v. (A) liver from adult 15 min and 1 hr after administration. (B) contents of the large intestine, bile and kidney 5 hr after administration. (C) whole body of fetus on the 12th and 18th days of gestation 5 hr after administration to mothers.

on the chromatogram, the peak reducing more rapidly than the other three peaks up to one hr after administration. Chemical compounds consisting of FIII were transferred to FII after hydrolysis by β-glucuronidase and aryl-sulfatase. It is thus presumed that BP metabolites in FIII are conjugates with glucuronic and/or sulfuric acids, and those in FII are hydroxy compounds after hydrolysis. Excreta in the gall bladder and the extract from the kidney consisted only of FIII chemical compounds and a main component of contents of the large intestine was FII as is shown in Fig. 2-B. These findings suggest that chemical compounds excreted into the gall bladder convert to hydroxy metabolites in the intestine. The metabolites in the pF peak remain unidentified, however, they were observed to remain in the tissues even after disappearance of other peaks.

The 4 peaks were also observed in the chromatograms of ethanol extracts of the whole body of fetuses on the 12th to 18th day of gestation as is shown in Fig. 2-C. The peak FIII was markedly dominant on the 18th day and showed a tendency to be divided into two. Such was the major component found in the intestinal tract. The peak FII, on the other hand, decreased in comparison to that of fetus on the 12th day. These findings suggest that the components of FII are changed to those of FIII in a near term fetus and are excreted through intestinal tract of the fetus.

DISCUSSION

As BP is insoluble in water, the microcrystals of this compound were suspended in the blood for intravenous application to mice. For this reason, radioactivity could be detected in the lungs where many emboli of the crystals appeared in the alveolar capillaries immediately after the application. High concentration of the carcinogen are attributed to the drug metabolizing activity of organs in the liver and kidneys and to lipo-solubility of BP in the adipose tissues. Autoradiographic findings herein also indicate that passage of the carcinogen is more easily facilitated through the placenta than through the blood brain barrier both in the adult and the fetus. This is supported by the higher incidence of tumors in the lungs with no incidence in the brain in both adults and fetuses at least by this route. Although the lung is the most susceptible organ in transplacental carcinogenesis of BP, the concentration of this chemical in the fetal lung was less than that in other organs. A direct proportion was observed between the concentration and the susceptibility to the carcinogen. These findings are very similar to those in 3-methylcholanthrene administration as described previously.[12]

In the liver and kidneys, the carcinogen which appears in the excreta consists only of conjugates of the compound with glucuronic and/or sulfuric acids. The conjugates in the bile split into acids and hydroxy compounds which appear in the feces. These findings are in parallel with those found in rats dosed with BP as reported by Falk *et al*[13] and those in mice dosed with 3-methylcholanthrene as described previously.[14] Intact, oxidized and conjugated BP was detected in the fetus on the 12th to 18th day of gestation. In an in vitro experiment carried out by Nebert *et al*,[15] it was clarified that aryl hydrocarbon hydroxylase was induced by BP in tissue culture from fetal hamster 4 days before delivery but not 6 days before. Therefore it is presumed that almost all of the metabolites of BP found in the fetus on the 12th day come from

the mother. However, the conjugated metabolites in the intestinal tract of the fetus on the 18th day are assumed to be products in the fetal liver, which would indicate the ability of the fetus near term to metabolize the excrete BP.

The correlation between occurrence of carcinogen-metabolizing ability and carcinogenesis in fetal tissue has yet to be fully elucidated and in vitro metabolism of BP is being further investigated in this laboratory.

SUMMNRY

The distribution of benzo(a)pyrene given i.v. into a pregnant mouse was observed by macro-autoradiography. Small amount of BP was transferred into fetus, indicating the highest concentration into the intestinal tract and next in the liver and kidneys in the fetus near term. However the lung was labeled very low in spite of the most susceptable organ in transplacental carcinogenesis.

Radiometric analysis revealed that intact BP and its metabolites were detected in the fetal tissues during various developmental stages. Hydroxy compounds were metabolized into conjugated materials and excreted through the intestinal tract of the fetus near term.

ACKNOWLEDGEMENTS

Thanks are due to Prof. K. Yasuhira Department of Pathology, Kyoto University for suggestions and guidance and to M. Ohara for assistance with the manuscript. This study was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Japan.

REFERENCES

1) Larsen, C. D.: Pulmonary-tumor induction by transplacental exposure to urethan. J. Natl. Cancer Inst., 8: 63–70, 1947.
2) Druckrey, H., Ivankovic, S., and Preussmann, A.: Teratogenic and carcinogenic effects in the offspring after single injection of ethylnitrosourea to pregnant rats. Nature, 210: 1378–1379, 1966.
3) Tomatis, L., Turusov, V., Guibbert, D., Duperray, B., Malaveille, C., and Pacheco, H.: Transplacental carcinogenic effect of 3-methylcholanthrene in mice and its quantitation in fetal tissues. J. Natl. Cancer Inst., 47: 645–651, 1971.
4) Whynder, E. L., and Hoffmann, D.: Ein experimenteller Beitrag zur Tabakrauchkarzerogeneses. Deuch. Med. Wochschr., 88: 623–628, 1963.
5) Lijinsky, W., and Schubik, P.: Benzo(a) pyrene and other polynuclear hydrocarbons in carcoal-broiled meat. Science, 145: 53–55, 1964.
6) Masuda, Y., *et al.*: Polycyclic aromatic hydrocarbons in common Japanese foods. I. broiled fish, roasted baley, shoyu, and caramel. Gann, 57: 133–142, 1966.
7) Cooper, R. L.: The determination of polycyclic hydrocarbons in town air. Analyst, 79: 573–579, 1954.
8) Bulay, O. M., and Wattenberg, L. W.: Carcinogenic effects of polycyclic hydrocarbon administration to mice during pregnancy on the progency. J. Nat. Cancer Inst., 46: 397–402, 1971.
9) Rigdon, R. H., and Rennels, E. G.: Effect of feeding benzpyrene on reproduction in the rat. Experientia, 20: 224–226, 1964.
10) Ullberg, S.: Autoradiographic studies on the distribution of labeled drugs in the body. Proc. Intern. Conf. Peaceful Uses At. Energy, Geneva, 24: 248–254, 1958.

11) Sims, P.: The metabolism of benzo(a)pyrene by rat-liver homogenates. Biochem. Parmacol., 16: 613–618, 1966.
12) Takahashi, G. and Yasuhira, K.: Macroautoradiographic and radiometric studies on the distribution of 3-methylcholanthrene in mice and their fetuses. Cancer Res., 33: 23–28, 1973.
13) Falk, H. L., *et al.*: Intermediary metabolism of benzo(a)pyrene in the rat. J. Nat. Cancer Inst., 28: 699–724, 1962.
14) Takahashi, G. and Yasuhira, K.: Excretion and conversion of 3-methylcholanthrene metabolites in the intestinal tract of the mouse. Cancer Res., 32: 710–715, 1972.
15) Nebert, D. W., and Bausserman, L. L.: Aryl hydrocarbon hydroxylase induction in cell culture as a function of gene expression. N. Y. Acad. Sci., 179: 561–579, 1971.