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Hepatoma induction in the rat by the subcutaneous administration of powdered 3′-methyl-p-dimethylaminoazobenzene

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Summary. A relatively safe and simple procedure was developed for the induction of hepatomas in the rat by the s.c. administration of powdered 3′-methyl-p-dimethylaminoazobenzene.

Hepatomas in rat liver are induced by the incorporation of azocarcinogens into semisynthetic diets[1-3]. Since the azocarcinogens were placed on the restricted list in the United States by the Federal Government[4], special facilities are required for the production of diets containing carcinogens. For investigators who do not have special facilities, a relatively simple and safe procedure is needed for the induction of liver tumors in the rat by azocarcinogens. This paper briefly describes such a method.

Material and methods. Inbred male Fischer rats (Charles River Breeding Laboratories, Wilmington, Massachusetts) 6–8 months old and weighing about 300 g were used. The azocarcinogen, 3′-methyl-p-dimethylaminoazobenzene (3′-Me-DAB) in powder form was purchased from Eastman Organic Chemicals, Rochester, N.Y.

The rats were anesthetized with ether and a 5–6 cm incision was made in the abdominal skin in a manner that the peritoneum was not damaged. The skin on one side of the incision was lifted and about 100 mg of powdered 3′-Me-DAB was spread evenly under the skin flap. Then the other side of the skin incision was raised and the rest of the 200 mg sample of azocarcinogen was evenly deposited under the skin flap. The skin was then sutured with metal wound clips (Clays Adams, Parsippony, N.Y.). This procedure for the administration of 3′-Me-DAB was carried out every 30 days for a period of 3–7 months unless liver tumors developed. After the administration of 300–600 mg of this azocarcinogen, the rats were examined for abdominal swelling, and if presence of tumors was suspected, laparotomy was carried out under ether anesthesia. If hepatomas were present, the liver was perfused under ether anesthesia with cold borate buffer, pH 8. The tumors and/or adjacent liver were excised and quickly frozen on the quick-freeze attachment of an International Cryostat, model CT1. Portions of the tumor and liver were fixed in formalin, embedded in paraffin, sectioned, and stained with haematoxylin and eosin. If the liver did not show gross pathological changes after laparotomy, the skin was sutured with metal wound clips and the s.c. administration of 3′-Me-DAB was continued after a period of 1–2 weeks.

Results and discussion. The rats tolerated the s.c. application of powdered 3′-Me-DAB very well, and none of this azocarcinogen was visible at the end of each treatment period. In 4 experiment, 67–82% of rats developed liver tumors following the s.c. disposition of 3′-Me-DAB (table). This tumor incidence is somewhat less than that produced by incorporation of azocarcinogens into the diet[2]. The tumors produced by the s.c. administration of 3′-Me-DAB were multiple and mixtures of hepatocellular carcinomas and cholangiocarcinomas such as have been found by the dietary administration of azocarcinogens[1,3,5-7]. The incidence of tumors produced by the s.c. administration of powdered 3′-Me-DAB only once a month averaged 71% whereas that obtained by the weekly s.c. injection of 3′-Me-DAB dissolved in cottonseed oil averaged 47%[7] and that of p-dimethylaminoazobenzene dissolved in olive oil averaged 31%[1] or lower[3]. Therefore, the s.c. procedure for the induction of liver tumors should be useful for investigators who do not have facilities for incorporation of 3′-Me-DAB into the diet. However, for personal safety an isolated laboratory might be needed for the monthly administration to rats of the powdered and potent azocarcinogen, 3′-Me-DAB.

Induction of liver tumors in rats by the s.c. administration of powdered 3′-Me-DAB

Experiment No.	No. of rats	No. of rats with tumors	Dosage of 3′-Me-DAB mg	Time of tumor appearance month	Incidence of tumors (%)
1	6	4	600–1400	3–7	67
2	7	5	800–1200	4–6	71
3	6	4	1200	6	67
4	6	5	600–1200	3–6	82

The induction of hepatomas by the s.c. application of 3′-Me-DAB avoids the problems caused by 1 scattering by rats of carcinogen-treated food out of their cages; 2 leaving disposal of bedding (and carcinogens) to ill-trained animal caretakers; and 3 leaving carcinogens unattended in cages after feeding.

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