EXPERIMENTAL TUMORS INDUCED IN RATS BY DICHLOROBENZIDINE

G. B. Pliss

From the Laboratory of Experimental Oncology (Head – Corresponding Member AMN SSSR L. M. Shabad), Institute of Oncology (Director – Active Member AMN SSSR A. I. Serebrov), AMN SSSR, Leningrad

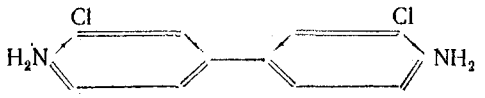
(Received May 16, 1958. Presented by Active Member AMN SSSR N. N. Petrov)

Benzidine and certain other polycyclic diamines are important intermediates in the production of synthetic dyes.

It is known that benzidine is a carcinogenic substance, and that to its action are due numerous cases of cancer of the bladder of workers in the aniline dye industry [1, 3, 5, 6, 9]. There are numerous references in the world literature to the blastomatogenic action of some benzidine derivatives (dianisidine, o-tolidine). Some authors believe that dianisidine may cause tumors of the urinary bladder [1, 10]. Since, however, the same individuals who are exposed professionally to dianisidine are also exposed to benzidine, there is as yet no conclusive evidence of the carcinogenic action of dianisidine. As for o-tolidine, this is not usually considered to be a carcinogen, although in experiments on rats it has been shown to cause tumors of the Zymbal glands [11] in 4.3% of cases.

No data have been published on the carcinogenic properties of dichlorobenzidine (DCB). It is known that DCB can be fairly readily absorbed through the undamaged skin, in particular under conditions of high environmental temperature and humidity [4, 7].

Physicochemical properties. 3,3'-dichlorobenzidine (3,3'-dichloro-4,4'-diaminodiphenyl) is a grey, odorless powder. It is sparingly soluble in water (100 g of water will dissolve 0.07 g of the substance at 15°).



EXPERIMENTAL METHODS

We used 111 sexually mature white rats, weight 110-130 g, in our experiments. The percentage incidence of spontaneous tumors in these rats is extraordinarily low. Only a few tumors were observed, these being sarcoma of the mesentery, and mammary gland tumors in aged virgin females. As controls, we took a group of 130 rats, which had over a period of 10 months received injections of octadecylamine or methylstearylamine into the subcutaneous areolar tissue. No tumors were found in this group over a period of 20 months of observation.

The animals were on an unrestricted diet. The diet was adequate with respect to animals proteins, carbohydrates, and vitamins. The DCB was supplied by the Voroshilov Scientific Research Institute of Organic Intermediates and Dyestuffs. The product was a paste, consisting of 45.3 parts of the substance to 50 parts of water. It was administered either subcutaneously, as a suspension in glycerol or sunflower seed oil, or per os. The organs of all animals dying or killed were subjected to macro- and microscopic examination.

Results of Experiments on Introduction of DCB, and Description of the Tumors

Mode of administration of DCB	Number of animals	Duration of administration of DCB (in mo.)	Total amt. of DCB administered (in grams)	Survived to appearance of the first tumor	Number of tumor-bearing animals	Situation of tumor: Zymbal's gland	Situation of tumor: mammary gland	Situation of tumor: connective tissue and muscles at the injection site	Situation of tumor: connective tissue and muscles distant*	Situation of tumor: liver	Situation of tumor: hemopoietic system	Situation of tumor: thyroid	Situation of tumor: urinary bladder	Remarks
Fed	50	12	4,53	29	12	2	4	—	2	1	3	1	2	3 rats had 2 tumors each
Injected	61	10—11	1,62—3*	35	18	9	7	5	—	1	2	1	—	4 rats had 2 tumors each, and one had 4 tumors
Total	111			64	30	11	11	5	2	2	5	2	2	

*The reason for the wide variations in dosage is that at the beginning of the experiment one group of rats was injected twice weekly with doses of 60 mg each.

Feeding of DCB. Fifty rats were taken for these experiments (see Table). From 0.5 to 1 ml of a 4.4% suspension of DCB in sunflower seed oil were added to the daily rations, except on Sundays and holidays, for a period of 12 months.

EXPERIMENTAL RESULTS

Acute enlargement of the spleen and liver of one rat was observed, over a period of 10 months from the beginning of the experiments. Microscopic examination of the organs showed the presence of myeloid leucosis.

After 11 months a mammary gland tumor was found in another rat. In all, 12 animals of this group developed tumors. These were variously situated, and 3 of the animals had two different types of tumor (sarcoma of subcutaneous connective tissue with a mammary gland tumor, a thyroid tumor with a hepatoma, a polymorphous cell sarcoma of the wall of a parasitary cyst with a papilloma of the urinary bladder).

Two of the bladder tumors were benign papillomas. One papilloma showed the beginnings of invasive growth into the submucosal layer. The papillomas were found 15 and 16½ months after the start of the experiments.

Tumors of the Zymbal glands (sebaceous glands of the external auditory canal [2]) had an organoid structure, with a preponderance of keratinized squamous tumor cells. The mammary tumors all had the structure of cystoadenocarcinomas; the liver tumors were hepatomas.

Injections of DCB. We used 61 rats for these experiments (see Table). From 20 to 120 mg of DCB were injected dialy into the subcutaneous connective tissue, for a period of 10-11 months. The injections frequently caused an excited state of the animals, with rigors of short duration. Because of the high mortality encountered, the dosage levels were later reduced, and, beginning from the 6th month of the experiment, the rats were given an injection of 0.5 ml of 8.8% DCB emulsion in glycerol once weekly, i.e. 20 mg per week.

A mammary gland tumor was found in one of the rats after 7 months of the injections. A second tumor, of the Zymbal glands, appeared in this same rat 5½ months after surgical removal of the mammary tumor.

In this series of experiments we found tumors in 18 rats, both at the site of injection and in various

internal organs. We shall describe some of the general features of these tumors, without entering into a detailed account of the histology of all the tumors. Multiple tumors were found in 5 rats (2 each in four, and 4 in the fifth rat). These were diagnosed as follows: a rhabdoblastoma at the injection site with a keratinized squamous cell carcinoma of Zymbal's glands; a hepatoma with a keratinized squamous cell carcinoma of Zymbal's gland; carcinomas of Zymbal's gland on the right and the left sides; a mammary adenocarcinoma with a Zymbal's gland carcinoma; a cystoadenocarcinoma and 2 fibroadenomas of the mammary gland with a thyroid adenoma. The Zymbal's gland tumors mostly had an organoid structure, and had in some cases caused erosion of the facial bone. The majority of the mammary tumors were cystoadenocarcinomas, with patches of consolidation.

We thus found that, of 64 rats which survived to the appearance of the first tumor, 30 (i.e. 47% of cases) developed tumors at various sites and of various morphological structure. In 5 rats tumors appeared not only at the injection sites but also in internal organs. Four of the tumors were sarcomas of different forms, and one was a rhabdomyoblastoma. It may be inferred from our findings that DCB is a relatively potent blastomatogenic agent. In considering the nature of the blastomatogenic action of this substance it is necessary to bear in mind the scanty information on the metabolism of DCB available in the literature. According to Meigs [8] DCB is excreted unchanged in the urine.

On the other hand, animal experiments have shown that it appears in the urine of rats exclusively in the form of metabolites.

Since DCB caused the appearance of tumors not only at the injection site and in parenchymatous organs, but also in the urinary bladder, where, according to the findings of our laboratory, it is not present, it may be supposed that the blastomatogenic effect is exerted both by dichlorobenzidine and by its metabolites.

Per os or subcutaneous administration of dichlorobenzidine to rats caused the appearance of tumors in a high proportion of cases. The tumors appearing after subcutaneous injection of DCB were situated not only at the injection site, but also in internal organs.

SUMMARY

Prolonged administration of dichlorobenzidine, per os, or by injection into the subcutaneous connective tissue led to appearance of tumors in a substantial proportion of cases. Of the original number of 111 white rats, 64 survived to the appearance of the first tumor, and of these rats 30 had tumors of various types of locations, including 5 sarcomas at the injection site. It appears that both dichlorobenzidine and its metabolites possess cancerogenic activity in the rat.

LITERATURE CITED

[1] I. S. Temkin, "Urinary Bladder Tumors Caused by Carcinogenic Amino-Compounds" (Moscow, 1957). [In Russian].

[2] W. E. Zymbal, Ztschr. Zellforsch. 1933, Bd. 18, S. 596-625.

[3] M. Barsotti and E. Vigliani, Brit. J. Indust. Med. 1950, v. 7, p. 96.

[4] J. M. Glassmann and J. W. Meigs, Arch. Indust. Hyg. 1951, v. 4, pp. 519-532.

[5] M. W. Goldblatt, Brit. Med. Bull. 1947, v. 4, pp. 405-416.

[6] W. C. Hueper, Occupational Tumours and Allied Diseases. Baltimore, 1942.

[7] J. W. Meigs, R. W. Brown and L. J. Sciarini, . Arch. Industr. Hyg. 1951, v. 4, pp. 533-540.

[8] J. W. Meigs, L. J. Sciarini and W. A. Van Sandt, Arch. Industr. Hyg. 1954, v. 9, pp. 122-132.

[9] A. Müller, Helv. chir. Acta. 1951, v. 18, pp. 1-42.

[10] S. Perlmann and W. Staehler, W. Ztschr. urol. Chir. 1932, Bd. 36, S. 139-164.

[11] S. Spitz, W. Maguigan and K. Dobriner, Cancer, 1950, v. 3, pp. 789-804.