Virchows Arch. A Path. Anat. and Histol. 370, 97—102 (1976)

Transmaternal Variation of the Berenblum Experiment with NMRI-Mice

Tumour Initiation with DMBA via Mothers Milk Followed by Promotion with the Phorbol Ester TPA*

Klaus Goerttler and Heinz Loehrke

Institut für Experimentelle Pathologie (Direktor: Prof. Dr. K. Goerttler)
am Deutschen Krebsforschungszentrum Heidelberg

Received December 30, 1975

Summary. DMBA which is applied transmaternally via the mothers milk can initiate tumour cells in the F-1 generation. Subsequent application of the tumour promoter TPA to the back skin of the young animals induced the formation of skin papillomas and carcinomas. Animals treated according to this scheme also developed malignant neoplasms in the other organs. Control animals treated with DMBA only, rarely developed tumours, whereas treatment with TPA alone had no effect.

This variation of the Berenblum-experiment suggests that the transfer of carcinogens in human milk should be considered in addition to transplacental carcinogen transfer as a potential hazard to the developing human infant.

Key words: Carcinogenesis — Transmaternal — DMBA-initiation — TPA-promotion.

Introduction

Experimental methods for the rapid induction of tumours by means of chemical carcinogens are becoming increasingly important in cancer research. In studies on skin carcinogenesis, tumours are normally induced either by repeated application of small doses of a primary carcinogen or by combined treatment with a primary carcinogen and cocarcinogens which cause hyperplasia (for review: Hecker, 1975). The present report deals with a transmaternal variation of the classical Berenblum experiment (Initiation with a carcinogen followed by promotion with a cocarcinogen; Berenblum, 1941), by which young sucking mice receive an initiating dose of the carcinogen dimethylbenzanthracene (DMBA) via their mothers milk. Two different doses of DMBA, neither of which is on its own sufficient to induce skin tumours during the 520 days of the experiment, were applied orally to different groups of mother animals. The young mice were subsequently treated repeatedly on the skin of the back with the tumour promoter 12-o-tetradecanoyl-phorbol-13-acetate (TPA; for terminology see Hecker, 1975).

Materials and Methods

Female mice of an NMRI strain which is bred and maintained under SPF conditions at the German Cancer Research Centre were used. Animals were kept in individual cages of the type Makrolon II and were fed Altromin ®-10 Standard, with water available ad libitum.

* Dedicated to Professor Dr. Heinrich Bredt on the occasion of his 70th birthday.

Injection against ectromelia (Vaccine was obtained from the Institut für Tropenhygiene, München) was carried out at the age of 8 weeks. Animals were paired at 12 weeks and the females with positive vaginal smears (sperma) were distributed randomly into the following groups:

Group I: Controls: 1 ml Livio® oil[1]/kg body weight = 5 mother animals.

Group II: DMBA 2 mg/kg body weight in Livio® oil$^+$ = 6 mother animals.

Group III: DMBA 4 mg/kg body weight in Livio® oil$^+$ = 6 mother animals.

The mother animals of each group received the appropriate doses intragastrically once a day from the 2nd–22nd day after giving birth (partus). The animals of groups II and III therefore received 40 and 80 mg DMBA respectively per kg body weight. After this treatment, the mother animals were observed in order to check the occurrence of tumours. After the 22nd day the young animals of each group were separated from the mothers and distributed randomly in the sub-groups Ia, Ib, IIa, IIb, IIIa, IIIb. Twice as many animals were assigned to group IIIa as to the other groups, since it was presumed that the animals of this group would have relatively low survival rates (see also Table 2).

Animals in subgroups Ia, IIa and IIIa (25, 20, 35 animals respectively) were treated on the skin of the back twice weekly between the ages of 8 and 28 weeks (corresponding to a total of 40 applications) with 12-o-tetradecanoyl-phorbol-13-acetate (TPA[2]) (0.01 µmole = 0.00615 mg TPA in 0.1 ml acetone). Animals of subgroups Ib, IIb and IIIb (20, 20, 16 animals respectively) remained untreated.

The occurrence of tumours was checked and registered once weekly over a period of 2 weeks after commencing treatment with TPA. All animals which died during the duration of the experiment (520 days) were autopsied to investigate cause of death and the presence of tumours in different organs.

Results

1. Animals of Groups Ia and Ib

None of the 45 young animals obtained from the 5 mothers treated with Livio oil alone developed any epithelial tumours during the experimental period. In addition, treatment with TPA (subgroup Ia, $n = 25$) did not have any effect on the induction of tumours.

During the experiment, 5 animals of group Ia died, one of which had a subpleural lung adenoma and another showed symptoms of preleukemia, a condition manifest histologically by hyperplasia or marked enlargement of lymphoid or myelogenic tissues, especially peribronchial, intestinal, intrarenal, or in lymphoid organs (lymphnodes, spleen, thymus), or as myelogenic nodules in liver, lymph nodes, bone marrow or spleen, as compared with similar human conditions described by Pierre (1974). In group Ib, 2 animals died, one of which had a lymphatic leukemia (Table 1).

2. Animals of Groups IIb and IIIb

In the total of 36 young animals from mothers treated with the 2 given doses of DMBA, no skin tumours developed during the experiment. 4 animals of group IIb died, one of which had symptoms of pre-leukemia in the thymus and lymph nodes whereas another had an adenocarcinoma of the large intestine. One of the 3 animals of group IIIb which died had a thymic lymphoma (Table 1).

1 Commercially available plant oil.

2 The substance was generously provided by Prof. Dr. E. Hecker, Biochemisches Institut, Deutsches Krebsforschungszentrum Heidelberg.

Table 1. Histologic type and localisation of tumours of animals which died during the experiment (0–520 days). Autopsy data

Animal group	Survival time	Tumours localisation	histology
Ia	276		
$n=25$	357		
	406	lungs	papillary adenoma
	459		
	459	lungs, spleen	preleukemia
Ib	358		
$n=20$	450	lymph-nodes, spleen, liver, kidney, lungs	lymphatic leukemia
IIa	271		
$n=20$	272		
	281		
	328	backskin	papillary fibroepithelioma
	419	fore-stomach	squamous cell carcinoma, liver metastases
		backskin	papillary fibroepithelioma
	434	backskin	squamous cell carcinoma, lymph node metastases, lung metastases
	476	backskin	squamous cell carcinoma
	508	backskin	broad-based papillary fibroepithelioma with transition to squamous cell carcinoma
		liver	hepatocellular carcinoma
	518	backskin	papillary fibroepithelioma
		lungs	subpleural adenoma
	518	backskin	squamous cell carcinoma, lung metastases
IIb	322	thymus, lymph nodes	pre-leukemia
$n=20$	381	colon	adenocarcinoma
	425		
	487		
IIIa	245		
$n=35$	274	backskin	papillary fibroepithalioma
	328	colon	adenocarcinoma
	434	backskin	spindle cell sarcoma
	435	backskin	papillary fibroepithelioma
		kidney	adenocarcinoma, lung metastases
	439	liver	hemangioepithelioma
	453	backskin	squamous cell carcinoma
		rectum	papillary adenoma
	456	backskin	squamous cell carcinoma
		lungs	papillary adenoma
	458	backskin	squamous cell carcinoma
		lungs	papillary adenoma
	460	lungs	adenoma with transition to adenocarcinoma
	461	backskin	squamous cell carcinoma
		lungs	subpleural adenoma

Table 1 (continued)

Animal group	Survival time	Tumours localisation	histology
IIIa	469	backskin	papillary fibroepithelioma
$n=35$		upper-arm	polymorph cellular sarcoma
	469	backskin	squamous cell carcinoma
		lungs	papillary adenoma
	510	backskin	hyperkeratotic papillary fibroepithelioma
		glandular stomach	adenocarcinoma
	511	mesenchyme	osteosarcoma, metastases in a branch of the vena porta and lungs
IIIb	42		
$n=16$	399		
	462	thymus	lymphoma

3. Animals of Groups IIa and IIIa

These groups contained a total of 55 animals, each of which was treated 40 times with TPA. In both sub-groups, papillary fibroepitheliomas (papillomas) were observed on the back skin as early as 6 weeks after commencing TPA treatment (i.e. at the age of 14 weeks) (Fig. 1). The number of tumours per animal was somewhat higher in those animals whose mothers had received the higher initiating dose of DMBA. This difference was observed until the end of the experimental period. The reduction in the number of papillomas at 38 weeks after starting treatment, which is particularly noticeable in group IIIa, is related to the simultaneous appearance of carcinomas. The animals treated with the higher DMBA dose developed malignant tumours earlier than those of the other group.

10 animals of group IIa died, of which 3 did not have any tumours. Of the others 2 had only benign papillomas.

15 animals of group IIIa died during the 520 days of the experiment. Only one of these did not have any tumours. Table 1 shows the results in detail. In addition to the tumours of the back skin, malignant neoplasms of the front limb extremity, bone, glandular stomach, large intestine, rectum, liver, kidney, and lung were also observed. 5 animals had lung tumours; 4 of these were papillary adenomas, and in one case a transition to an adenocarcinoma was observed. Multiple tumours were developed by 8 animals. It was noticeable that neoplasms of the haematopoetic and lymphatic systems were not present.

4. Survival Rates and Incidence of Tumours in Surviving Animals

The details of the tumours developed by the surviving animals of each group after 520 days are shown in Table 2. It can be seen that after 520 days, the percentage of survivors in the group of animals treated with DMBA/TPA is less than 50% (26 from 55) whereas 67 animals survived from the total of 81 in the groups Ia, Ib, IIb and IIIb. Moreover, only 4 animals of groups IIa and IIIa did not have visible tumours. In contrast, the surviving animals of the other groups were practically free of visible tumours.

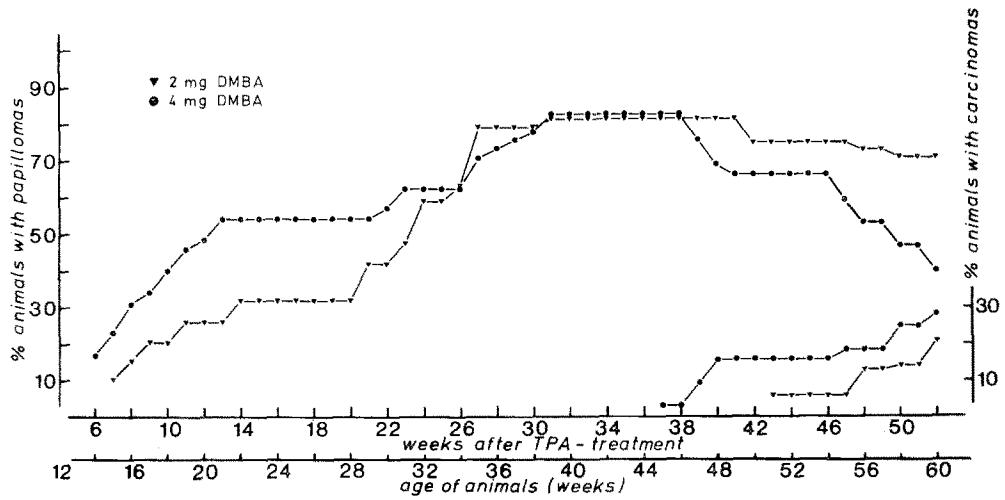

Fig. 1. Appearance of papillomas and carcinomas after transmaternal induction with DMBA (2 and 4 mg) and postnatal promotion with TPA (×40) in the experimental groups IIa and IIIa. ($n = 20$ and 35 animals)

Table 2. Survival times in individual experimental groups and externally visible tumours in 520 day survivors. For comparison with autopsy data see Table 1

Experimental group and type of treatment	No. of animals at beginning of experiment	Survivors after 520 days no. totals/no. with visible tumours
Ia Controls	25	20/0
Ib Controls + TPA	20	18/0
IIa DMBA 2 mg + TPA	20	10/8[a]
IIb DMBA 2 mg	20	16/0
IIIa DMBA 4 mg + TPA	35	20/18[b]
IIIb DMBA 4 mg	16	13/0

[a] Including 7 animals with macroscopically recognisable carcinoma and one with a papilloma
[b] Including 12 animals with macroscopically visible carcinomas and 6 with several papillomas

Discussion

The most important results of these investigations are:

1. It is possible to transform the cells of young animals into tumour cells by transmaternal treatment, i.e. via the mothers milk, with the carcinogen DMBA.

2. The relatively low doses of DMBA used (2 and 4 mg/kg body weight, applied to the mother animal) 20 times between the 2nd and 22nd day after giving birth (partus) were not sufficient to induce epidermal tumours. Tumours of the skin of the back were only observed when the transmaternal initiation was followed by treatment with the promoter TPA.

3. Of the animals of group Ia, which were treated with TPA alone, the only tumorous conditions observed were a subpleural lung adenoma and a preleukemia.

Those animals treated only with DMBA (groups IIb and IIIb) developed one preleukemia, one lymphoma of the thymus and one adenocarcinoma of the rectum.

4. In addition to the induction of benign fibroepitheliomas of the back skin, treatment with either dose of DMBA followed by TPA led to the formation of malignant tumours of the back skin and of other organs. Malignant tumours were also observed in organs which could not have been exposed directly to the effect of TPA. Although the number of lung tumours, which are also observed spontaneously in this strain of mice, was increased, neoplasms of the haematopoetic and lymphatic systems were completely absent.

These preliminary studies, which were carried out with a relatively small number of mother animals and their litters, has therefore provided surprisingly clear results, in spite of the fact that only very small amounts of the carcinogen DMBA were effectively applied via the mothers milk to the young animals. It must be assumed that the young animals are particularly sensitive to carcinogen treatment, as can be seen from the very early incidence of tumours. The basic Berenblum concept of a 2-stage mechanism of tumour formation (Berenblum and Shubik, 1947) has therefore been shown also to be applicable in this case.

The co-carcinogen TPA, however, obviously has direct or indirect effects on organs other than the skin, since an increased incidence of malignancies was observed in various parts of the body. The number of neoplasms of the haematopoetic and lymphatic systems was not increased, although transformations of this type occurred both spontaneously in the controls, and in animals which were not treated with TPA.

The possibility of transmaternal application of carcinogen via the mothers milk has already been described by Shay et al. (1950). Our studies involve a new variation of the Berenblum experiment in which transmaternal initiation with DMBA is followed by local promotion with the carcinogen TPA. The next logical step in this series is the development of a transmaternal diaplacental variant of the Berenblum experiment with prenatal initiation and postnatal promotion of tumours in the young animals. These investigations have now been successfully completed and will be reported elsewhere (Goerttler, Loehrke, unpublished results).

References

Berenblum, I.: The mechanism of carcinogenesis. A study of significance of cocarcinogenic action and related phenomena. Cancer Res. **1**, 807–814 (1941)

Berenblum, I., Shubik, P.: A new quantitative approach to the study of the stages of chemical carcinogenesis in the mouse's skin. Brit. J. Cancer **1**, 383–391 (1947)

Hecker, E.: Cocarcinogens and Cocarcinogenesis. In: Handbuch der allgemeinen Pathologie (ed. E. Grundmann), Bd. 6, S. Teil 6, 651–676. Berlin-Heidelberg-New York: Springer 1975

Pierre, R. V.: Preleukemic states. Seminars in Hematology **11**, 73–92 (1974)

Shay, H., Weinhouse, S., Gruenstein, M., Marx, H. E., Friedman, B., Glazer, L.: Development of malignant lymphoma in some of the young rats suckled by mothers receiving methylcholanthrene by stomach tube only during the lactation period. Preliminary report. Cancer (Philad.) **1950**, 891–895

Prof. Dr. Klaus Goerttler
Institut für Experimentelle Pathologie
Deutsches Krebsforschungszentrum
Im Neuenheimer Feld 280
D-6900 Heidelberg 1
Federal Republic of Germany