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Positive Two-Stage Carcinogenesis in Female Sprague-Dawley Rats Using 7,12-Dimethylbenz(a)Anthracene (DMBA) as Initiator and 12-O-Tetradecanoylphorbol-13-Acetate (TPA) as Promotor

Results of a Pilot Study*

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Summary. In contrast to a previous report by Shubik, the validity of the 2-stage skin carcinogenesis experiment was demonstrated in the rat. The modified experiment was carried out in female Sprague-Dawley rats using intragastrically administered DMBA as a carcinogen and the topically applied phorbol ester TPA as a promoter.

Seven groups of animals were used. Two groups were treated with TPA only, two groups were initiated only with DMBA, two further groups were both initiated and promoted, and one group served as a control. Each of the initiated/promoted groups or only initiated or promoted groups contained one sub-group in which the animals had been bilaterally ovarectomized prior to the experiment.

Hyperplasia of the dorsal epidermis occurred only in the promoted and in the initiated/promoted groups. Tumors of the back skin were observed exclusively after initiation/promotion. Ovarectomy – leading to a prolonged survival time of the animals – seems to be crucial for the manifestation of malignant skin tumors. Initiation/promotion also gives rise to tumors of the forestomach, the small intestine, the liver and the colon. Tumors in other organs (especially in the mammary gland and the Zymbal gland) were also be observed after initiation alone.

Key words: Tumor promotion – Tumor initiation – DMBA – TPA – Two-stage carcinogenesis experiment – Carcinogenesis – Cocarcinogenesis – Rat.

Introduction

The classical 2 stage carcinogenesis experiment introduced by Berenblum (Berenblum, 1941) and Mottram (Mottram, 1944) is routinely carried out on the back skin of mice. Single painting of dorsal skin with a carcinogen, i.e. DMBA

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(initiation) followed by repreated treatment with a cocarcinogen, i.e. croton Oil or TPA (promotion) consistently gives rise to the early appearance of a significantly higher number of both benign and malignant skin tumors than painting of the skin with the initiating dose of the carcinogen alone. As a rule, the cocarcinogen is not carcinogenic by itself.

The 2-stage carcinogenesis experiment has been the subject of various modifications regarding the mode of application of both the initiator and the promoter. Recently we were able to demonstrate its effectiveness even in cases were the initiating carcinogen was applied orally (Goerttler et al., 1979) or had reached the animal either via mothers milk (Goerttler and Loehrke, 1976a) or by the diaplacement route (Goerttler and Loehrke, 1976b).

For some time the main objection to general acceptance of the 2 stage principle of carcinogenesis was the failure to reproduce the experiment in species other than the mouse (including the rat, rabbit and guinea pig; Shubik, 1950) and it thus appeared as if the experiment was species and organ specific. This situation has, however, changed dramatically during the last two years. 2-stage mechanisms of carcinogenesis in the internal organs of a variety of species of animals have been reported from several laboratories, using initiator-promoter combinations other than DMBA/TPA (for review, see Slaga, Sivak, Boutwell (eds.), 1978). Using the DMBA/TPA combination in the mouse we have also been able to demonstrate that in addition to the dorsal epithelium a variety of internal organs were susceptible to tumor formation by a 2-stage mechanism (Goerttler and Loehrke, 1977). After appropriate application procedures for both the initiator and the promoter, the forestomach epithelium (Goerttler et al., 1979) and the vaginal epithelium (Goerttler et al., unpublished results) in particular exhibited the same high selectivity with regard to tumor formation as the dorsal epithelium in the classical experimental design. Moreover, recently we demonstrated a DMBA/TPA induced 2-stage mechanism for malignant melanoma development in the Syrian golden hamster (Goerttler et al., in press). The present paper deals with the results of a pilot study on DMBA/TPA mediated 2-stage carcinogenesis in rat dorsal skin.

Materials and Methods

Female Sprague-Dawley rats, bred under SPF conditions were purchased from the Süddeutsche Zuchtanstalten, Tuttlingen, Germany. 135 animals were assigned to 7 experimental groups by random distribution (I(15); II(15); III(15); IV(15); V(15); VI(30); VII(30); see Table 1). The animals, weighing on an average 240 ± 10 g, were maintained singly in Macrolon type III cages and fed with Altromin®-10 Standard (Lage/Lippe, Germany) with water available ad libitum.

Since the female rat is known to develop mammary gland tumors following systematically administered DMBA (Archer and Orlando, 1968; Engelbart and Gericke, 1964), we included groups of ovarectomized animals in order possibly to prolonge their life expectancy. Therefore, at the age of 12 weeks the animals of groups II, V and VII were bilaterally ovarectomized. Animals of groups IV–VII (Table 1) were initiated at the age of 14 weeks with 100 mg/kg body weight of 7,12-Dimethylbenz (a) anthracene (Fluka, Buchs, Switzerland, dissolved in sesame oil DAB7; 10 mg/ml). The carcinogen was administered intragastrically by means of a stomach tube.

Starting from week 15, animals of all experimental groups were routinely shaved once per week on the back skin. Promotion with 12-O-Tetradecanoylphorbol-13-acetate (the substance was

Table 1. Histological findings in the back skin after DMBA/TPA mediated 2-stage carcinogenesis in the rat

Histological Findings	Group I (acetone)	Group II (TPA)	Group III (Ovar-ectomy/ TPA)	Group IV (DMBA)	Group V (Ovar-ectomy/ DMBA)	Group VI (DMBA/ TPA)	Group VII (Ovar-ectomy/ DMBA/TPA)
Hyperplasias	–	12/15	14/15	–	–	25/30	23/30
Papillomas	–	–	–	–	–	3/30	3/30
Adenomas of the sebaceous glands	–	–	–	–	–	–	1/30
Squamous carcinomas	–	–	–	–	–	–	2/30
Basal cell carcinomas	–	–	–	–	–	–	2/30
Mean survival time (days)	331[a]	331[a]	331[a]	176	204	154	226

[a] For details, see Results

kindly provided by Prof. E. Hecker and his group, Institute of Biochemistry) was carried out in the non-initiated groups II and III and in the initiated groups VI and VII (Table 1). Twice per week each animal was treated with 100 nMol = 0.0615 mg TPA (dissolved in 0.5 ml acetone) on the back skin until the death of the animal. Animals of group I received only 0.5 ml acetone.

Animals which died in the course of the experiment were autopsied and organs or body sites showing pathological alterations were histologically investigated. The last animal of groups IV-VII died after 321 days; at that time, control animals and animals of groups II and III still alive were killed and investigated for tumors.

Results

Group I. Control group; $n=15$

At the time of sacrifice (i.e. 331 days, corresponding to the longest survival time of an animal of groups IV–VII), the control animals showed neither epidermal hyperplasias nor benign or malignant tumors in any of the organs investigated.

Group II. Promotion with TPA; $n=15$

12 of the 15 animals showed a marked epidermal hyperplasia in the TPA treated areas. The normally 3–4 layer thick dorsal epidermis had consistently increased in thickness up to 5–8 layers, including a pronounced granular layer. Apart from sporadically occuring hyperplasias and hyperkeratoses of the forestomach epithelium no benign or malignant alterations were found in any of the organs investigated. Only one animal had died within 331 days.

Group III. Ovarectomy prior to promotion with TPA; $n=15$

14 of the 15 animals showed hyperplasia of the dorsal epidermis. Hyperkeratoses of the forestomach were found in 3 animals. No other morphological alterations could be observed. None of the animals died within 331 days.

Group IV. Initiation with DMBA; $n=15$

Morphological alterations of the dorsal epidermis were absent from all animals. As expected the tumor incidence in the mammary gland was extremely high (15/15) and also the Zymbal gland proved to be very sensitive to neoplastic transformation (13/15). The epithelium of the forestomach frequently showed hyperkeratoses. Apart from an adenoma of the adrenal medulla, tumors were absent from other organs. Due to the high number of mammary gland tumors the average survival time was reduced to 176 days.

Group V. Ovarectomy prior to initiation with DMBA; $n=15$

By analogy with group IV, no alterations of the dorsal skin could be seen. Compared with the non-ovarectomized animals the tumor incidence of the mammary gland was drastically reduced (1/15). Hyperkeratoses of the forestomach epithelium were in the same order of magnitude as in group IV. The same holds true for tumors of the Zymbal gland (10/15). In addition, an oligomorphous glioma had developed in the central nervous system. Despite the large reduction in the number of mammary gland tumors, the average survival time (204 days) was only slightly higher than in group IV.

Group VI. Initiation with DMBA and promotion with TPA; $n=30$

25 animals developed marked hyperplasia of the dorsal epidermis. In addition we observed 3 papillomas in the TPA treated areas and a further papilloma of the upper lip. Whereas the number of mammary gland tumors was high as expected (32/30; two animals had developed 2 tumors) the tumors of the Zymbal gland (3/30) did not reach the high incidence observed in groups IV and V. Tumors also occurred in the forestomach (1/30), the liver (1/30), the small intestine (1/30) and 3 animals had developed leukemias. The average survival time in this group was by far the lowest (154 days).

Group VII. Ovarectomy prior to initiation with DMBA and promotion with TPA; $n=30$

As in group VI, the majority of the animals of this group showed a marked hyperplasia of the dorsal epidermis (23/30) along with the occurrence of 3 papillomas. In addition, an adenoma of the sebaceous gland, 2 squamous carcinomas and 2 basal cell carcinomas were observed. The number of tumors of the Zymbal gland was high, as in group VI (14/30) and despite ovarectomy, 6 tumors of the mammary gland had developed. The tumor incidence in the forestomach, the liver, the small intestine as well as the number of leukemias was in the same range as in group VI. Finally, the occurrence of a meningeal

sarcoma and an adenocarcinoma of the colon should be mentioned. The average survival time in this group was significantly higher (226 days) when compared with the non-ovarectomized animals of group VI.

Discussion

It was our aim to reassess the validity of the Berenblum/Mottram experiment in the rat, especially in view of the negative result reported by Shubik (Shubik, 1950). The findings of the present pilot study allow the conclusion that the DMBA/TPA mediated 2-stage carcinogenesis experiment is valid not only for the mouse but also favours the development of benign and malignant skin tumors in the rat.

As in the mouse (Slaga et al., 1976), the formation of benign or malignant skin tumors seems to go along with the induction of an epidermal hyperplasia by the promoter. Our experiment in the rat also shows that the initiating step can take place in a body site other than the subsequently promoted one (Goerttler and Loehrke, 1976b). In view of the initiation procedure used by Shubik (3 applications of a 1.5% DMBA solution at twice weekly intervals, Shubik, 1950), the negative outcome of his experiment cannot be explained by insufficient dosage of the initiator. It seems likely that the use of croton oil instead of TPA is the cause of the failure to produce tumors. In contrast to Shubiks findings with croton oil, long-term application of TPA did not lead to the formation of eczematoid skin lesions in the treated areas. The effect of TPA was mainly restricted to the epidermis and resulted in the development of a pronounced hyperplasia. More importantly, ovarectomy prior to initiation and promotion – leading to a dramatic reduction of mammary gland tumor incidence and a considerable increase in the mean survival time of the animals – seems to be crucial for the manifestation of malignant skin tumors.

In addition to the production of skin tumors in terms of a 2-stage mechanism, it seems likely that organs which are exposed first to the orally administered initiator (i.e. forestomach, small intestine, liver, colon) are also influenced in the same manner. In previous experiments in mice we have shown that the promoting ability of TPA is not restricted to the TPA-treated areas but that the substance also promotes initiated cells of internal organs (Goerttler and Loehrke, 1977). However, the low incidence of tumors in body sites such as the forestomach, the liver, the small intestine and the colon in the present experiment does not allow a definite statement.

Apart from the mouse and the golden syrian hamster, the rat represents the third species of animals in which the effectiveness of DMBA/TPA mediated 2-stage skin carcinogenesis can be demonstrated. From experiments in progress we can stage that the combination DMBA/TPA is also efficient in rabbits. There is every reason to believe that in the human organism tumors can also be induced by initiation-promotion processes (Weber and Hecker, 1978). In the light of the potential menace to the human biosphere by environmental carcinogens, 2 stage carcinogenesis experiments should attract our close attention.

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