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Enhancement of Tumor-Initiating Activity of DMBA by the Carbamate Fungicide Mancozeb

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Mancozeb is a protective fungicide and a polymeric complex of ethylene bis (dithiocarbamate) manganese with zinc salt. It is reported to be a skin irritant in rats after topical application (The Pesticide Manual, 1983), and considered by the working group of the International Agency for Research on Cancer, Lyon, France as one of the high priority chemicals to be tested for its tumorigenic activity (IARC, 1984). Recently, mancozeb has been reported by our group to act as a tumor initiator on the mouse skin in two stage protocol of study for its carcinogenic potential (Mehrotra et al. 1987).

In view of these reports and its increasing demand in India as a fungicide, mancozeb (Technical grade) has been tested in the present study for its cocarcinogenic activity on the mouse skin using 7,12-dimethylbenzanthracene (DMBA) as tumor initiator and 12-O-tetradecanoyl phorbol-13-acetate (TPA) as a promoter.

MATERIALS AND METHODS

Chemicals. Mancozeb (Technical grade, minimum purity 95%) was obtained from Bharat Pulverizing Mills, Bombay, India. 7,12-Dimethylbenzanthracene (DMBA), and 12-O-tetradecanoyl phorbol-13-acetate, (TPA) were obtained from Sigma Chemical Co., USA. Dimethyl sulfoxide (DMSO), and other reagents of analytical grade were arranged locally.

Bioassay protocol. Female Swiss albino mice (weighing 12-15 g) were taken for the study, and kept on synthetic pellet diet and water ad libitum. Animals were randomly divided into seven groups, each comprising of 20 animals. Hair was clipped initially and thereafter every week on the interscapular region over an area of 2 cm^2 with the help of an electrical

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hair clipper and treatment was topically provided on the shaved dorsal skin as per schedule given in Table 1.

Animals were watched for gross and microscopic changes including the development of tumors during the period of study locally on the skin. Body weight of all the animals was recorded every 15th day. Surviving animals from all the groups were sacrificed at the end of the study period of sixteen weeks.

Skin from the treated areas, with or without tumor growth, was taken and fixed in 10% formaldehyde solution and paraffin blocks were prepared. Tissue sections (5 μm) were cut and studied histopathologically after staining with hematoxylin and eosin.

Statistical Analysis. The body weight and tumor data were statistically analysed according to the method of Fischer (1950).

RESULTS AND DISCUSSION

Gross changes. Mice belonging to the Groups IV (DMBA + TPA) and V (DMBA + Mancozeb + TPA) showed distinct finger like or flat papillomatous growth on the dorsal skin, while mice belonging to other groups (groups I, II, III, VI and VII) did not show any such growth till the termination of experiment (Table 2). The average body weight of the animals belonging to various groups was compared during the period of treatment. It was seen that only the mice belonging to groups IV and V showed lowered body weight, after 75 days of treatment.

Repeated topical application of mancozeb on the interscapular skin of mice belonging to the groups III, IV and V, resulted in local baldness which was reversible. Loss of fur was initially noticed on 5th day after the first application and persisted upto 2 weeks after the last application of mancozeb. A normal hair growth pattern was observed thereafter.

Tumor development was observed for the first time after 46 days of TPA application in group IV, in 1/15 animals. By the 57th day of promotion, about 50% of the animals (7/15) of group IV developed tumor(s) on their back. By the 81st day of TPA application, 100% tumorigenesis was recorded in all the surviving (12/12) animals of group IV (Figure 1).

In group V the first tumor was encountered in 1/18 animals after only 36 days of TPA application. More than 50% animals (10/18) had developed tumor(s) on

Table 1. Treatment schedule of mancozeb as coinitiator with DMBA on mouse skin promoted by TPA

Group	Treatment	Initiator	Co-initiator	Promotor
I	Control (untreated)	-	-	-
II	Control (Vehicle)	Acetone	DMSO	Acetone
III	DMBA & Mancozeb	DMBA	Mancozeb	Acetone
IV	DMBA & TPA	DMBA	DMSO	TPA
V	DMBA & Mancozeb & TPA	DMBA	Mancozeb	TPA
VI	TPA	Acetone	DMSO	TPA
VII	Mancozeb	Acetone	Mancozeb	Acetone

a-Initiation was provided by a single application of 52 µg DMBA dissolved in 100 µl acetone in the begining of experiment.
b-Co-initiation was provided by painting mancozeb (100 mg/kg body weight) dissolved in 50 µl DMSO, 1 hr after the DMBA application and thrice per week for a total of 9 applications.
c-Promotion was provided with 5 µg TPA dissolved in 100 µl acetone. TPA was painted 1 week after the last application of co-initiator and twice per week for 12 weeks (24 applications).
d-Acetone and DMSO are solvents used to apply materials.

their back on 46th day and by the 71st day of TPA application 100% tumorigenesis was recorded in the surviving animals (18/18) (Figure 1).

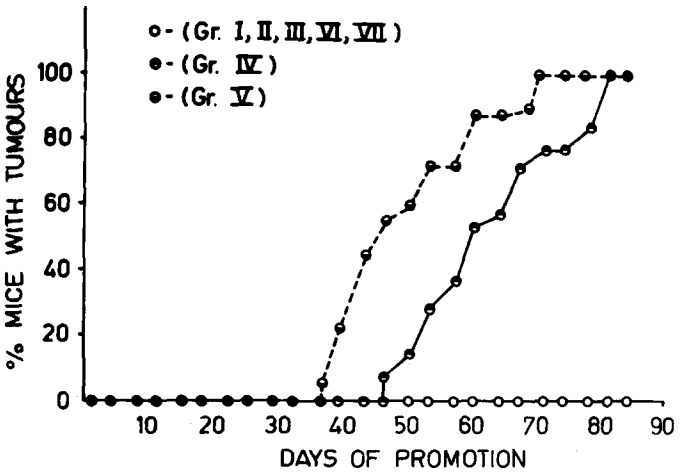

Figure-1 Incidence of tumors in various groups, details of group I to VII are described in Table 1.

These tumors in both the groups (i.e. groups IV and V) started as miniature excrescences firmly attached to skin, which grew into finger like or flat processes. Some tumors, had fragile top, and few tumors were hard in consistency with black areas over the surface representing old hemorrhages. In some animals which had more than one tumor grown on the painted areas, as they increased in size, a few of these tumor coalesced.

The cumulative number of tumors was much higher in group V (123/17) in comparison to the group IV (55/12). The average number of tumors per mouse was also higher in group V (7.23) over group IV (4.58).

Microscopic changes. The histopathological study of the skin tumors revealed them as benign squamous cell papillomas (pedunculated or flat type) and mixed tumors. The squamous cell papillomas were mostly pedunculated with a well defined and vascularised fibrotic inner core around which squamous epithelial cells were arranged in an arboreal pattern. Sometimes these papillomas were broad based and hence designated as flat type. In few cases the tumors had both the arboreal pattern growth of squamous epithelial cells and whorled horny masses of keratin developed from the hair element of the skin (acanthomatous changes) and hence were designated as mixed tumors.

Table 2. Coinitiating activity of mancozeb

Group	Treatment	Total No. of mice bearing tumors (after 12 wks of promotion)	Cumulative number of tumors (CMT)	Number of tumors/ mouse (Mean)	1st Induction of tumor (in days)	100% Tumorigenesis (in days)
I	Control (untreated)	0/18	-	-	-	-
II	Control (Vehicle)	0/18	-	-	-	-
III	DMBA & Mancozeb	0/16	-	-	-	-
IV	DMBA & TPA	12/12	55	4.58	46	81
V	DMBA & Mancozeb & TPA	17/17	123	7.23*	35	70
VI	TPA	0/17	-	-	-	-
VII	Mancozeb	0/16	-	-	-	-

* $p < 0.01$ when compared with group IV.

These results indicate that the limited application of mancozeb in the manner described above, for a three weeks period was able to enhance the DMBA initiated and TPA promoted carcinogenesis over mouse skin. The dose and frequency of application of mancozeb with DMBA (group III) however did not seem to warrant tumor development during the entire period of study i.e. upto 16 weeks.

The tumor induction time was reduced in 2-stage protocol when mancozeb was added in the treatment schedule along with DMBA and TPA. The first tumor appeared in group V after 35 days of TPA treatment (cf. 46 days of TPA treatment in group IV). Similarly, the average number of tumors per mouse was less in the DMBA + TPA treated animals in comparison to DMBA + Mancozeb + TPA treated animals. The 100% tumorigenesis was also recorded in group V earlier than in group IV. There was significant weight loss in animals belonging to groups IV and V as compared to the controls (group I).

These findings suggest that the mancozeb has some cocarcinogenic properties, when topically applied over mouse skin, as it enhances the effect of DMBA initiation subsequently followed by TPA promotion in two stage carcinogenesis protocol. To a greater or lesser degree, it could be due to the complete carcinogenic activity of its unit constituent maneb, which is reported to induce a variety of tumors including fibrosarcomas, tumors of pulmonary system and thyroid in rats (Balin, 1970; Andrianova and Alokseav, 1970). The cocarcinogenic activity of mancozeb over mouse skin may also be due to its tumor initiating ability *per se* (Mehrotra et al. 1987). So the cocarcinogenic role of mancozeb in the present study may be due to the presence of its constituent ingredient maneb and/or any other agent which might be present as an impurity in the technical grade mancozeb. The detailed chemical analysis of technical grade mancozeb would reveal the presence or absence of any such carcinogenic compound as impurity. In the present study mancozeb was applied for a limited period of 3 weeks, which may be only sufficient to co-initiate the mouse skin basal cell population in the mouse epidermis. In the animals belonging to group V the incidence of tumors per mouse was higher because mancozeb, in view of the above discussed possibilities could enhance the process of initiation in skin cell population. There is yet another possibility that mancozeb may act as phase I promoter and TPA both first and second stage promoter in multistage carcinogenesis (Slaga et al. 1980).

Thus it can be concluded that the mancozeb has some cocarcinogenic activity, which enhances the process of

tumor induction in DMBA initiated and TPA promoted mouse skin.

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