Histo-Pathological Study: Skin Cancer VarietiesDue to a Compound of Coal Tar & of Vinegar

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Objectives: To see skin cancer varieties due to coal tar & vinegar, the present studies, using mice as a model, evaluated histo-pathological and certain biochemical changes induced in skin by repeated topical application of their compounds, DMBA and TPA.

Materials & Methods: Hundred mice distributed into four groups of twenty five mice each were utilized. Two separate control groups were maintained. In the first experimental group, DMBA alone and in the second experimental group, TPA after single application of DMBA were applied on the skin of mice. Histo-pathological characterizations and cellular DNA, RNA and proteins molecules concentrations' estimation of the lesion's cells were evaluated through the comparative optical density change of the absorbance for DNA, RNA and Proteins molecules of different samples with that of their control throughout this twenty weeks study.

Results: It was observed that the repeated application of DMBA alone and repeated application of TPA after single application of DMBA produced both epidermal and dermal tumours like papilloma, dysplasia, keratoacanthoma, squamous cell carcinoma *in situ*, extensive squamous cell carcinoma, and fibrosarcoma. The malignant varieties of skin tumors were 30 % more with the repeated application of DMBA alone than that by repeated application of TPA after single application of DMBA. The statistical analysis of cellular DNA, RNA and proteins concentrations estimates were found comparatively significant in experimental groups.

Conclusion: The contact of DMBA (a compound of coal tar) can act as initiator as well as promoter for skin tumourigenisis.

Abbreviations:
¤ **DMBA** (Dimethyl Benz anthracene) is derived from anthracene i.e. poly-aromatic hydrocarbon found in commonly used coal tar.¤¤ **TPA** (tetradecanyl phorbol acetate) is derived from acetate that is salt or ester of ethanoic acid manufactured by oxidation of ethanol and commonly used for production of vinegar.

Introduction

Tumor is un-controlled abnormal production of cells. It is produced due to irreversible changes in the genes that regulate growth and differentiation of normal developing tissue[1]. A number of initiating inherited or exogenous (carcinogennic) factors for this genetic abnormality are identified[2]. Tumorigenesis by carcinogens usually occurs in multi-steps. The first two steps are known as initiation and promotion[3]. A final step is progression during which the transformed cell develops into a malignant tumor[4].

In the present studies therefore, varieties of induced tumour and multi-steps tumorigenesis of skin cancer due to the application of DMBA were evaluated through a complete follow up studies of histo-pathological and certain biochemical changes of cells in the developing lesions.

Materials and Methods

Hundred albino mice (*Mus musculus*) of both genders, 5-6 week old and weighing between 22 to 25 gm were distributed in four "A," "B," "C" and "D" groups, twenty five mice in each group. (Table 1). They were kept in separate iron cages under controlled conditions of a 12-h light / 12-h dark cycle at the animal house of Zoology Department Government College University Lahore. Air conditioning was used to keep temperature $22 \pm 2\ c^{o}$. Cages and animals were labeled with waterproof marker. The animals quarantined / acclimatized for a week before start of the experiment and their backs were shaved with electric clippers 3 days prior to the first skin treatment and thereafter shaved when needed. Commercial national mice food and tap water regularly provided to mice were changed with fresh one each day. The particulars of each animal such as the name of the group, age at start of the experiment, period for keeping the animal alive, gender, weight in grams at start and at the end of the experimentation, the period of application / administration of the carcinogen, diluent and vaccine for each animal were recorded throughout the length of experiment for the objective of correlation. Gross tumor appearing features were observed, counted and measured by Vernier Caliper weekly. The lesions and the surrounding skin were also examined macroscopically with magnified lens. The required skin tissues of all animals were taken by needle biopsy at first, fifth and tenth week of the study while all animals of each group at end of twenty weeks were sacrificed by decapitation after resection of the required tissue under anesthesia.

Carcinogens: Both carcinogens DMBA and TPA were obtained from Sigma Chemical Company (St. LOUIS, MO,

Table 1: *Purpose and Dose Schedule of Different Groups in the Present Twenty Week Studies.*

Group	Purpose	Dose schedule		
		Twice a week topical application of 20*u*g **DMB** per 0.2ml of acetone	Twice a week topical application of 2*u*g of **TPA** per 0.2 ml of acetone	Twice a week topical application 0.2 ml of acetone (Solvent)
A	Control	-	-	-
B	Placebo Control	-	-	20 Weeks
C	To test DMBA as skin cancer initiator as well as promoter agent.	20 weeks.	-	-
D	To test DMBA and TPA as skin cancer initiator and promoter agents respectively.	Initial single application.	20 weeks except ist. One.	-

Table 2: *Statistical Analysis For Estimates of DNA and RNA Proteins Concentrations for a 20-Week Application of DMBA (20 Mg / 0.2 Ml Acetone) on the Skin of Mice (Group "C") Compared with that of Control (Group "A").*

Weeks	DNA			RNA			PROTEINS		
	Group A	Group C	"t" test	Group A	Group C	"t" test	Group A	Group C	"t" test
5	2.79± 0.02	2.86*± 0.04	p< **0.05**	12.7 ± 0.04	12. 9*± 0.05	p< **0.05**	11.01± 0.007	12.812*± 0.009	p< **0.05**
10	2.79± 0.02	2.93**± 0.08	p< **0.01**	12.7 ± 0.04	13.36**± 0.030.04	p< **0.01**	11.01± 0.007	12.813**± 0.009	p< **0.01**
20	2.79 ± 0.02	3.65***± 0.09	p< **0.001**	12.65 ± 0.04	16.481***± 0.004	p< **0.001**	11.01± 0.007	14.717***± 0.009	p< **0.001**

USA). 100 mg of DMBA (available in ampoule of dried powder) was dissolved in one liter of acetone to make concentration of 100 µg per ml of acetone while 1mg dry powder of TPA was dissolved in 100 ml of acetone to get concentration 10µg per ml of acetone. The working solutions of both DMBA and TPA were prepared just before the use. Stock and working solutions of both carcinogens were kept at 20c^{o}. Automatic pipettes were used for topical application.

Light Microscopy and Spectro-photometry: The tissues obtained by biopsy / resection were given 2-3 washes with 0.85% saline solution. Each specimen of the tissues was then divided into two parts, one part stored in the frozen nitrogen while the other piece kept in Neutral buffered formaline solution for twenty-four hours. Subsequently the frozen tissues were subjected to standard spectrophotometric procedures. The estimates for concentrations of the DNA, RNA and proteins molecules were measured thro-ugh the comparative optical density change of the absorbance for DNA, RNA and Proteins molecules of different samples with that of the control. Then they were analyzed statistically using "t" test. The paraffin sections 1-5 *u* of formaline fixed tissues were prepared by standard histological techniques and stained with Haemotoxylin & Eosin (H& E).

Results

Effects of Carcinogens: The repeated topical application of DMBA alone produced skin cancer of the following types with frequencies (Table 3): papilloma 10% (Fig. 1), dysplasia (Fig. 3), 10%, keratoacanthoma 10%, squamous cell carcinoma *in situ* 10%, extensive squamous cell carcinoma, 40%, and fibrosarcoma (Fig. 2), 20%, in addition to some areas of chronic inflammation and of precancerous changes, such as pleomorphism etc. The frequency of benign epidermal tumour (papilloma) were more than malignant epidermal tumour (squamous cell carcinoma) in early period of experiment unlike the number of dermal tumour (fibrosarcoma i.e. malignant tumour of fibroblast) (table 3). The sequence of formation and progression of gross tumour findings in the skin observed are described below:

- Initial incomplete loss of hair restricted to the site of the application observed at 2 weeks, post-application. At 10 weeks, post-application, complete loss of hair at the site occurred and this loss progressed to adjacent 0.5 – 1 mm area beyond the treated skin area. In the remaining observation period, further enhancement in alopecia observed was insignificant.

Table 3: *varities and prgression of skin cancer for a 20-week application of dmba (20 µg / 0.2 ml acetone) alone or with tpa on the skin of mice*

Group	The Week of Examination	% of varieties of skin tumour						
		Epidermal					Dermal	
		Benign			Malignant		Benign	Malignant
		Papilloma	Keratocanthoma	Dysplasia	Sq. cell carcinoma *In situ*	Sq. cell carcinoma *extensive*	Osteoma	Fibrosarcoma
"C" (treated with DMBA alone)	5	60%	10%	10%	-	-	-	20%
	10	40%	10%	10%	10%	10%	-	20%
	20	10%	10%	10%	10%	40%	-	20%
"D" (treated with TPA after single dose of DMBA)	5	50%	-	30%	-	-	20%	-
	10	40%	-	30%	10%	-	20%	-
	20	30%	-	30%	20%	-	20%	-
"A" (Control)	1,5,10 & 20	No abnormality is detected						
"B (Placebo Control)	1,5,10 & 20	No abnormality is detected						
During 1 week, Precancerous changes like hyperplasia, pleomorphism etc. in the applied skin area of each mice of the group "C" & "D" were observed.								
Remarks	Progressive Development of squamous cell carcinoma (malignant tumour) from the benign tumour Papilloma reflected from the Result of Group "C" in th present study exhibits model of multisteps mechanism of tumourigenisis .							

- At 2 weeks, post-application, superficial scaly crescents, ovoid or irregular skin ulceration with punched, inverted or everted margins of about 1 mm length appeared in the applied area of the carcinogen. At 10 weeks, post-application, crusting or slight bleeding with depth of ulceration progressing from 0.1-0.5 mm was observed. Slight progression of ulceration was noted in the remaining period of the study.
- At 5 weeks, post-application, 1 to 2.
  pin-head sized smooth to roughened, sessile, soft to rubbery, tan to grayish white in color outgrowths were observed in the treated area. These outgrowths, at 12 weeks, post-application became adherent to the underlying tissue, increased in size and took the shape of wheat to maize grain. Hemorrhagic and necrotic foci occurred in the remaining period of observation.

The repeated application of TPA for 20 weeks after single application of DMBA in the present studies produced skin cancer varieties with frequencies (Table 3) : 30 % papilloma, 30% dysphasia, 20 % Osteoma, 20% squamous cell carcinoma in situ, in addition to some areas of acute inflammation and of precancerous changes like hyperplasia, pleomorphism etc. The frequency of benign epidermal tumour (papilloma) observed here was relatively more. Morever, the repeated application of TPA resulted into conversion of dermis fibroblast into osteoma (benign tumour) unlike the repeated application of DMBA that resulted into fibrosarcoma.

The loss of hair, ulceration and outgrowth in skin observed here displayed the sequence and progression resembling that observed with repeated application of DMBA alone.

Effects on body weight: Topical application of these carcinogens had no effect on the body weight of the treated mice. (Table 2).

Statistical Analysis: The estimates of the cellular concentrations of DNA, RNA and Proteins molecules of samples of DMBA alone or TPA after DMBA treated animals were found statistically significant for various levels of significance compared with that of control ($p < 0.05$, $p < 0.01$, $p < 0.001$: Table 3).

Discussion

The present studies show that repeated topical application of DMBA alone produce both benign and malignant epidermal (epithelial) tumors, which indicates that DMBA is initiator and promoter for epidermal cancer. These findings are in

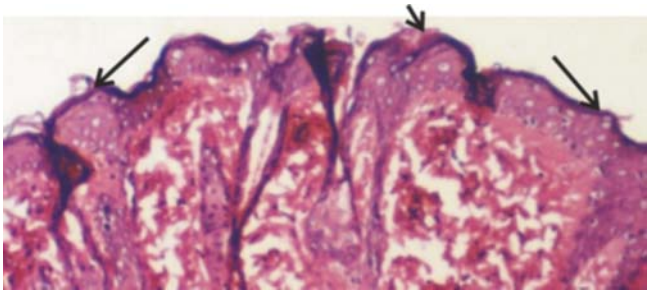

Fig. 1: *Photograph showing Microscopic structural characterizations squamous cell papilloma in the skin of Mice exposed to DMBA (H & E, 100 x). Black Arrows pointing to papilloma.*

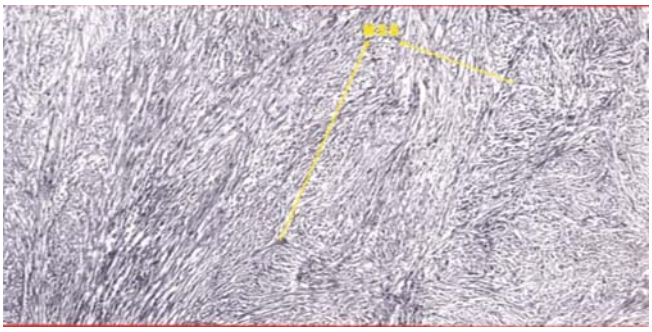

Fig. 2: *Photograph showing microscopic structural characterizations of fibrosarcoma in the skin of mice exposed to DMBA. (H & E, 400 x). MSB pointing malignant spindle cells bundles interlacing.*

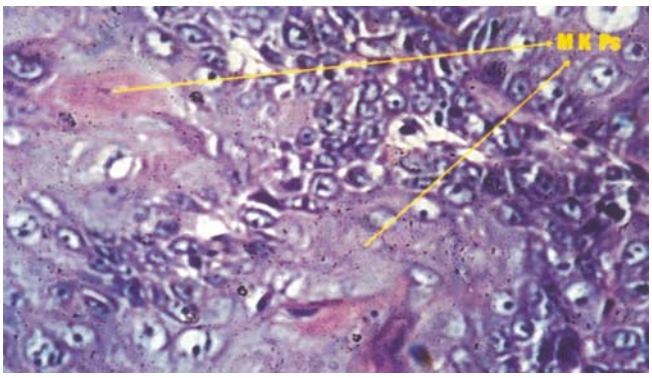

Fig. 3: *Photograph showing microscopic structural characterizations of invasive squamous cell carcinoma in the skin of mice exposed to DMBA. (H & E, 400 x). MKPs pointing Malignant Karatoma hyaline epithelial pearls.*

agreement with those of Gijare[4]. Another inference of the present investigations is that all epidermal tumors remain benign during the early period of the experiment but some papilloma become malignant progressively (Table-3), showing that chemical carcinogenesis occurs as a multistage process in epithelial tissue leading to malignant tumor This bservation is consistent with reports of Lakshma-Reddy[5], Hennings[6], and **Skarin**[7]. The present observation, "DMBA induces more tumour of epidermal than dermal origin"(table 3), indicates that epidermal cells are more likely get affected by DMBA, probably because they are more in ready contact and therefore more prone to the action of DMBA than the underlying dermal cells. However early development of malignancy in dermal cells indicates that if the dermal cells happen to be exposed to DMBA, the cancerous changes develop comparatively more rapidly in the dermal cells unlike to the multistage process of epithelial carcinogenesis. Deep ulceration of skin in some cases seen in the present study shows one way of penetration of DMBA to dermis. In the present investigations, DMBA is also found to be a skin irritant as it caused inflammation of the skin. This finding is not in line with **Lewis**[8] who reported that cancer promoter and not the cancer initiator causes skin inflammation in mice. This study has also shown that repeated application of TPA produced benign skin tumors during the early period and very few papilloma progressed into squamous cell carcinoma *in situ* in late phase of the experiment (Table 3) exhibiting that malignant conversion is slower with the repeated application of the cancer promoter (TPA). This is consistent with the findings of Hennings [6] who reported that the malignant conversion of the skin tumors is enhanced by repeated application of tumor initiator and not by repeated application of tumor promoter. Inflammation of the skin with repeated TPA application noted in the present study agrees with observations of **Lewis**[8] indicating the promoter's irritating ability of causing inflammation. The biochemical estimates of concentrations of DNA, RNA and Proteins in the skin exposed to DMBA and TPA in the present studies and those of Manjanatha[9] are significantly higher indicate that repeated application of carcinogens causes stimulation of more DNA synthesis in the exposed cells leading uncontrolled multiplication of cells representing the mechanism of carcinogenisis by these carcinogens. The finding "no weight loss in DMBA / DMBA & TPA treated mice" of the present research studies is consistent with finding of jiska[10]. Because of this feature, DMBA more commonly is used as cancer initiator than other cancer initiators. On the basis of our findings, we suggest the wearing of skin cancer protective screener and other defensive measures for the people/ laborers who work in the environment contaminated with these carcinogens or coal tar etc.

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