

Full Length Research Paper

Successful treatment of benign lesions by bis 3-azophenyl-4-hydroxy-6-methyl-pyran-2-one cobalt (II) in albino rats

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The study of the effect of bis 3-azophenyl-4-hydroxy-6-methyl-pyran-2-one cobalt (II) as chemotherapeutic agent was undertaken with the intention of studying the effects in 7,12-dimethylbenz[a] anthracene (DMBA) and tetradecanoyl phorbol-13-acetate (TPA) induced chemical carcinogenesis on skin of 5 - 7 weeks old healthy albino rats. The animals were divided into 5 groups (A, B, C, D and E) of twenty each. The rats were treated with carcinogens for 15 weeks and then the curative effect of bis 3-azophenyl-4-hydroxy-6-methyl-pyran-2-one cobalt (II) was observe. The bis 3-azophenyl-4-hydroxy-6-methyl-pyran-2-one cobalt (II) prevents malignant conversion of chemically induced benign tumors and did not kill cancer cells but change them to normal cells. It was concluded that if the complex is given in the pre-malignant phase of tumor development, it decreases the risk of malignant transformation.

Key words:7,12-Dimethylbenz[a] anthracene (DMBA), tetradecanoyl phorbol-13-acetate (TPA), complex {bis-3-azophenyl-4-hydroxy-6-methyl-pyran-2-one cobalt (II)}, skin cancer.

INTRODUCTION

Cell division or cell proliferation is a physiological process that occurs in almost all tissues under different circumstances. Normally, the balance between proliferation and programmed cell death is tightly regulated to ensure the integrity of organs and tissues. Mutations in DNA that lead to cancer disrupt these orderly processes. Leong and Leong (1989) realized that tumor in body behaved in different ways. The uncontrolled and often rapid proliferation of cells can lead to either a benign tumor or a malignant tumor (cancer). Benign tumors do not spread to other parts of the body or invade other tissues, and they are rarely a threat to life unless they extrinsically

compress vital structures. Malignant tumors can invade other organs, spread to distant locations (metastasize) and become life threatening.

Tumorigenesis by carcinogens usually occur in multi step, the first two steps are known as initiation and promotion, while the third step is progression during which the transformed cell develops into malignant cells. The most widely used drugs in chemotherapy (Rosenberg and Camp, 1970) are metal-based drugs. There are many other transition metals that exhibit role of powerful anti-cancer agent.

Pyrones and their derivatives have been widely acknowledged compounds, an account of the fact that they are natural products. Pyrones has displayed remarkable utility for antibacterial and antifungal (Rehman et al., 2005). The transitional metal complexes with hetrocyclic systems containing nitrogen and sulphur atoms have been studied extensively because of antitumor (Rosonberg and Camp, 1970; Bhatti et al., 2001; Chohan et al., 2003, Dollet and Sorenson, 1985) activities. The study was undertaken with the intention of studying the effects of

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Abbreviations: DMBA, 7,12 Dimethyl benz(a) anthracene; TPA, 12-O-tetradecanoyl-1-phorbol-13-acetate; DMF, dimethyl-formamide.

Table 1. The division of animals and the dose schedule of chemical carcinogens and bis 3-azophenyl-4-hydroxy-6-methyl-pyran-2-one cobalt (II).

Group (30 weeks)	Carcinogen (For 15 weeks)				Complex (For 15 weeks)	
	DMBA in acetone (100 µg/ml)		TPA in acetone (10 µg/ml) after 2 weeks of DMBA till 15 weeks		Complex in DMF (10 µg/ml) after 15 weeks of carcinogenesis till 30 weeks	
	Route	Schedule	Route	Schedule	Route	Schedule
A	Nil	Nil	NIL	Nil	Nil	Nil
B and C	Nil	Nil	NIL	Nil	Nil	Nil
D	Topical	Single dose	Topical	Twice a week till 15 weeks	Nil	Nil
E	Topical	Single dose	Topical	Twice a week till 15 weeks	Topical	Twice a week till 30 weeks

DMBA = 7,12-dimethylbenz[a] anthracene, TPA = tetradecanoyl phorbol-13-acetate.

chemical carcinogens on the skin of rats with initiation-promotion protocol and the use of previously reported complex bis-3-azophenyl-4-hydroxy-6-methyl-pyran-2-one cobalt (II) (Shahid et al., 2005) as chemotherapeutic agent.

MATERIALS AND METHODS

7,12-Dimethyl benz(a) anthracene (DMBA), used as initiator and 12-O-tetradecanoyl-1-phorbol-13-acetate (TPA) were purchased from Sigma Chemical Company and the study was done at the Postgraduate Medical Institute Lahore, Pakistan. Hundred albino rats (twenty each) of A, B, C, D and E. The animals used in the experiment were healthy and adult were kept under optimal atmospheric and hygienic conditions. All the five groups were kept in separate iron cages fitted with removable gauze lids and were labeled with their respective identification markings.

The animals were allowed and facilitated to acclimatize in the house for one week before the experiment was started. After one week of acclimatization of the rats, the back of each rat was shaved off hair (5 x 5 cm area) with electric clipper, this was done three days before the first dose was administered. Two chemicals were used as carcinogens, DMBA was used as initiator and TPA was used as promoter in the experimental study. Freshly prepared solution of DMBA in acetone (100 µg/ml) as a single dose was applied on the skin of the albino rats (Mughal, 2005). Insulin syringes were used for the application of solution. After two weeks of application of DMBA, TPA (10 µg/ml in acetone) was applied twice, a week after (Slaga et al., 1990; Bukhari et al., 2002).

Group A

The animals of group A acted as control and were not given any treatment throughout the experimental period (30 weeks).

Groups B and C

The animals of groups B and C also acted as control. However, these animals received doses of acetone and dimethylformamide (DMF), the vehicles used for dilution of carcinogens and bis 3-azophenyl-4-hydroxy-6-methyl-pyran-2-one cobalt (II) (Table 1).

Group D

The animals of this group were administered with DMBA at 100 µg/ml topically, as single dose on the shaved dorsum of the albino rats' skin, after two weeks. TPA was applied twice a week till 15 weeks to test the cancer induction effects of DMBA and TPA (Table 1).

Group E

Animals of group E were given carcinogens (DMBA and TPA) in the same schedule as for other animals (Table 1). In this group, complex was given locally after 15 weeks of carcinogenesis in a dose of 10 µg/ml twice a week for the next 15 weeks to observe the response of locally applied given complex against chemical carcinogens (Table 1). After completion of 30 weeks, biopsies were taken to see the chemotherapeutic response of locally applied given complex against chemical carcinogens.

Particulars of lesion recorded

Every week, loss of hair and gross morphological features such as ulcers were closely observed in each animal and if found, were measured carefully with Vernier calipers throughout the experiment. After the completion of fifteen weeks, the lesions and the surrounding skin of each animal was also closely examined (by a true cut fine needle biopsy) with a microscope to determine the extent of histopathological changes, such as papiloma, squamous cell carcinoma, malignant fibrous histiocytoma, atrophy, fibrosarcoma, chronic inflammation, squamous cell carcinoma *in situ* and osteoma at the end of the experiment. The lesions were then diagnosed according to the histopathological changes by using ether to anesthetize the rats. All the animals were sacrificed and after removing sections of the rats' dorsal skin, sections that contained or surrounded the lesion were removed for further cutting. The cancerous and surrounding tissues were washed two to three times with 10% formalin and were then used for further studies.

Histopathological studies

Histopathological examination was done according to Hopwood et al. (1990), Gorden et al. (1990) and Steven, (1990), the following steps were taken: Fixation stages in tissue processing, gross

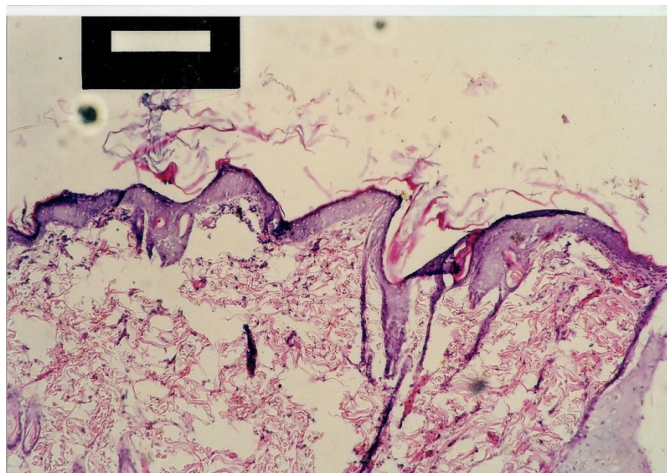


Figure 1. Photomicrograph showing a papilloma on the skin of rat (H & E, X 100).

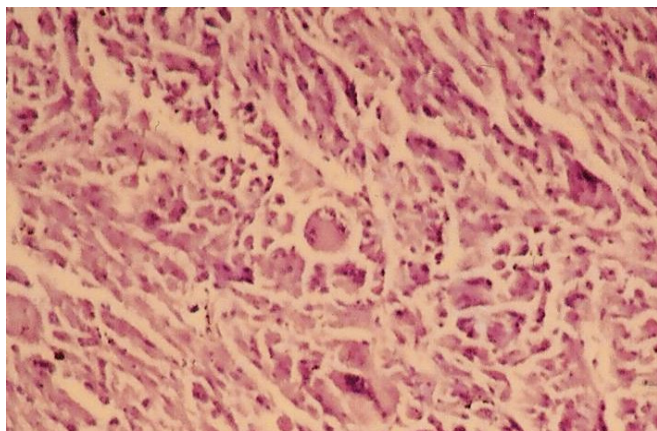


Figure 2. Photomicrograph showing histology of malignant fibrous histiocytoma.

examination, sectioning of the tissues, processing for the section, clearing: wax impregnation, embedding, storage of blocks, trimming of blocks, section cutting, removal of paraffin wax, hydration, staining, oiling and clearing and mounting.

RESULTS AND DISCUSSION

The maximum number of lesions were found in group D (100%), while no tumor was observed in the control groups (A, B and C) (Figure 3). All animals received topical application of DMBA and TPA. In group D, 14 animals developed benign lesions which were epidermal hyperplasia (07), osteoma (01), dysplasia (03), papiloma (03) (Figure 1). There were (06) malignant lesions which were squamous cell carcinoma *in situ* (02), squamous cell carcinoma (02) and malignant fibrous histiocytoma (02) (Figures 1 and 2). Most of the rats had chronic inflammation and precancerous changes in early weeks.

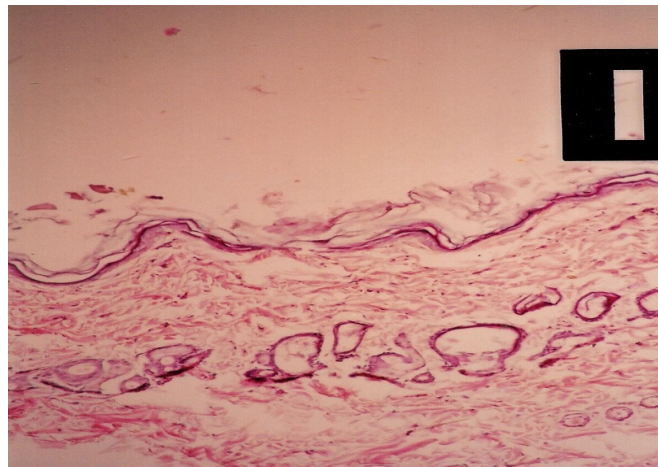


Figure 3. Photomicrograph showing normal epidermal and dermal layers (H & E X 100).

Hair loss was observed on specific areas on the third week where DMBA and TPA were applied locally. Post application of DMBA and TPA showed slight bleeding and ulceration which was not too deep, and this was observed at 14 weeks. Small size out growths (pinkish white color) were also observed (papilloma) at 15 weeks in the treated area.

In group E, 14 animals developed benign lesions which were epidermal hyperplasia (08), papilloma (03) and dysplasia (03). Five animals developed malignant lesions squamous cell carcinoma (02), squamous cell carcinoma *in situ* (02) and malignant fibrous histiocytoma (01) (Table 1) after 15 weeks of carcinogenesis. When the chemotherapy was given to this group, all the benign lesion were cured and the hair of the rats grew back after some time, while malignant lesions remained the same as they were before chemotherapy (Table 2). Our findings are consistent with Limtrakul et al. (2003) and Richardson et al. (2006) who used iron complex as an antitumor.

The role of metallic compounds as anti-tumor agent has well been established. Cis-platinum complexes are particularly effective in combination with other drugs in the treatment of other tumors of testes, ovary, head and neck and lung. Platinum complexes with amino ligands and steroid derivative show anti-tumor activity in which the metal is coordinated by dehydrocholic acid and a phosphine. Similarly, bleomycin is a clinically used anti-tumor agent. It is isolated from *Streptomyces verticillus* as a copper complex and is a compound used in many tumors, e.g. lymphoma and testicular cancers. The use of iron chelators as clinical agents against cancer is an area of growing interest. Previously, the di-2-pyridylketone isonicotinoyl hydrazone analogues were identified as ligands with potent and selective antineoplastic activity that can induce the expression of molecules involved in cell cycle arrest. This study also showed that these

Table 2. Distribution of lesions obtained in different animals with carcinogens and bis 3-azophenyl-4-hydroxy-6-methyl-pyran-2-one cobalt (II).

Group	Benign lesion					Malignant lesion				Total lesion (%)
	EPA	Pap	Dys	OST	Total	SQCCIS	SQCC	MFH	Total	
A (control)	-	-	-	-	-	-	-	-	-	0%
B	-	-	-	-	-	-	-	-	-	0%
C	-	-	-	-	-	-	-	-	-	0%
D	7	3	3	1	14	2	2	2	6	20 (100%)
E (before complex)	8	3	3	-	14	2	2	1	5	19 (95%)
E (after complex)	-	-	-	-	-	2	2	1	5	5 (25%)

EPH, Epidermal hyperplasia; Pap, papilloma; Dys, dysplasia; SQCCIS, squamous cell carcinoma *in situ*; SQCC, squamous cell carcinoma; MFH, malignant fibrous histiocytoma; OST, osteoma. N = 20.

chelators have high chelation efficacy, indicating a potential mechanism of anti-tumor activity. The di-2-pyridylketone isonicotinoyl hydrazone chelators with greatest anti-proliferative activity effectively entered cells, bound iron, induced iron mobilization and prevented iron uptake from transferring. The results suggested that the antiproliferative effects of these chelators relates to intracellular iron chelation, followed by the stimulation of iron mediated free radical generation via the so formed iron complex.

Our study was designed to see the effect of bis 3-azophenyl-4-hydroxy-6-methyl-pyran-2-one cobalt (II) (complex) in DMBA, followed by TPA, induced chemical carcinogens on the skin of albino rats. No tumor developed in the first and second group. However, rats belonging to the third group to whom no chemotherapy was given developed malignant tumors which became worse and two animals bearing these malignant tumors died before the completion of the experimental period. Our study suggested that use of iron complex decreases the risk of malignant conversion of benign tumors, because all benign tumors and premalignant lesions were cured with the use of bis 3-azophenyl-4-hydroxy-6-methyl-2h-pyran-2-one cobalt (II). Our findings are consistent with Richardson et al. (2006). They also used the iron complex (diphenyl thiosemicarbazone) as an anti-tumor agent.

This study is encouraging as it suggests that if the complex is given in the pre-malignant phases of tumor development, it decreases the risk of malignant transformation. There is immense scope for further research on these complexes as anti-tumor agents.

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