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## Anti-inflammatory Drug (Indomethacin) and its Effect on Liver Tumor Induced by DMBA

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### Abstract

Liver tumors were induced in the toads, *Bufo regularis*, in 16 out of 50 cases by the administration of 0.2 mg DMBA/toad, 3 times/week for 12 weeks. Light and electron microscopic photographs demonstrate that these hepatocytes of DMBA-treated toads showed criteria of malignancy. In contrast, toads treated with DMBA at the same dose level and 0.005 percent w/w indomethacin/toad, 3 times/week for 12 weeks showed a lower incidence of liver tumors, 8 out of 50 cases. The biochemical analysis showed that the activity of G6PD, LDH, acid phosphatase and alkaline phosphatase enzymes were decreased in animals treated with DMBA and indomethacin in comparison with toads treated with DMBA alone. It is concluded that indomethacin has an inhibitory effect on hepatocarcinogenesis in toads.

### Introduction

Epidemiologic studies have demonstrated the influence of the environment on the development of certain forms of cancer. Therefore, it is important to discover naturally occurring or synthetic compounds which can suppress or prevent the process of carcinogenesis (Boone *et al.*, 1990; Kulkarni *et al.*, 1992). During the last decades, tumor growth in a number of experimental models is inhibited by non-steroidal anti-inflammatory drugs such as aspirin and indomethacin (Tsavaris *et al.*, 1990; Marnett 1992) but the mechanism of action is still unclear. On the other hand, others reported that indomethacin did not inhibit tumor (Hofer *et al.*, 1980; Feldman and Hilf 1985).

Toads have been used as an advantageous model to study the development of tumors in relation to carcinogens (El-Mofty *et al.*, 1997), co-carcinogen (Sadek and Abdelmeguid 1986) and vitamins (Sadek and Hayat 1996). It is worth mentioning that similarities in cytological characteristics between tumors in toads and human have been documented (Abdelmeguid *et al.*, 1997).

Little attention has been given to the effect of indomethacin on liver tumor. The present investigation is undertaken to determine whether administration of indomethacin will have any inhibitory effect on toad's liver tumor incidences induced by 7, 12-dimethylbenz (a) anthracene. Also, it will be of interest to measure the level of some enzymes in blood and serum to shed more light on the mechanism of action of indomethacin.

### Materials and Methods

Sexually mature male and female toads, *Bufo regularis*, (about 30 gm, each) were used. The experimental animals were collected by a regular supplier from El-Nozha district, Alexandria, Egypt. The toads were maintained in glass tanks at a temperature of 20-22°C. The experimental animals were divided into four groups (50 toads/group) and treated as follows:

1. Animals of group A were force fed with 7,12-dimethylbenz(a)anthracene (DMBA) (Sigma Chemical Company, St. Louis, MO, USA) at a dose level of 0.2 mg/toad, 3 times/week for 12 weeks.
2. Toads of group B were given the same dose of DMBA

as in group A and fed with 0.005 percent w/w indomethacin (Sigma Chemical Company, St. Louis, Mo, USA), 3 times/week for 12 weeks according to (Noguchi *et al.*, 1991).

3. Animals of group C were given the same dose of indomethacin as in group B, 3 times/ week for the same period.
4. Toads of group D received no treatment and used as control.

The body weight of toads in each group was observed before and after the experimental period. The percentage of mortality was recorded.

At the end of 12 weeks, all animals were killed and all organs including the liver were carefully examined macroscopically. Tumors were appeared in the liver of some animals. These tumors were greyish-white in colour. Also, dead toads were examined. For histological evaluation, the liver tissue was fixed in carnoy, dehydrated, embedded in paraffin, sectioned and stained with haematoxylin and eosin. For electron microscopic studies, specimens from each group were fixed in 2.5 percent glutaraldehyde for 1 hr, then rinsed in 0.1 M phosphate buffer. This was followed by post fixation using 1 percent Os O4 for 2 hr at 4°C, then the specimens were dehydrated through graded ethanol and treated with propylene oxide and embedded in Araldite-Epon mixture. Semithin (1 µm) and ultrathin (50 nm) sections from selected areas were cut with a glass knife on LKB ultramicrotome. After being double stained with uranyl acetate and lead citrate, the sections were examined by Jeol 100 CX electron microscope.

The activities of glucose 6-phosphate dehydrogenase (G6PD), in blood, Lactic dehydrogenase (LDH), acid and alkaline phosphatases in serum were detected in blood samples of each group. G6PD enzyme activity was determined according to Sigma procedure which is a modification of the spectrophotometric methods of Kornberg and Horecker (1955) and Lohr and Waller (1974). LDH was determined according to Buhl and Jackson (1978) procedure. Acid and alkaline phosphatase enzymes were determined by calorimetric method (Belfield and Goldberg 1971).

Statistical analysis using t-test was performed to determine the level of significant difference between tumor incidence and enzyme activities in toads treated with DMBA alone when compared with toads treated with DMBA and indomethacin.

## Results

Liver tumors (Fig. 1) were recognized in toads fed with 0.2 mg DMBA/toads, 3 times/week for 12 weeks, 16 cases out of 50 (Table 1). The tumor was diagnosed microscopically as hepatocellular carcinoma possessing criteria of malignancy (Fig. 2, 3). Also this group of animals showed marked increase in the rate of mortality. Electron microscopy demonstrate that these hepatocytes of DMBA-treated toads showed an irregular dilated nuclear envelope, displacement of nucleolus and segregation of its components (Fig. 4). Also, an increase in number of pleomorphic-shape mitochondria and dilation in intercellular spaces were observed in comparison with control.



Fig. 1: Whole mount of liver of toad treated with DMBA. showing numerous small-sized nodules (arrows) [x6].

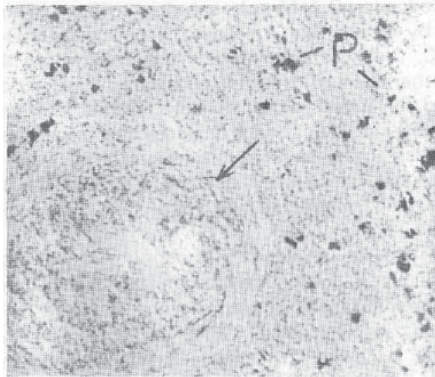


Fig. 2: Section of liver of toad treated with DMBA. showing a nodule encased by fibrous capsule (arrow), pigments (p) [x200]

However, toads treated with DMBA at the same dose level and 0.005 percent w/w indomethacin/toad, 3 times/week for 12 weeks showed a lower incidence of liver tumors, 8 cases out of 50. The animals of this group showed a marked decrease in mortality rate than control (Table 1). The electron micrographs of this group demonstrate that indomethacin decrease the irregularities of nuclear envelope. Also, the mitochondria appeared highly organized and were evenly distributed in the cytoplasm.

No tumor incidences were detected in toads which treated with 0.005 percent w/w indomethacin/toads 3, times/week for the same period. The electron micrograph of these cells showed normal nuclear envelope and normal mitochondria.

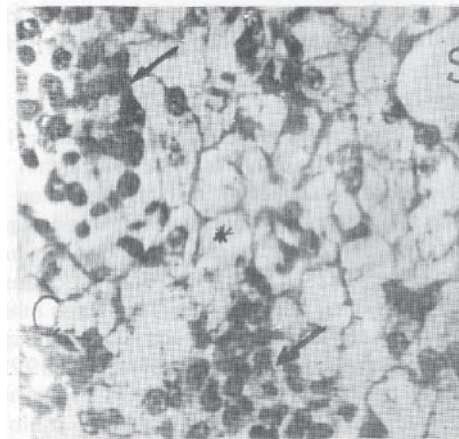


Fig. 3: Section of liver of toad treated with DMBA. Showing vacuolated cytoplasm (\*), lymphocytic infiltration (arrow), dilated sinusoids (s), congested capillaries (c). [x800].

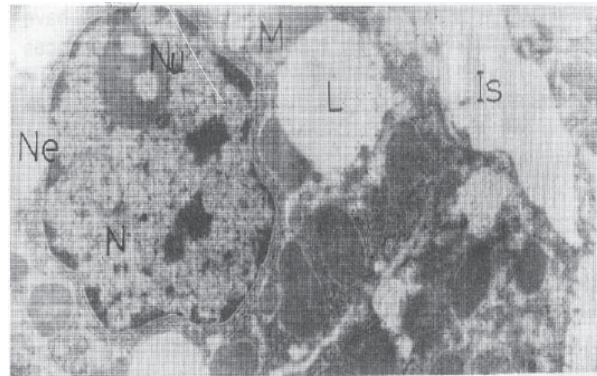


Fig. 4: Electron micrograph. Liver of toad treated with DMBA. Showing an irregular nuclear envelope (Ne), nucleolus (Nu) contains holo-centered spheres, mitochondria (M), Lipid droplets (L), dilated intercellular space (IS) [x15,000]

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Table 1: Effect of indomethacin on liver tumor incidence

Group	Treatment	Dose/toad	Total number of toads	Number of toads bearing liver tumor
A	DMBA	0.2 mg	50(22)	16
B	DMBA + Indomethacin	0.2 mg + 0.005% w/w	50(14)	8
C	Indomethacin	0.005% w/w	60(11)	0
D	Control	0	50(0)	0

( ) : Number of dead toads

Table 2: Effect of indomethacin on activities of G6PD, LDH, acid and alkaline phosphatase enzymes

Enzyme unit	DMBA	DMBA + Indomethacin	Indomethacin	Control
G6PD $\mu$ /g hb	23.4 $\pm$ 1.66	17.6 $\pm$ 0.62*	19.0 $\pm$ 0.94	19.9 $\pm$ 1.64
LDH $\mu$ /l	89.5 $\pm$ 39.60	662.7 $\pm$ 7.56**	726.7 $\pm$ 64.06	889.7 $\pm$ 68.92
Acid phosphatase $\mu$ /l	188.7 $\pm$ 30.99	44.7 $\pm$ 7.32*	68.0 $\pm$ 19.25	85.3 $\pm$ 4.46
alkaline phosphatase $\mu$ /l	192.0 $\pm$ 20.53	161.0 $\pm$ 5.44*	108.7 $\pm$ 5.46	178.7 $\pm$ 47.32

\*Significant  $p < 0.05$ , as compared with the DMBA alone group \*\*Non significant difference as compared with the DMBA alone group

The biochemical analysis showed that the activity of G6PD, LDH, acid phosphatase and alkaline phosphatase enzymes were decreased in animals treated with DMBA and indomethacin in comparison with toads treated with DMBA alone (Table 2).

### Discussion

The results of the present investigation confirm our previous studies by light and electron microscope (Sadek and Abdul-Salem, 1994a, b; Abdelmeguid *et al.*, 1997) which showed that DMBA induces liver tumor. Also, the present study clearly indicated that indomethacin decreases the incidence of liver tumor in the Egyptian toad. Many investigators have been reported that indomethacin suppressed tumor growth in the colon (Reddy, 1992), in bladder (Cohen *et al.*, 1981), in tongue (Tanaka *et al.*, 1989) or in pancreas (Takahashi *et al.*, 1990) in rodents. Also indomethacin exerts antineoplastic properties in rats (Tanaka *et al.*, 1991).

The present study has been shown that indomethacin suppress the hepatic damage caused by DMBA in the Egyptian toad on the ultrastructural level. Nothing is known of the ultrastructure effect of indomethacin on liver tumor. Barriault *et al.*, 1994 reported that indomethacin prevent the hepatotoxicity of phalloidin in mice. The biochemical data of the present study showed that indomethacin decrease significantly the G6PD enzyme activity in blood of toads previously treated with DMBA. the inhibition of tumor induction may be due to failure of G6PD-deficient cells to metabolize chemicals to an ultimate form which is able to, induce cancer (Pascale *et al.*, 1987). It is worth to mention that there is, a positive correlation between G6PD enzyme activity; cell proliferation and cancer (Dessi *et al.*, 1984). Also, the present results revealed an increase in the level of LDH enzyme in toads treated with the chemical carcinogen (DMBA), while its activity was decreased in significantly after treatment with indomethacin. Also, it has been shown that the activity of LDH was increased in men colon cancer (McGinty *et al.*, 1973). However, the decrease in LDH enzyme was observed after the effect of antitumor drug (5-Fluorouracil) in colon adenocarcinoma (Ismail *et al.*, 1978). The present data showed that indomethacin decreased acid and alkaline phosphatase enzyme activities in toads previously treated with DMBA, Similar results showed that indomethacin decrease the activity of acid phosphatase

enzyme in rat gastric. mucosa (Navarova and Nosalova 1994).

The mechanism (s) by which indomethacin modulates tumorigenesis are unknown but there are several possible mechanisms. One of these various possibilities is that inhibition of hepatic cytochrome P-450. This enzyme is activated by many chemical carcinogens to become capable of binding to DNA and initiating the carcinogenic process (Pelkonen, 1992). It is worthy to mention that indomethacin prevent the proliferative activity due to inhibition of DNA synthesis (Kanzaki *et al.*, 1979). Add that, indomethacin inhibits cell proliferation in a variety of normal and neoplastic mammalian cells (Fulton 1984).

Other possible mechanism was undertaken by Vane and Botting (1990) who reported that indomethacin is a potent inhibitor of prostaglandin (PG) synthetase. We cannot exclude the possibility that PG exert an inhibitory effect on natural killer (NK) cells, the components of the defense system (Goodwin and Cauppens, 1983) and the NK cells in toads have an inhibitory effect against tumor cell line (YAC-1) (Ghoneum *et al.*, 1990). Much additional study is required to determine the relationship between indomethacin and immune system.

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