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Curcumin and its Effect on Cytochrome P450 and GST in Toad Liver Tumor Induced by DMBA

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Abstract

Curcumin has been used as a colouring agent and spice in many food. The present results clearly demonstrated that toads received 0.5 mg curcumin and 0.5 mg DMBA/toad, 3 times/week for 12 weeks, showed a significant decrease in tumor incidence (3 out of 50 cases) in comparison with that treated with DMBA alone (12 out of 50 cases). At the same time, curcumin reduce the activity of liver microsomal cytochrome P450s and cytosolic GTSS enzymes in toads previously treated with DMBA. The present data suggest that curcumin decrease incidence of liver tumor in toads through inhibition of cytochrome P450 and GST activities. The present report was undertaken for two reasons. First to determine whether curcumin, which are widely used in food have anticarcinogenic effect on the liver of the Egyptian toad. Second, are curcumin effect on microsomal cytochrome P450 and GST activities to shed more light on the mechanisms (s) of action.

Introduction

Studies on the effect of diet and cancer have had an increasing influence on public cancer policy and recommended life style changes to reduce the risk of cancer. Tumeric has been used as a coloring agent and spice in many foods (Tonnesen *et al*., 1994). Turmeric, powdered rhizome of the plant *Curcuma longa* Linn., has been widely used for the treatment of a variety of inflammatory conditions and other diseases (Nadkarani 1976; Ammon and Wahl, 1991). Curcumin (difleruloylmethane), a phenolic compound that has been identified as the major pigment in turmeric possesses both anti-inflammatory (Srimal and Dhawen, 1973; Satoskar *et al*., 1986. and antioxidant (Sharma, 1976; Toda *et al*. 1985) Properties. Dietary administration of 2 percent turmeric inhibited 7, 12-dimethylbenz (a) anthracene (DMBA) induced skin and Benzo (a) pyrene-induced forestomach tumors (Azuine and Bhide, 1992), but the mechanism of action is still unclear.

As yet, no data are available concerning possible inducing or inhibitory properties of curcumin towards cytochrome P450s nor towards Glutathione S-Transferase GSTs, two of the most important enzyme systems involved in the bioactiviation and bioinactivation of xenobiotic compounds (Vermeulen *et al*., 1992). Toads have been used as models to study the development of tumors in relation to carcinogen (El-Mofty *et al*., 1987), a co-carcinogen (Sadek and Abdul-Salam, 1994), vitamins (Sadek and Hayat, 1996) and substances originating from plant (Sadek *et al*., 1995). It is worth to mention that similarities in cytological characteristics between tumors in toads and humans have been documented (Abdelmeguid *et al*., 1997). The influence of curcumin on experimental hepatocarcinogenesis has received little attention.

Materials and Methods

Toads: Sexually mature male and female toads, Bufo regularis, were used. The average weight per experimental animals was 40 g. The experimental animals were collected by a regular supplier from El-Noha district, Alexandria, Egypt. The toads were maintained n glass tanks at a temperature of 20-22°C and fed equal meal of earth worms, once per week. The experimental animals were divided into 4 groups [50 toads/group] and treated as follows:

1. Toads of the first group (group A) were given 0.05 ml of olive oil and used as control.
2. The second group (group B) was injected with DMBA into the dorsal lymph sacs (Sigma Chemical Company, St. Louis, Mo, USA) at a dose of 0.5 mg/toad, 3 times/week for 12 weeks.
3. Animals of group C were given curcumin (Sigma Chemical company, St. Louis. MO, USA) at a dose of 0.5 mg/toad, 3 times/week for the same period.
4. Toads of group D were given the same dose levels of DMBA and curcumin/toad, 3 times/week for the same period.

Growth and histopathological observations: At the ed of 12 weeks all animals were killed and all organs including the liver were carefully examined microscopically. Tumors appeared in the liver of some animals. These tumors were grayish white in color. For histological evaluation the liver tissue was fixed in Bouin and embedded in paraffin. The sections were stained with hematoxylin and eosin.

Biochemical assays: After 12 weeks, liver specimens from all groups were taken for biochemical assays. Frozen liver specimens from each group were homogenized by sanification. Determination of protein was carried out as described by Lowry *et al*. (1951) with serum albumin (BSA) as standard. cytochrome P450-mediated 0-dealkylation of EROD and PROD were measured according to Reiners *et al*. (1990), using ethoxy-and pentoxy resorufin as substrates and nicotinamide adenine dinculeotide phosphate (NADPH) generating system. Fluorescence was recorded at a wavelength of 530 n and an emission wavelength of 586 nm with a Perkin Elmer Model 3000 fluorescence spectrometer.

Table 1: Effect of curcumin on DMBA-induced liver tumor in toads

Group	Treatment	Dose	Total No. of toads	No. of toads bearing liver tumor	Tumor incidence (%)
A	Olive oil	0.1 ml	50 (2)	0	0
B	DMBA	0.5 mg	50 (4)	12	24
C	Curcumin	0.5 mg	50 (2)	0	0
D	DMBA	0.5 mg	50 (3)	3	6*
	Curcumin	0.5 mg			

() No. of dead toads *Significant $p < 0.05$, as compared with DMBA

Table 2: Effects of curcumin on the activities of microsomal Cytochrome P450-dependent EROD and PROD as well as cytosolic GST activities in toad liver functions.

Treatments	Microsomes/EROD activity	Microsomes/PROD activity	Cytosolic GST activity
	pmol/min/mg protein		nmol/min/mg protein
Control (group A)	0.838 ± 0.076	0.733±0.081	0.854±0.047
DMBA (group B)	2.451 ± 0.278	1.193±0.196	3.099±0.145
Curcumin (group C)	0.484 ± 0.055	0.670±0.094	1.458±0.109
DMBA + Curcumin (group D)	1.248 ± 0.068	0.866±0.099	2.357±0.232

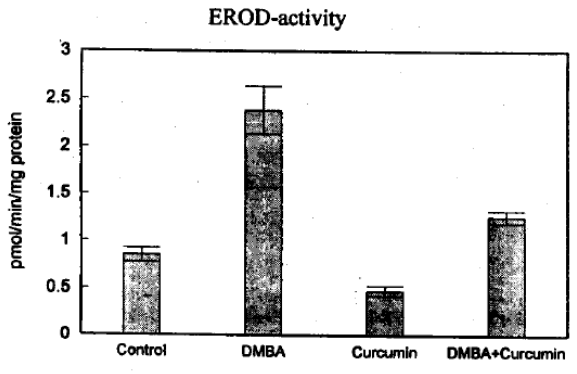

Fig. 1: The inhibition of cytochrome P450-mediated EROD activity in liver microsomes from DMBA-induced toads by curcumin.

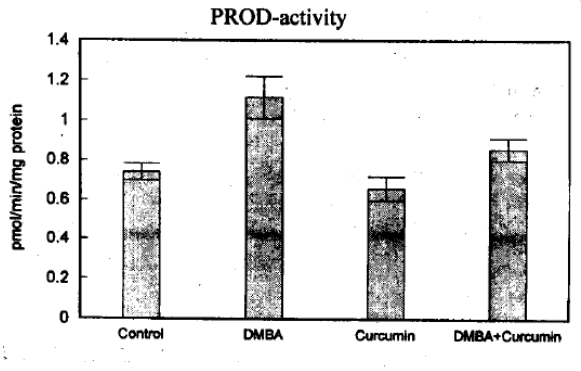

Fig. 2: The inhibition of cytochrome P450-mediated PROD activity in liver microsomes from DMBA-induced toads by curcumin.

GST activities towards 1-chloro-2, 4-dinitrobenzene (CDNB) were measured spectophotometrically according to the method of Habig *et al*. (1974). The absorption differences (•abs/min) were recorded on a Philips PU8720 UV-VIS scanning spectrophotometer at 340 nm. All the above determinations were carried out in triplicate and performed under conditions leading to linear reaction rates with time and protein concentration.

Statistical analysis: 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 toad treated with DMBA and curcumin.

Results

Hepatocellular carcinoma were recognized in toads which received 0.5 mg DMBA/toad, 3 times/week for 12 weeks. This resulted in a tumor incidence of 24 percent (Table 1). Changes in the expression of microsomal cytochrome P450s and cytosolic GSTs were also recognized in this group. Cytochrome P450 IAI/IA2 measured as EROD activity, cytochrome P450 2B1/2B2 measured as PROD activity and Glutathione (GSH) conjugation of CDNB for GST activities were significantly increased by 2.92 times, 1.62 times and 3.62 times respectively (Table 2).

Toads treated with DMBA at the same dose level in group B and 0.5 mg of curcumin/toad, 3 times/week

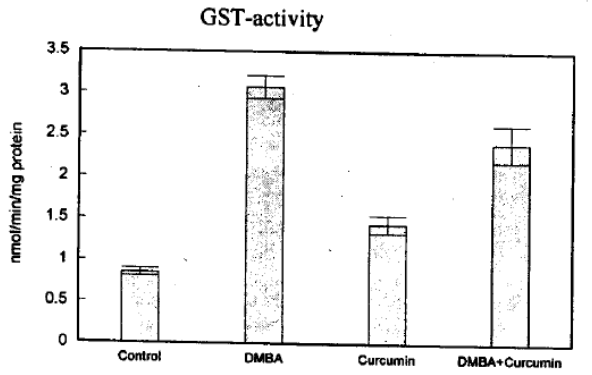

Fig. 3: The inhibition of GST-mediated CDNB GSH-conjugation activity in liver cystol from DMBA-induced toads by cui cumin.

for 12 weeks showed a significant decrease in tumor incidence 3 out of 50 cases (Table 1). This group of toads showed a significant inhibition on EROD and PROD activities by 49 percent and 27 percent respectively (Fig. 1,2). The CDNB-conjugation activities of GST were also significantly decreased in this group by 24 percent in the case of cystole from toads treated only with DMBA (Fig. 3). Neither tumor growth nor neoplastic changes were detected after 12 weeks in the liver of toads which given curcumin alone or olive oil (Table 1).

Discussion

Dietary administration of 2 percent turmeric inhibited DMBA-induced skin and Benio (a) pyrene-induced forestomach tumors (Azuine and Bhide, 1992). Also, curcumin inhibits colon tumorigenesis induced by azoxymethane in rat (Rao *et al*., 1995). It is well known that curcumin is a phenolic compound that has been identified as the major pigment turmeric. The results of the present investigation suggest that curcumin posses inhibitory effect against toad liver tumor induced by DMBA. Curcumin may exist its inhibitory effect upon chemical carcinogenesis by numerous possible mechanisms (S). One of these various possibilities is an inhibition of 5-lipoxygenase, 12-lipoxygenase and cycloxygenase (Ammon *et al*., 1993). Another possibility is that curcumin inhibits the microsomal-mediated mutagenicity by benzo (a) pyrene (Nagabhushan *et al*., 1987). Recently, topical application of curcumin was reported to inhibit B[a]P-DNA adducts and to protect against tumorigenic activities of B[a]P and DMBA in the epidermis of female CD-1 mice (Huang *et al*., 1992). Mukundan *et al*. (1993), found that 0.03 percent curcumin in the diet for 4 weeks also significantly reduced the levels of BLaJP-DNA adducts in the liver of rats. Soudamini and Kuttan (1992) suggested that the mechanism of action of curcumin as an inhibitor of chemical carcinogenesis involved scavenging of peroxides and superoxides as a result of its antioxidant capacity. The carcinogen B[a]P, however, requires oxidative bioactiviation to B[a]P-7,8-dihydrodio1-9, 10-epoxide, the ultimate carcinogen known to bind, to DNA (loannides and Parke 1993). B[a]P is bioactivated by cytochrome P450 1A1 (Guengerich and Shimada, 1991).

In the present study, we found that in DMBA induced mcirosomes, curcumin is an extremely potent inhibitor of cytochrome P450 1A1/1A2 and is slightly inhibitor of cytochrome P450 2B1/2B2. Similar results have been obtained by Oetari *et al*. (1996) in liver systole from rats previously treated with B[a] P. We cannot exclude the possibility hat, a part from antioxidant activity of curcumin my act as anticarcinogen due to its strong and specific inhibitory activity towards Cytochrome P450 1A1/1A2 (Donatus and Vermeulen, 1990).

Curcumin has structural similarities to ferulic acid, which is also known as an alkaline degradation product of curcumin (Tonnesen *et al*., 2002). Plant phenols such as ferulic acid is shown to posses inhibitory properties towards GST activity from rat liver (Oetari *et al*., 1996; Das *et al*., 1984). Also, our results showed that curcumin is a potent inhibitor of GSTs in liver of toads pretreated with DMBA.

It is worthy to mention that amphibians have a well developed immune system that consist of major cellular and humoral components (Cooper 1976). Some studies showed that the immune system of frog posses inducable killer (IK) cells (CTL-like) and spontaneous (SK) cells (NK - like) which effect responses against allogenic cell (Ghoneum and Cooper 1987) and the tumor cell line YAC-1 (Ghoneum *et al*., 1990).

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