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Effect of dietary fat on toad liver tumor induced by DMBA: Ultrastructural studies

I.A. Sadek and F. Abdul-Salam
Department of Zoology, Faculty of Science, Kuwait University, Kuwait

Summary. Toads injected with 2 mg 7,12-dimethylbenza(a)anthracene (DMBA)/toad, 3 times/week for 12 weeks induced liver tumors in 12 out of 50 cases. The electron micrograph of toad liver tumor showed disorganization of the rough endoplasmic reticulum which encircles or partially surrounds the mitochondria. Cristae mitochondrialis are rare in comparison with control. Enhancement of liver tumor incidences (29 out of 50 cases) by DMBA at the same dose level plus 2cc corn oil/toad, 3 times/week for 12 weeks was detected. Electronmicrograph of this group showed the same criteria of malignancy as in the first group. No tumor incidences were detected in toads fed corn oil only. The electronmicrograph of liver cells showed a high increase in glycogen and lipid droplets.

Key words: DMBA, 7,12-dimethylbenz(a)anthracene, Toads, *Bufo viridis*

Introduction

There is a continuing interest in the relationship between dietary fat and tumorgenesis. Feeding the poly-unsaturated dietary fat to mice increases the incidence of methylcholanthrene-induced tumors in these animals (Mertin and Hunt, 1976). Fats such as corn oil, containing large amounts of poly-unsaturated fatty acids, enhanced mammary tumor growth in rats treated with DMBA (Hopkins et al., 1976). Also, corn oil enhanced liver tumor induced by para-dimethylamino-azobenzene (Kline et al., 1946) and 7,12-dimethylbenz(a)anthracene (DMBA) (Sadek, 1986a). The mechanism(s) by which corn oil enhance(s) carcinogenesis has not been established yet.

Toads have been used as models to assay the development of tumors in relation to carcinogens (Sadek, 1981), co-carcinogen (Sadek, 1986a,b) and vitamins (Sadek, 1984). It is worth mentioning that similarities in cytological characteristics between tumors in frogs and humans have been documented (Deyree et al., 1960).

It was, therefore, of interest to examine the effect of dietary fat with or without DMBA on the liver of toad by using electron microscopy to shed more light on the mechanism(s) of action.

Offprint requests to: Dr. I.A. Sadek, Department of Zoology, Faculty of Science, Kuwait University, Kuwait

Materials and methods

Sexually mature male and female toads, *Bufo viridis*, were used. The average weight per experimental animal was 30 g. The experimental animals were collected by a regular supplier from El-Wafra district, Kuwait. The toads were maintained in glass tanks at a temperature of 20-22 °C. The experimental animals were divided into 4 groups (50 toads/group) and treated as follows:

1. Group A was injected into the dorsal lymph sacs with 7,12-dimethylbenz(a)anthracene (DMBA) (Sigma Chemical Company, St. Louis, MO, USA) at a dose of 2 mg/toad, 3 times/week for 12 weeks.
2. Toads of group B were given the same dose of DMBA and fed with 2cc commercial corn oil (Dalal, Kuwait Company), 3 times/week for 12 weeks.
3. Animals of group C were given 2 cc of corn oil, 3 times/week.
4. Group D was untreated and used as control.

At the end of 12 weeks, all animals were killed and the organs were examined by the naked eye. It was noticed that tumors had appeared in the liver of some animals. These tumors were small nodules, greyish-white in colour. For histological evaluation the liver was fixed in Bouin's solution and embedded in paraffin. The sections were stained with haematoxylin and eosin.

After 12 weeks, liver specimens from each of the four groups were fixed for 3 h in 2.5% glutaraldehyde-cacodylate buffer (pH 7.4) and postfixed for 2 h in 2% OsO_4. Specimens were then washed in phosphate buffer, dehydrated in a graded ethanol series, followed by propylene oxide, and embedded in Epon. Sections were cut on an LKB-III ultra-microtome, stained with uranyl acetate and lead citrate, and were examined in a Jeol

X100 electron microscope.

Statistical analysis for χ^2 test was done according to Steel and Torrie (1960) to clarify the impact of corn oil on tumor incidence.

Results

It was observed that toads of group A, which received 2mg DMBA/toad, 3 times/week for 12 weeks, induced liver tumors in 12 out of 50 cases (Table 1). The electron micrograph of toad liver tumor showed disorganization of the rough endoplasmic reticulum. The granular profiles were scattered throughout the cytoplasm. The rough cisternae entered into close contact with mitochondria, which they encircled or partially surrounded (Fig. 1). The cisternae of rough endoplasmic reticulum were dilated and started to lose their ribosomes. Mitochondrial cristae were rare, with nearly complete loss of intra-mitochondrial granules in comparison with control (Fig. 2). Also, only a little amount of glycogen was observed.

Toads of group B, which were given the same dose of DMBA and fed 2 cc corn oil/toad, 3 times/week for 12 weeks, showed a higher incidence of liver tumor (29 out of 50 cases). According to χ^2 test it was highly significant (P>0.01). Electron micrograph of this group (Fig. 3) showed the same criteria of malignancy as in Fig. 1.

No tumor incidences were detected in toads of Group C which were fed 2cc corn oil/toad, 3 times/week for the same period. The electron micrograph of these cells showed normal rough endoplasmic reticulum and increased number of mitochondria. Also, a high increase in glycogen and lipid droplets was observed (Fig. 4).

Table 1. Liver tumor incidence in the toad, *B. viridis*, treated with DMBA, corn oil or both.

GROUP TREATMENT	DOSE/TOAD	TOTAL NUMBER OF TOADS		TOADS WITH LIVER TUMORS	
		Initial	Final	n	%
DMBA	2 mg	50	48	12	24
DMBA + Corn oil	2 mg + 2 cc	50	48	29	58
Corn oil	2 cc	50	50	0	0
Control	0	50	50	0	0

Discussion

The results of the present investigation confirm our previous studies which showed that corn oil enhanced toad liver tumor incidences induced by DMBA (Sadek, 1986a). The ultrastructure of the liver cell of treated toads with DMBA showed disorganization of rough endoplasmic reticulum. Similar findings in rat hepatoma cells has been shown by Pitot and Peraino (1964). In rare cases one may observe concentric lamellar formation of

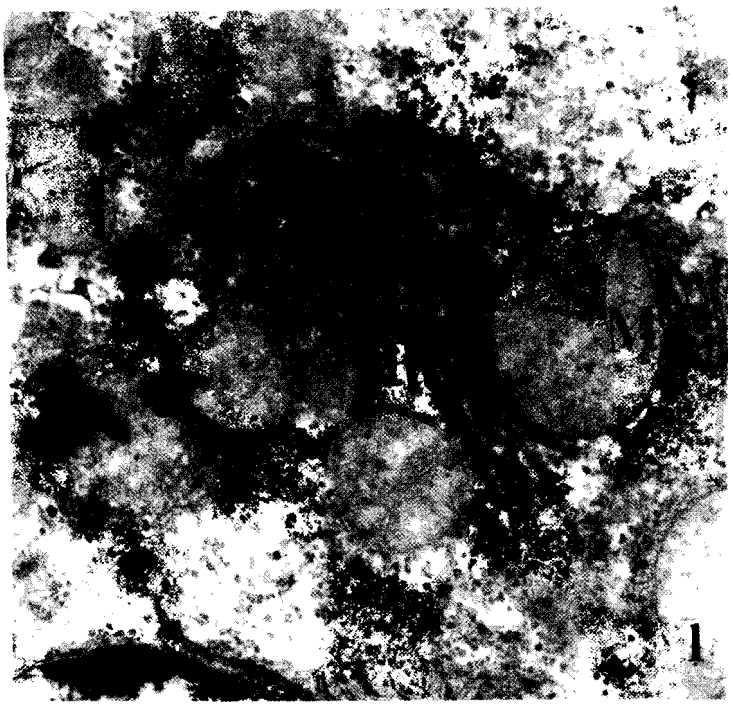

Fig. 1. Electron micrograph of liver of toad treated with 2 mg DMBA/toad, 3 times/week for 12 weeks. Note mitochondria (M), without cristae. The rough endoplasmic reticulum (RER) is scattered throughout the cytoplasm and is in close contact with mitochondria, which it encircles or partially surrounds. Note concentric lamellar formations of the rough cristae. Poor glycogen (G) x 10,000

Fig. 2. Electron micrograph of untreated toad liver. Note mitochondria (M) with cristae, normal endoplasmic reticulum (RER). Glycogen (G). x 10,000

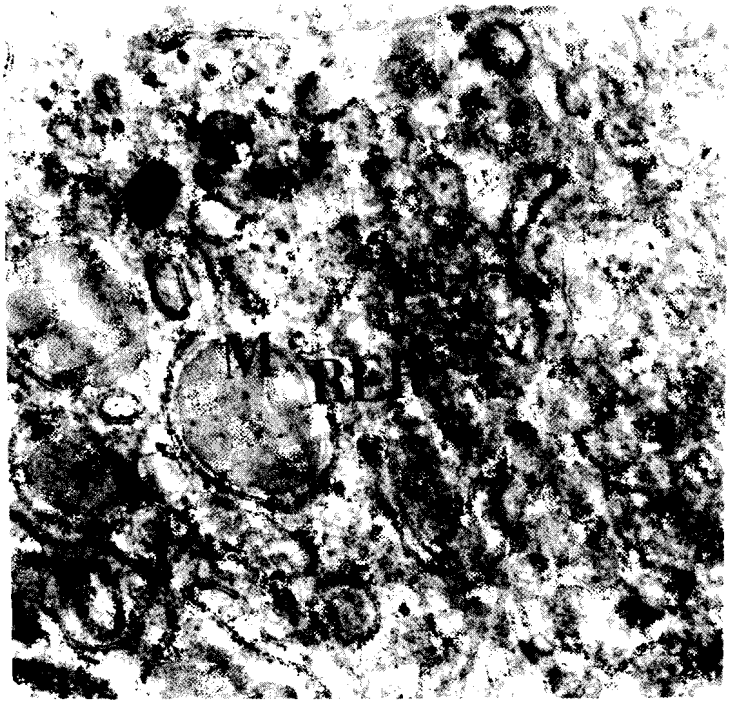

Fig. 3. Electron micrograph of liver of toad treated with 2 mg DMBA/toad plus 2 cc corn oil/toad, 3 times/week for 12 weeks. Note mitochondria (M) without cristae. RER is scattered throughout the cytoplasm and is in close contact with mitochondria, which it encircles or partially surrounds. Glycogen (G). x 10,000

Fig. 4. Electron micrograph of liver of toad treated with 2 cc corn oil/toad, 3 times/week for 12 weeks. Note mitochondria (M), rough endoplasmic reticulum (RER). Abundance of glycogen (G). Lipid droplet (L). x 10,000

the rough cisternae (Fig. 1). These membranous formations were never observed in normal liver cells, but have been described earlier for hepatoma cells (Fawcett, 1955). The loss of mitochondrial cristae are common in almost all mitochondria in the liver cell of toads treated with DMBA or DMBA plus corn oil. This phenomenon was observed in liver of rat after exposure to carcinogenic substances like N-nitrosomorpholine (Bannasch, 1968), N-hydroxy-2-acetyl-aminofluorene (Hartman, 1965) and dimethylnitrosamine (Mukherfee et al., 1963). It is well known that mitochondrial functional or structural abnormality is one of the most sensitive indicators of cell injury (Roadyn and Wilkie, 1968). The morphology of mitochondria in normal cells varies in different cell types but is usually constant in cells of the same type. The observations of Lenk and Penman (1971) showed that normal mitochondria synthesize proteins that either form an integral part of mitochondrial cristae or act as anchor proteins to hold the cristae intact. The inhibition of their synthesis results in the reduction of mitochondrial cristae.

In the present results, the rarity of mitochondrial cristae by DMBA plus corn oil was observed. Also, moderate amounts of glycogen and lipid droplets were detected. We cannot exclude the possibility that corn oil increases DMBA inhibition of mitochondrial protein synthesis due to the fact that mitochondrial proteins are involved in cristae formation (Clarke-Walker and Linnane, 1967). The electron micrograph of liver cells of toads fed with corn oil alone showed increases in glycogen and lipid droplets. Another point of consideration is that the co-carcinogenic effect of corn oil may be due to increases in glycogen in the liver cells of toads. It is worth mentioning that there have been several indications in humans (Bannasch et al., 1984) and in rodents (Bannasch, 1968; Seelman-Eggebert et al., 1987) that deviations in glycogen metabolism are closely associated with hepato-carcinogenesis.

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