Effect of Fish Oil on Liver Tumorigenesis and Biochemical Perturbations in Toads Treated with 7,12-Dimethylbenz (a) anthracene

Ismail A. Sadek, Kamal S. Ghazaly, Amina E. Essawy and Atif M. Al-Attar

The effect of fish oil on 7,12-dimethylbenz(a) anthracene (DMBA) - induced liver tumors in toads *Bufo regularis* was investigated. Feeding toads with DMBA 0.5 mg/toad twice weekly for 14 weeks, induced liver tumors (hepatocellular carcinomas) in 32 % of the treated animals. No tumors were detected in toads received DMBA plus 0.1 ml fish oil. Biochemical estimation of several parameters; total lipids, cholesterol, triglycerides, glutamic pyruvic acid transaminase (GPT) and glutamic oxaloacetic acid transaminase (GOT), demonstrated a significant effects of fish oil on the alterations induced by DMBA. The obtained results collectively revealed the anticancer activity of fish oil as well as its pronounced ameliorative effect against the toxic effects of DMBA on the haematochemical parameters in toads *Bufo regularis*.

Key words: Fish oil, toads, hepatocellular carcinomas

1Department of Zoology, Faculty of Science, 2Department of Biology, Faculty of Education, Alexandria University, Alexandria, Egypt

3Department of Biology, Faculty of Education, El-Taif, Saudi Arabia
Sadek et al.: Fish oil, toads, hepatocellular carcinomas

Introduction
Marked variations in dietary habits among population of different cultures and life styles have been associated with a risk for the development of cancer. Among the dietary habits, dietary fat has received considerable attention as a risk factor in the etiology of colon and mammary cancer (Carroll and Braden, 1984; Reddy, 1986). Increasing dietary intake of plant polysaturated fatty acids rich in the essential fatty acid linoleate, has shown to enhance tumor development in chemically induced mammary carcinogenesis (Ip et al., 1986). Studies in laboratory animals demonstrated that, high fat diets containing corn oil enhanced the chemically induced colon tumors (Holt et al., 1986), mammary tumors (Welsch, 1992 and 1994), Pancreatic tumors (Birt el al., 1990) and liver tumors (Sadek, 1989; Sadek and Abdul-Salam, 1994).
Polyunsaturated fatty acids in the form of marine oil, contain a large proportion of n-3 long chain fatty acids, were found to inhibit the growth and metastasis of tumors (Hubbard et al., 1986; Hamid et al., 1999). Epidemiological studies have shown that, the Japanese and Greenland Eskimos have a very low mortality from breast cancer because they consume large amounts of fat derived from marine oils.

As a matter of fact, blood serves as the most convenient indicator of the general condition of the animal body. Haematological and histopathological parameters give a valuable information on the physiological reactions of animals to carcinogens (Shimkawa et al., 1977; Burt and Brennan, 1980). Blood enzymes such as GOT and GPT play an important role in the metabolic function and have been used in the diagnoses of animal diseases as well as in the detection of tissue damage caused by carcinogenic agents (Casillas and Amesz, 1968).

In this aspect, the research work was planned to study the possible inhibitory effects of fish oil on toad's liver tumors induced by 7,12-Dimethylbenz(a)anthracene, which is a well known potent carcinogen in mammals and many animal species (Iversen, 1991). The experiments here were designed also to study the possible role played by the supplementation of fish oil against the toxic effect of DMBA on the haematological parameters in the Egyptian toads, Bufo regularis, which have been recommended for testing the carcinogens (El-Mofty et al., 1997; Abdel Maguid et al., 1997) and co-carcinogens (Sadek and Abdul-Salam, 1994).

Materials and Methods
Sexually mature male and female toads, Bufo regularis weighing approximately 40 g each were used. The animals were collected by a regular supplier from El-Nozha District in Alexandria, Egypt. They were kept in a large glass aquarium with small amounts of dechlorinated water that was changed twice daily and were fed with earthworms once every 3 days.

The toads were divided into five groups of 50 toads each. Each toad of the first group (group I) was fed with 0.5 mg DMBA in 0.1 ml olive oil twice a week for 14 weeks. DMBA was purchased from Sigma Chemical Company, ST. Louis, Mo., U.S.A.

Toads of the second group (group II) were given the same dose of DMBA as in group I, and fed also with fish oil (Sigma Chemical Company, ST. Louis, Mo., U.S.A) at a dose level of 1 ml/ toad, twice weekly for 14 weeks. Experimental toads of the third and fourth groups (group III and group IV) served as control groups for toads in group I and group II and force fed olive oil 0.1 ml/toad and fish oil 1 ml/toad, respectively. Toads in the fifth group (group V) were untreated and used as controls for all previously mentioned treated groups.

Histopathological studies: At the end of experimental period (14 weeks) all alive animals were autopsied and all organs were examined by the naked eye. It was noticed that tumors had appeared only in the liver of some DMBA-treated animals. Livers bearing tumors were photographed and removed for fixation. For histological evaluation, the liver tissue was fixed in 10% formalin and embedded in paraffin. Sections 4-5 μm were cut and stained with haematoxylin and eosin.

For electron microscopy, the liver was removed quickly and dropped immediately into 2% OsO4 fixative. Small pieces of the liver (1 mm) including tumor nodules were cut within 1-2 min while immersed in the fixative. The fixed samples were postfixed in 1% OsO4 for 2 h, dehydrated in graded ethanol, treated with propylene oxide and embedded in Epon. Ultrathin sections (50 nm) were cut on an LKB ultratome with a glass knife, double stained with uranyl acetate and lead citrate, and examined with a Jeol 100 CX electron microscope.

The X2 test (Steel and Torrie, 1960) was used to assess the significant change in tumor incidence.

Biochemical studies: At the termination of the 21st, 6th, 10th and 14th experimental weeks, blood samples were taken by heart puncture and collected in non heparinized tubes. The blood samples were centrifuged at 8000 r.p.m for 15 minutes. Serum activities of aspartate aminotransferase (AST or GOT) and alanine aminotransaminase (ALT or GPT) were determined according to the procedures of Raitman and Frankel (1967). Total lipid, cholesterol and triglycerides were measured as described by Zollner and Kinoshita (1962), Richmond (1973) and Trinder (1969), respectively. Data were statistically analyzed using the student's t-test.

Results
Effect of DMBA and DMBA plus fish oil on the histological and subcellular structures of liver cells: Liver tumors were only found in toads (21 out of 86 survivors), which had received DMBA 0.5 mg/toad twice weekly for 14 weeks. No tumors were detected in the liver of toads received DMBA plus fish oil (group II). Also no tumors or other pathological changes were observed in the organs of the control groups (group III, IV and V).

The tumor nodules observed in the liver of DMBA treated toads (Fig. 1) were diagnosed as hepatocellular carcinomas, in which the nodules appeared as localized masses of tumor cells, circumscribed with a fibrous capsule (Fig. 2). The architecture of the fibrous capsule was scanty and mingled with the tumor cells in a diffuse and disorderly manner. The tumor cells exhibited loss of polarity and displayed varying degrees of pleomorphism. The nuclei were pleomorphic and some of them were hyperchromatic. The nuclei are prominent and mitotic divisions of the hepatocytes were abundant (Fig. 3).

The structure of liver in the toads received DMBA plus fish oil showed the normal appearance of liver architecture (Fig. 4). Electron micrographs of the tumor cells in toads fed DMBA, showed some important criteria of malignancy such as pleomorphic nuclei, irregular nuclear envelope and clefted nucleus (Fig. 5). The most predominant nuclear abnormality was the frequent presence of nuclear pycnotic (Fig. 6). Some nuclei contained nuclear pseudoinclusions (Fig. 7). These intranuclear inclusions were demarcated by a nuclear envelop and associated heterochromatin mass. As regards cytoplasmic abnormalities the cytoplasm contained disorganized, giant and pleomorphic mitochondria (Fig. 8), cytoplasmic vacuoles (Fig. 6) and autophagosomes (Fig. 9). Pleomorphic fibroblasts and collagen fibers were observed in tumor capsule (Fig. 10).

Electron micrographs of hepatocytes of toads treated with DMBA plus fish oil illustrated the normal appearance of the regular nuclear envelop with distinct nuclear membrane, oval mitochondria with disemblile cristae and dense matrix, and normal rough endoplasmic reticulum (Fig. 11).

Effect of DMBA and DMBA plus fish oil on some biochemical parameters: Feeding of DMBA induced a marked elevation (P < 0.05) in serum GOT, GPT, total lipids, cholesterol and triglycerides, throughout the periods of treatment (Figs. 12 and 16).

Administration of DMBA plus fish oil provoked significant increases (P < 0.05) in the biochemical parameters. However, the magnitude
Fig. 1: Liver of a toad treated with DMBA. T, Tumor nodules. (X 3)

Fig. 2: Liver section of a toad treated with DMBA, showing tumor nodule (T) surrounded by fibrous capsule (C). Note the necrosis of central area of tumor nodule (arrow). (X 130)

Fig. 3: Higher magnification of the previous section, showing pleomorphic nuclei with mitotic figures (X 1150)

Fig. 4: Liver section of a toad received DMBA plus fish oil, demonstrating the normal liver architecture. The hepatic acini are nearly circular in outline and the hepatocytes contain a eccentric basophilic nucleus. (X 600)

Fig. 5: Electron micrograph of hepatocyte of a toad treated with DMBA, showing disorganized nucleus with irregular nuclear envelope. Note the mostly absence of mitochondria and rough endoplasmic reticulum (X 15000)

Fig. 6: Electron micrograph of hepatocyte of a toad treated with DMBA, illustrating irregular nuclear envelope (NE), nuclear pocket (NP), and vacuolated cytoplasm (V). (X 15000)
Fig. 7: Electron micrograph of hepatocyte of a toad treated with DMBA, demonstrating pseudoinclusion (P) inside the nucleus. (X 10000)

Fig. 9: Electron micrograph of hepatocyte of a toad treated with DMBA, illustrating autophagosomes (arrows), vacuolated cytoplasm and numerous free ribosomes (R). (X 16000)

Fig. 10: Electron micrograph of liver tumor capsule of a toad treated with DMBA, demonstrating fibroblast (FB) and collagen fibers (CF). (X 10000)

Fig. 11: Electron micrograph of hepatocyte of a toad treated with DMBA plus fish oil, illustrating regular nuclear envelope (NE) with distinct nuclear membrane, normal mitochondria (M) with numerous cristae, normal rough endoplasmic reticulum (RER) and lipid droplets (L). (X 10000)

Fig. 12: Effect of DMBA, DMBA plus fish oil, fish oil and olive oil on the activity of glutamic pyruvic acid transaminase (GPT) in serum of the toad, B. bufo regular treated for 14 weeks.
Sadek et al.: Fish oil, toads, hepatocellular carcinomas

Fig. 13: Effect of DMBA, DMBA plus fish oil, fish oil and olive oil on the activity of glutamic oxaloacetic acid transaminase (GOT) in serum of the toad, Bufo regularis treated for 14 weeks.

Fig. 14: Effect of DMBA, DMBA plus fish oil, fish oil and olive oil on the serum level of cholesterol of the toad Bufo regularis treated for 14 weeks.

Fig. 15: Effect of DMBA, DMBA plus fish oil, fish oil and olive oil on the serum level of triglycerides of the toad Bufo regularis treated for 14 weeks.

Fig. 16: Effect of DMBA, DMBA plus fish oil, fish oil and olive oil on the serum level of total lipid of the toad Bufo regularis treated for 14 weeks.

Discussion

The results obtained in this research work revealed that, treating the toads with fish oil significantly prevented the incidence of liver tumors induced by DMBA. These results are in agreement with the work of Reddy et al. (1991), who reported that, incidences of colon tumor were significantly reduced in rats fed the high fish oil diet as compared with those fed the high corn oil diet. Jiang et al. (1997) showed that dietary fish oil inhibited Sprague-Dawley rat colon tumorigenesis. Also, Singh et al. (1997) found that the high fish oil diet inhibited azoxymethane (AOM) – induced rat colon tumorigenesis compared with high corn oil diet.

The literature supports an association of chronic inflammation with the development of tumors. The colon contains a specialized lymphocyte population that may influence various stages of colon carcinogenesis. Results of a study carried out by Kuratko (2000) indicated that fish oil (high in n-3 fatty acids) slows the inflammatory response in the colon as compared to corn oil (high in n-6 fatty acids). Moreover, polyunsaturated fatty acids in fish oil have been shown to inhibit the growth and metastasis of breast tumors (Hubbard et al., 1998).

The precise mechanism by which fish oil inhibits toad liver tumor is not known. However, it has been found that, fish oil may inhibit prostaglandin synthesis. Rao and Reddy (1993) demonstrated that the high fat fish oil and high fat olive oil inhibited rat colon carcinogenesis and significantly suppressed the prostaglandin level in plasma. Liver and colon mucosa of rats compared with high fat corn oil.

Large number of human cancers and experimentally induced cancers in animals contain and/or produce large quantities of prostaglandins, particularly E series (Appel and Woutersen, 1994; Kargman et al., 1996).

The biosynthesis of prostaglandins through the cyclooxygenase system and hydroxy fatty acids via lipoygenase pathway from arachidonic acid exert a variety of biological activities. Several studies have shown that cyclooxygenase metabolites, particularly prostaglandins of type -2 series, modulate cell proliferation, tumor growth, and immune responses (Marnett, 1992; Smith, 1992), whereas lipoygenase metabolites can influence various biological responses including chemotactic responses, hormone secretion, ion transport, tumor cell adhesion, stimulation of tumor cell spreading, and regulation of tumor cell metastatic potential (Honn and Tong, 1992; Timer et al., 1992).

Hamid et al. (1999) demonstrated the presence of two immunoreactive isoforms of cyclooxygenase (COX-1 and -2), and the modulating effects of n-3 fatty acids on their expression, in N-nitrosomethylurea (NMU) – induced rat mammary tumors. They
Sadek et al.: Fish oil, toads, hepatocellular carcinomas

concluded that, high fish oil diet (rich in n-3 fatty acids) significantly suppressed both COX-1 and COX-2 protein levels when compared to the high corn oil diet.

It can not exclude the possibility that prostaglandins exert an inhibitory effect on natural killer (NK) cells; components of the defense system (Breda et al., 1980; Goodwin and Ceuppens, 1983). Prostaglandin E2 is known to suppress a variety of immune responses including T-cell functions (Fulton and Levy, 1980), cytokine production (Renz et al., 1988) and macrophage / natural killer cell-mediated cytotoxicity (Young et al., 1986). Natural killer cells are considered to be a possible first line of defense in the host’s antitumor immuno-surveillance (Herberman, 1983). Clearly, amphibians have a well developed immune system that consists of major cellular and humoral components (Cooper, 1976). In addition, frogs possess cytotoxic cells which appear functionally similar to mammalian killer cells (Ghose et al., 1990).

In addition to the previously discussed antitumor activity of fish oil, this study showed its effect in reducing the DMBA induced changes in the haematocological parameters. Insuring to our results, Castillo et al. (1999 and 2000) demonstrated that, dietary fish oil reduces cholesterol and triglycerides in chick plasma. Hyperlipidaemia, hypercholesterolaemia and hyperglycaemia are previously known to be associated with liver tumors in rat (Jao et al., 1997) and human (Ghose and Bacon, 1993). Results obtained by Tarao et al. (1997) demonstrated an intimate relationship between liver tumorigenesis and the elevation in human blood activities of GPT and/or GOT.

In conclusion, data revealed that the anticancer activities of fish oil as well as its pronounced ameliorative effect against the toxic effects of DMBA on the haematocological parameters. These results are in agreement with the previous studies in supporting the hypothesis that fish oil possesses chemopreventive properties and validate its potential usefulness in the field of cancer prevention.

References


Sadek et al.: Fish oil, toads, hepatocellular carcinomas


MS received 22th April, 2002; accepted 26th May, 2002