ANTI-CARCINOGENIC EFFECT OF VIRGIN OLIVE OIL ON DMBA-INDUCED SALIVARY GLANDS CARCINOGENESIS IN RATS

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SUMMARY : The anti-carcinogenic effect of virgin olive oil (VOO) on 7, 12-dimethyl benzanthracene (DMBA)-induced salivary glands carcinogenesis was studied histologically in Wistar rats. Experimental animals were prefed before, and postfed after, DMBA implantation a standard chow diet supplemented with 30% VOO. The histological observations on biopsied specimens of rats salivary glands showed delayed onset and decrease in the severity of carcinogenesis in the prefed group compared with the postfed group or control group of animals which clearly showed early onset and extensive carcinogenesis. This suggested that VOO can provide a dietary chemoprotective effect against DMBA-induced salivary glands carcinogenesis in rats. The probable mechanisms of this anti-tumorigenic effect of VOO is discussed implicating antioxidant functions and enhancement of cell-mediated immunity by the dietary components of VOO.

Key Words : Cancer, tumor, olive oil, salivary gland, DMBA.

INTRODUCTION

Although epidemiological and experimental studies implicate dietary fat as a factor in the etiology of certain cancers (1-3), results of experiments using rodent model systems of chemically induced and transplantable mammary tumors suggested that both the quantity and quality of the dietary fat can influence the incidence and growth of these tumors (1-3). A diet containing more polyunsaturated fat than normal requirement has been reported to be an effective promoter of carcinogenesis in rats and this seems to be linked to the presence of essential fatty acid particularly linoleic acid at 4% (weight/weight) or more (5-7). However, the chemoprotective effect of dietary constituents has recently received a great attention in cancer prevention (8,9). Experimental studies in rats with various edible oils as sources of dietary fat, such as extra Virgin (not purified) olive oil (VOO) has been shown to induce longer tumor free time, fewer tumors per rat, and lower tumor incidence using 7,12-dimethyl benzanthracene (DMBA) as a carcinogen (1,10). Analysis of fatty acid composition of VOO showed that oleic acid is present in highest amount (73.2%) followed by palmitic acid (13.7%), linoleic acid (8.6%) and other fatty acids in trace quantities (11). The superiority of VOO in preventing experimental tumor is possibly due to the presence of large quantities of naturally occurring antioxidants such as vitamin E (12).

Inhibition of malignancies using pure vitamin E (tocopherol) as an antioxidant has been studied in several tissues (13,14), but not in salivary glands and also not via natural dietary intake of vitamin E, especially since VOO is commonly used for cooking purposes in middle eastern countries. The purpose of this study is to : (a) investigate the effects of VOO supplemented diet on chemically induced squamous cell carcinoma in sub-maxillary salivary glands of Wistar rats and compare that with other groups

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of rats fed with standard chow diet and (b) compare the effect of VOO supplemented diet feeding before or after carcinogenesis induction.

MATERIAL AND METHODS

Thirty male Wistar rats of 10-12 weeks age and weighing approximately 100 gms each, were randomly divided into three equal groups designated A, B and C.

The DMBA implantation was made into left and right submaxillary salivary glands by placing 5 mg pellets via an asceptic surgical incision. After exposing the glands the DMBA pellet was placed through a cannula, the skin was then repositioned and closed with silk sutures. Among the animals, Group A served as control in which DMBA was implanted at the beginning and was fed with standard chow diet for the entire length of the experiment of 8 weeks. Group B was prefed a modified diet containing 30% VOO for 3 weeks followed by DMBA implantation and continued on the diet until the termination of the experiment, and Group C was postfed the same diet containing 30% VOO at 4 weeks after DMBA implantation and continued on the diet for the remaining period of 8 weeks of the experiment.

At 4 weeks and 8 weeks after DMBA implantation, the submaxillary salivary glands in all animals of Group A, B and C were surgically exposed, examined and then removed under anesthesia with diethyl-ether. The glands were fixed in 10% formalin, serially sectioned in paraffin and stained with hematoxylin and eosin. The stained sections were then examined under the light microscope. The following criteria were examined and compared among all groups including : inflammation, fibrosis, cystic degeneration, ductal cell proliferation and squamous metaplasia. Tumorigenic changes in the glandular zones were evaluated as described previously by Mohammad *et. al.* (15).

Histological changes were statistically analyzed by Wilcoxon rank sum test and Wilcoxon signed rank test (16).

RESULTS

The results of microscopic observation on histological changes in salivary glands of various animal groups at 4 weeks and 8 weeks of experimental treatments are shown in Table 1. The statistical significance of these results evaluated by Wilcoxon rank sum test and Wilcoxon signed rank test are also shown in Table 1.

Salivary glands tissue from animals of Group A showed the expected pattern of DMBA induced carcinogenesis. All of those removed at the 4th week showed

Table 1: The average histological changes (i.e. inflammation, fibrosis, cystic degeneration, ductal cell proliferation and squamous metaplasia and tumorigenic changes) observed in various animal groups at 4th week and 8th week of experimental treatment.

Animal groups and salivary glands zones*	Average histological changes observed at*											
	4th Week						8th Week					
	1	2	3	4	5	6	1	2	3	4	5	6
Group A												
CZ	+	c	c-	d+++	c+++	c++	d-	c-	++	d+++	c+++	c+++
IZ	+	d+	-	d+++	d++g	d++g	d-	c-	d++	d+++	c+++	c+++
PZ	+	-	-	d+++	d++g	d++g	d-	d-	d++	d+++	c+++	c+++
AZ	+	-	-	d+++	d++g	+g	d-	-	c++	d+++	c+++	c+++
Group B												
CZ	a+++	a+++	a++	a-	a+++	a-	a+++	a+++	a+	a+	a-	a-
IZ	b++g	b+++	-	a+	b+	a-	a+++	a+++	a-	a+	b+	a-
PZ	b++g	a++g	-	a+	a-	a-	a+++	a+++	a-	a-	a-	a-
AZ	b++g	-g	-	a+	a-	a-	b++	a++	a-	a-	a-	a-
Group C												
CZ	e+	f++	f+++	e++	+	+	f++	f++b	e+++	f++	e++	e++
IZ	f+	f++	+g	f++	+	+	f++	f++b	e++	++	++	e++
PZ	f+	f+g	+g	f++	+	-	f++	f++b	+	f++	+	+
AZ	-	-g	f-g	++	+	-	+	e-	-	e++	+	-

*CZ : central zone, IZ : intermediate zone, PZ : peripheral zone, AZ : adjacent zone, 1 : inflammation, 2 : fibrosis, 3 : cystic degeneration, 4 : duct cell proliferation, 5 : squamous metaplasia, 6 : tumorigenesis, - : no change, + : mild change, ++ : moderate change, +++ : severe change, a : $p<0.01$ (significantly different from group A, b : $p<0.05$ (significantly different from group A, c: $p<0.01$ (significantly different from group C), d : $p<0.05$ (significantly different from group C), e : $p<0.01$ (significantly different from group B), f : $p<0.05$ (significantly different from group B), g : $p<0.05$ (significantly different from the respective CZ).

extensive squamous metaplasia and keratinization in the central area of the implant site. A pronounced degree of malignant change and necrosis was evident in some metaplastic islands and some indication of infiltration of adjacent glandular tissue. Glandular tissue harvested at the 8th week showed marked metaplasia and carcinogenesis with more extensive areas of infiltration of the surrounding tissue.

Salivary glands removed from rats in Group B 4 weeks after the DMBA implant showed remarkable signs of repair in the central area of the implant site. Fibrosis and regeneration of the salivary gland tissue surrounded by lymphocytosis was present. Only in a few serial sections did the regenerating glandular tissue show signs of squamous metaplasia and some areas of necrosis in the implant sited. Extensive hemorrhage was present in glandular tissue adjacent to the implant site. At 8 weeks after the DMBA implant lymphocytosis was present in the central zone of the implant site which was separated from the normal glandular wall by an extensive fibrotic wall. Overall the reaction site appeared to be decreasing in size and the changes in the adjacent glandular tissue were less pronounced. The metaplastic changes were much less in the glands harvested at the 8th week than removed at the 4th week after the DMBA.

Glands taken from rats in Group C 4 weeks after DMBA implantation showed similar reactions to those in Group B but to a lesser degree. The glandular tissue exhibited a moderated amount of squamous metaplasia and keratinization in the center of the implant area and there was little evidence of metaplastic and carcinomatous change. Glands removed 8 weeks later showed increased lymphocytes when compared with those harvested in the 4th week but less than glands taken from animals in Group B, 8 weeks after implantation. Metaplastic and carcinomatous changes were more evident than that seen in the specimens of Group B.

DISCUSSION

Previous studies have shown that the VOO-based diet was able to provide some protections against DMBA-induced mammary carcinogenesis in rats (1,10). The present study also showed that VOO-based diet reduced tumor producing ability of DMBA in rats salivary glands; however prefed rats (Group B) were better protected than postfed rats (Group C). The obvious question that arises is how VOO functions as a chemoprotective agents against DMBA-induced tumorigenesis. A recent hypothesis is that various chemical initiators and promoters of carcinogenesis act via the generation of activated forms of oxygen and associated lipid peroxidation (17-19). It will then be reasonable to assume that antioxidants may provide protection against carcinogenesis. This has been proven to be true as it is known that antioxidants suppress lipid peroxidation and that they stop the action of promoters of carcinogenesis (20,21). Therefore, a probable mechanism for the chemoprotective role of VOO might be that it contains chemoprotective substances which inhibit the process of carcinogenesis. VOO is known to contain sufficient quantity of vitamin E (12), a proven efficient biological antioxidant (22,23) and thought to influence efficiently drug detoxification in rats (24). Another possibility is that VOO may help to enhance cell-mediated immunity (CMI) which is an important factor in determining the outcome of exposure to a chemical carcinogen (25,26). This function of CMI-enhancement by VOO may also be mediated through vitamin E, which has been reported to exert a positive role in enhancing the immune response mechanisms (27,28). A specific protective immune response occurs due to highly complex membrane interaction among macrophages (antigen presenting cells), T-lymphocytes and B lymphocytes involving Ia-antigen producing T-effector cells and antibodies (29). The amount of linoleic acid in the diet is known to influence the composition and fluidity of tumor cell membranes (30,31) and host immune cell membranes which may affect the function of these cells (32,33). Thus, it seems that the linoleic acid content and the appropriate ratio of saturated fatty acids to unsaturated fatty acids, particularly palmitic acid: oleic acid : linoleic acid, may be responsible for the chemoprotective effect observed with VOO in salivary glands carcinogenesis as well as in other studies with mammary carcinogenesis (10,33-35). Moreover, vitamin E has been shown to play an important role in stabilizing cell membrane fluidity (22,36). The finding of Laseken *et. al.* (10) that protection by VOO against DMBA-induced mammary carcinogenesis in rats fails if the dietary level of linoleic acid reaches $\geq$34% (wt/wt) can therefore be explained as possibly due to overloading of the biological antioxidant system and effects on cell membrane fluidity. This clearly indicates that a com-

plex interrelationship among the antioxidant, dietary linoleic acid and carcinogen exists and carcinogenesis may be due to imbalance of it.

However, to attain 34% linoleic acid level in human diet, one needs to use about 37-40% of VOO which usually may not occur. Therefore, it is difficult to extrapolate the observations of Laseken et. al. made in rats to human. This suggested that further investigations are warranted about the exact mechanism of the above mentioned complex interaction among anti oxidant, carcinogen and lipids. Although the precise mechanism of interaction remains uncertain, this study has shown for the first time that VOO exert a chemoprotective effect on chemically induced squamous cell carcinoma in rats salivary glands and prefeeding seems to have a better prophylactic effect compared to post feeding the diet before and after DMBA implantation respectively.

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