THE EFFECT OF GARLIC OIL (Allium Sativum) ON DMBA INDUCED SALIVARY GLAND TUMORIGENESIS IN RAT

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SUMMARY: The chemoprotective effect of garlic oil on 7,12-dimethylbenzanthracene (DMBA) induced carcinogenesis in sub-maxillary salivary glands of male wistar rats was studied. Animals were equally divided into five experimental groups: Group A: Given garlic oil intra peritoneally daily for three weeks prior to DMBA implantation. Group B: Given garlic oil intra peritoneally daily for four weeks after DMBA implantation. Group C: Given DMBA only. Group D: Given daily dose of garlic oil only. Group E: Normal control receiving chow diet only. The histological parameters in sub-maxillary salivary glands and serum beta-carotene levels were analyzed at 4, 8 and 12 weeks after DMBA implantation. The histological studies revealed that there was delayed onset and decreased severity of carcinogenesis in Group A, compared to Group B or Group C which showed early onset and extensive carcinogenesis. The serum beta-carotene levels in Group A and B were significantly higher compared to Groups C and E at all levels. Interestingly, Group D had the highest serum beta-carotene level, and Group A and Group B had significantly lower serum beta-carotene compared to Group D. The Group A animals had even significantly lower serum beta-carotene levels compared to Group B.

These results indicate that garlic oil may have an adjuvant effect on various defense mechanisms of the host against DMBA-induced carcinogenesis in sub-maxillary salivary glands of rat through increased availability and utilization of beta-carotene. There is evidence that beta-carotene can indeed function as a non-conventional anti-oxidant at low oxygen pressures and thus render protection against cancer by preventing lipid peroxidation in vivo.

Key Words: Garlic oil, tumorigenesis, salivary glands, DMBA.

INTRODUCTION

The importance of garlic (Allium sativum) was recognized many centuries ago in early Chinese, Egyptian and Indian civilizations as a herbal or traditional medicinal agent. Garlic is still employed in folk medicine today in many parts of the world, both for prophylaxis and for the cure of a variety of diseases including acute and chronic infections, gastritis, dysentery, typhoid fever, cholera, tuberculosis, pneumonia, diabetes-mellitus, heart disease and hypertension (1-3). Prevention of cardiovascular disorders and retardation of hyperglycemia by, and antiseptic activities of garlic have been well documented (4-8). One of the most important biological effects observed recently with garlic is prevention of cancer which is reported in various experimental systems with mouse and rat (9-12). Furthermore, there is a report from China concerning the preventive effect of garlic on human gastric cancer (13). Since garlic is widely consumed with the diet, it appears to be a promising source of chemoprotective agents against cancer and thus, further studies are warranted in this field. Literature survey of reported studies has indicated that garlic exhibits inhibits carcinogenesis in many tissues, e.g. skin, colon, lung, liver fore-stomach and esophagus (9-13). However literature survey revealed that no study had been reported on the chemoprotective effects of garlic oil on

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cancer of salivary glands. Therefore, the present study was initiated to investigate the chemoprotective effect of garlic oil on DMBA-induced sub-maxillary salivary glands carcinogenesis in rats. Serum beta-carotene levels and histopathological parameters in sub-maxillary salivary glands were followed as markers for protection against carcinogenesis.

MATERIALS AND METHODS

Sixty male Wistar rats of 10-12 weeks age and weighing approximately 100 gms each were randomly divided into five equal groups designated as Group A, Group B, Group C, Group D and Group E. Groups A and B were the experimental groups while the control groups were included Group C, Group D and Group E.

Group A : The garlic oil was administered intra peritoneally daily (50 mg/kg body weight) for three weeks prior to DMBA (5 mg pellet) implantation into sub-maxillary salivary glands as previously described (14,15) and continued receiving garlic oil for 3 months.

Group B : Administrated garlic oil intra peritoneally daily (50 mg/kg body weight) for 4 weeks after receiving DMBA, and continued on garlic oil for 3 months.

Group C : Received only DMBA (5 mg pellet) implanted into the sub-maxillary salivary glands as previously described (14,15).

Group D : Received daily dose of garlic oil (50 mg/kg body weight) only for 3 months.

Group E : Normal controls receiving only standard chow diet for the entire length of the experiment.

All animals were given standard chow diet and drinking water *ad libitum*.

At 4 weeks, 8 weeks and 12 weeks after DMBA implantation, 4 animals from each group were sacrificed respectively. Blood was collected in tubes without anticoagulant and the separated serum was kept frozen at -30°C until analyzed for the beta-carotene levels. The serum beta-carotene level, a marker for protection against carcinogenesis, was estimated in all sera in duplicate adopting the spectrophotometric method of Neeld and Pearson (16). The sub-maxillary salivary glands from all animal groups were removed and evaluated for carcinogenesis histologically under light microscopy as described previously (14,15).

The results were evaluated statistically by student's t-test and Wilcoxon Rank Sum Test (17).

RESULTS

The results of histological studies of sub-maxillary salivary glands from Groups A, B and C showed significant variations in onset and severity of carcinogenesis. Salivary glands removed at 4 weeks from Group C animals showed early onset and 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. Salivary glands harvested at the 8th and 12th weeks showed marked metaplasia and carcinogenesis with more extensive areas of infiltration of the surrounding tissue. Salivary glands removed from Group A (pre-fed) and Group B (post-fed) animals at 4 weeks of DMBA implantation showed signs of repair in the central area of the implant site. Fibrosis and regeneration of the salivary gland tissue surrounded by lymphocytes was present. The signs of repair and regeneration of the salivary gland tissue was more evident in glands from Group A and Group B harvested at 8 weeks and 12 weeks of DMBA implantation. However, pre-feeding with garlic oil before DMBA-implantation (Group A) seemed to be superior than post-feeding (Group B) in protecting against the malignant changes induced by DMBA implantation.

The results of serum beta-carotene analysis (Table 1) showed that Group A initially had a higher serum level of beta-carotene compared to group B at 4 weeks. At 8 weeks and 12 weeks, Group A showed statistically significant lower serum levels of beta-carotene compared to Group B suggesting better utilization of beta-carotene in pre-fed (Group A) than post-fed (Group B) animals. The comparison of experimental groups (A and B) with control groups (C and E) revealed significantly higher levels of serum beta-carotene in both Group A and Group B at all levels. Interestingly, Group D which had received garlic oil only had the highest serum beta-carotene level compared to all the other animal groups.

DISCUSSION

Although an extensive literature exists concerning medicinal uses of garlic since ancient times, the tumor-inhibitory effects of garlic have been reported only recently in various experimental models including human (9-13). The results of our present study (Table 1) demonstrated for the first time that garlic oil exerts chemoprotective effect against DMBA-induced sub-maxillary salivary glands carcinogenesis in rats. However, the mechanisms by which the chemoprotective effect of garlic oil is achieved are not clear although many hypothesis are proposed.

Fujiwara and Natata reported that mice injected with ascites tumor cells that were pretreated with an extract of garlic developed strong immunity against the

Table 1: Serum beta-carotene levels (μg/L) in different animal groups at 4, 8 and 12 weeks of DMBA implantation and their statistical comparison by student's t-test.

ANIMAL GROUP	Serum β-carotene level (μg/L) after DMBA implantation		
	4 weeks*	8 weeks*	12 weeks*
Group A (Pro-fed)	48 ± 12	80 ± 18	120 ± 24
Group B (Post-fed)	27 ± 10	124 ± 12	156 ± 25
Group C (DMBA only)	28 ± 12	25 ± 10	24 ± 11
Group D (Garlic oil only)	420 ± 36	432 ± 25	451 ± 30
Group E (Normal control)	26 ± 10	36 ± 12	35 ± 11
Student's t-test:**			
Group A vs Group B ('P' value):	<0.05	<0.02	<0.05
Group A vs Group C ('P' value):	<0.05	<0.01	<0.001
Group A vs Group D ('P' value):	<0.001	<0.01	<0.001
Group A vs Group E ('P' value):	<0.02	<0.01	<0.001
Group B vs Group C ('P' value):	>0.5	<0.001	<0.001
Group B vs Group D ('P' value):	<0.001	<0.001	<0.01
Group B vs Group E ('P' value):	>0.5	<0.01	<0.001
Group C vs Group D ('P' value):	<0.001	<0.001	<0.001
Group C vs Group E ('P' value):	>0.5	>0.5	>0.5
Group D vs Group E ('P' value):	<0.001	<0.001	<0.001

* Each value is the Mean ± SD of duplicate observation on four serum sepicimens.

** 'P' value >0.05: Not significant; 'P' value 0.05; Significant; Degree of freedom: 8+8-2=14.

same type of tumor cells (18). As it is known that garlic contains many organosulphur compounds which react with sulphydryl groups of tumor cell proteins (19,20), they suggested that the tumor cells are probably attenuated by the similar action of garlic extract. Working with a transitional cell carcinoma (MBT-2), Lau *et. al.* showed that garlic extract exhibited significant effectiveness in inhibiting tumor growth in mice through elicitation of activated macrophages and lymphocytes (21). It is, therefore, not improbable that garlic extract played a dual role, inactivating sulphydryl compounds of tumor cells (18) and cytotoxic destruction of tumor cells by activated macrophages and lymphocytes leading to inhibition of tumor growth (21). Induction of increased glutathione-S-transferase activity, an enzyme which assists in the detoxification of many chemical carcinogens, by organosulphide compounds from garlic was implicated as a probable mechanism for chemoprotective effect of garlic against benzopyrene (BP)-induced carcinogenesis in mice (12). Further, garlic extract was reported to increase the life span of tumor-bearing animals that were treated with cyclophosphamide (22). Perhaps one of the most important activity of garlic extract is in the reduction of free radicals, as Uni Krishnan et. al. showed significant reduction in the cyclophosphamide-induced lipid peroxidation in mice liver by garlic extract leading to protection against chemotoxicity (22). This chemoprotective action of garlic extract against cyclophosphamide-induced chemotoxicity can therefore be attributed to its free radical scavenging role as antioxidant.

The results of our present study indicated that the antioxidant function of garlic oil is most likely performed through increased availability of beta-carotene. There is ample evidence that beta-carotene is a very effective quencher of singlet oxygen (23,24). Burton and Ingold reported further evidence that beta-carotene can indeed function as a non-conventional antioxidant at low oxygen pressures and plays an important role in protecting lipid tissue from peroxidation *in vivo* (25). The chain breaking action of beta-carotene complements that of vitamin E, since beta-carotene is effective at low oxygen pressure and vitamin E is effective at high oxygen concentrations (25). Human cancer risks are reported to be inversely correlated with blood retinol and dietary beta-carotene (26,27). Our results with Group A, Group B and Group D animals (Table 1) indicated that probably beta-carotene which is absorbed unchanged from the intestine is chiefly responsible for the chemoprotective effect of garlic oil. The reduced levels of serum beta-carotene observed in Group A and Group B animals were suggestive of increased utilization of it in breaking peroxidation chain reaction and conversion to vitamin A also to some extent. The utilization of beta-carotene and chemoprotective effect of garlic oil was more pronounced in Group A (pre-fed) as compared to Group B (post-fed) animals. This seemed to indicate that garlic oil exerts its tumor-inhibitory effect by acting at the initial stage of the cancer promoting processes and this view was supported by the reported studies of Nishino *et al.* (9). It is known that various initiators and promoters of carcinogenesis act via generation of activated forms of oxygen and associated lipid peroxidation and reported protec-

tion against carcinogenesis by antioxidants supports this view (28-30).

In conclusion, our results presented here provide a rational for the anti-tumor activity of garlic oil against DMBA-induced sub-maxillary salivary glands carcinogenesis in rats. The garlic oil seems to exert adjuvant effect on various defense mechanisms of the host against chemically-induced carcinogenesis through increased availability and utilization of beta-carotene. However, other events may be involved which warrants further investigation to elucidate them.

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