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Lack of inhibitory effect of lycopene on dysplastic lesions induced by 7,12-dimethyl-benz[*a*]anthracene in hamster buccal pouch

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Abstract

It is widely known that oxidative damage caused by free radical imbalance in tissues plays a major role in the carcinogenesis processes of initiation and promotion. Natural antioxidants present in the diet are useful protectors against lipid, protein, and DNA oxidative damage. In this study, we evaluated the effects of lycopene, a red pigment largely found in tomatoes, on dysplastic lesions induced by 7,12-dimethyl-benz[*a*]anthracene (DMBA) in hamster buccal pouch mucosa. The extent of lipid peroxidation and liver reduced glutathione concentrations were used to assess overall oxidative levels. Thirty animals received topical application of a 0.5% solution of DMBA in mineral oil in the left buccal pouches, 3 times per week for 10 weeks. Ten animals were euthanized 1 day after the last DMBA application and the remaining 20 were divided into 2 groups, which received a standard AIN-93M diet or this diet supplemented with 20 ppm lycopene for the next 11 weeks. Ten animals remained untreated throughout the entire experiment as negative controls. Levels of thiobarbituric acid–reactive substances were significantly lower in the group that received DMBA and lycopene as compared to the group treated with DMBA but did not significantly alter liver levels of glutathione. Incidences of carcinoma were 80% and 70% ($P > .05$), respectively, in groups B (DMBA only) and C (DMBA and lycopene). The present data suggest that the antioxidant activity of lycopene may not be sufficient to suppress tumor formation in a postinitiation phase.

Keywords: Lycopene; Experimental oral carcinogenesis; Hamster; 7,12-dimethyl-benz[*a*]anthracene; Oral dysplasia

1. Introduction

Worldwide, oropharyngeal cancer ranks sixth in the list of the most frequent cancers and, although its incidence in Europe and the United States is relatively low (around 5%), some developing countries may have incidences as high as 25% to 30% [1]. Among other factors, late diagnosis of the disease has an important role in treatment outcomes, which have not improved significantly in the last decades. Moreover, the surgical management of oral cancer often results in face deformation and oral cavity dysfunction [2]. Increasing attention has been given to the potential protective roles of specific antioxidant nutrients found in fruits and vegetables [3,4]. Antioxidant nutrients may help to prevent cancer and other chronic diseases by protecting lipids, proteins, and DNA against damage by reactive oxygen species, which are continuously generated in cells either through endogenous or exogenous sources [5].

Lycopene is the predominant carotenoid found in tomatoes and tomato products, and its antioxidant capacity is the highest among all dietary carotenoids. The biological

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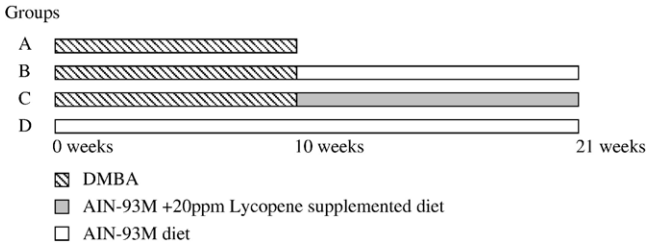

Fig. 1. Experimental design. Ten hamsters were assigned to each group. 7,12-Dimethyl-benz[*a*]anthracene at 5% in mineral oil was painted 3 times per week on the animals' cheek pouches. Group A was euthanized at 10 weeks and the remaining groups at 21 weeks (group D, no DMBA).

function of lycopene has been attributed in part to their ability to scavenge free radicals and physically quench singlet molecular oxygen [6]. Nonoxidative effects of lycopene, such as the ability to improve cell to cell communication, to enhance cell-cycle regulation, and to induce detoxification enzymes, have also been reported [7-10]. Epidemiological observations on normal and at-risk populations have demonstrated an inverse association between lycopene and tomato-derived products and the risk of various types of cancer, including oral cancer [11,12]. There is good evidence for the chemopreventive activity of lycopene in rodent models, in which it has been shown to inhibit mammary tumor formation [13], liver preneoplasia [14], lung neoplasia [15], and buccal pouch squamous cell carcinomas in hamsters [16]. In a recent study, the intake of daily lycopene capsules effectively improved oral leukoplakia lesions in a group of patients [17].

Based on the knowledge that cancer is a process that depends on cumulative action of multiple events and that reactive oxygen species can stimulate cancer development in all 3 stages (initiation, promotion, and progression) [18], we conducted a study to investigate the effects of lycopene on DMBA-induced mild dysplasia in hamster buccal pouch. We also evaluated the levels of lipid peroxidation and reduced glutathione (GSH), a nonenzymatic endogenous antioxidant, as biomarkers of oxidative stress and antioxidant defense. To our knowledge, this is the first attempt to verify the effect of lycopene in previously induced mild dysplasia and how this natural dietetic antioxidant modulates plasma levels of GSH and lipid peroxidation subproducts. The results of this study should contribute for a better understanding of the chemopreventing properties of lycopene and whether its beneficial effects are present in precancerous lesions.

2. Methods and materials

The experiment was carried out in 40 male 12-week-old Syrian hamsters, obtained from the Animal House of the Faculty of Medicine of Ribeirao Preto, University of Sao Paulo (Sao Paulo, Brazil). The study was previously approved by the ethics committee on animal experimentation of the faculty. The animals were maintained in a room with controlled temperature and a 12-hour light/dark cycle. Food and water were offered ad libitum.

Fig. 1 illustrates the experimental protocol. Thirty animals had their left buccal pouches painted with 0.5% solution of 7,12-dimethyl-benz[*a*]anthracene (DMBA, Sigma Chemical, St Louis, MO) in mineral oil using a paintbrush, 3 times per week for 10 weeks. Approximately 5 μL of DMBA was used in each application. During this period, all animals were given AIN-93M [19], a purified rodent diet (Table 1). The animals were then divided into 3 groups of 10 each. One group was euthanized 1 day after the last DMBA application (group A) and used as a control for the initial DMBA-induced changes.

2.1. Treatment group (B)

Ten animals (group B) continued in the experiment for another 11 weeks with access to the same diet.

2.2. Control group (C)

Group C continued in the experiment for the same period and received a 20-ppm lycopene (Roche Vitamins, Basel, Switzerland)–supplemented AIN-93M diet.

Ten hamsters remained untreated for the entire experiment and were used as a negative control (group D). The experiment was terminated at week 21.

All animals were anesthetized with sodium pentobarbital (40 mg/kg) and were euthanized during blood collection by cardiac puncture. Plasma was separated by centrifugation at 4°C and frozen until analysis. The cheek pouches were excised, rinsed with phosphate-buffered saline (PBS) solution twice, flattened on expanded polystyrene board, and fixed in 10% PBS-buffered formalin for 24 hours. A liver lobe was dissected out, weighed, and cooled to liquid nitrogen temperature.

2.3. Biochemical estimations

Plasma lipid peroxidation was determined by high-performance liquid chromatography according to the method based on the formation of thiobarbituric acid–reactive substances (TBARS) [20]. One milliliter of plasma was mixed with 2.0 mL of TCA-TBA-HCl (15% wt/vol trichloroacetic acid, 0.375% wt/vol thiobarbituric acid, and

Table 1
List of ingredients contained in the AIN-93M diet

	g/kg diet
Cornstarch	465.692
Casein (>85% protein)	140
Cornstarch	155
Sucrose	100
Soybean oil	40
Fiber	50
Mineral mix (AIN-93G-MX)	35
Vitamin mix (AIN-93-VX)	10
L-Cystine	1.8
Choline bitartrate (41.1% choline)	2.5