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Research Article

Anti-lipid peroxidative potential of glycyrrhetinic acid in 7,12-dimethylbenz(a)anthracene induced hamster buccal pouch carcinogenesis

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ABSTRACT

Present study investigated the effect of glycyrrhetinic acid on lipid peroxidation and antioxidants status in 7,12-dimethylbenz(a)anthracene (DMBA) induced hamster buccal pouch carcinogenesis. Topical application of DMBA for 14 weeks in the buccal pouch of hamsters resulted in well-developed squamous cell carcinoma. The status of lipid peroxidation and antioxidants were measured in the plasma and buccal mucosa of hamsters treated with DMBA alone and DMBA + glycyrrhetinic acid treated hamsters. Altered levels of lipid peroxidation by-products [Thiobarbituric acid reactive substances (TBARS)] and disturbances in antioxidants status [vitamin E, reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)] were noticed in hamsters treated with DMBA alone. Oral administration of glycyrrhetinic acid at a dose of 45 mg/kg b.w restored the status of lipid peroxidation and antioxidants in hamsters treated with DMBA. Present study thus suggests that glycyrrhetinic acid improved the status of lipid peroxidation and antioxidant defense mechanism during DMBA induced hamster buccal pouch carcinogenesis.

Keywords: oral cancer; lipid peroxidation; antioxidants; hamsters; DMBA.

INTRODUCTION

Oxidative stress occurs in the cell when there is an imbalance in the status of oxidant and antioxidant. Oxidative stress, due to overproduction of reactive oxygen species (ROS) or insufficient antioxidant potential has been implicated in the pathogenesis of several pathological conditions including oral cancer (Ishii, 2007). Previous studies from our laboratory have reported altered status of lipid peroxidation and antioxidants in human and experimental oral cancer (Manoharan, et al., 2005; Kavitha & Manoharan, 2006).

18ß- glycyrrhetinic acid, a pentacyclic triterpenoid derivative and the active aglycone of glycyrrzin, has diverse pharmacological effects including anti-ulcer, anti-inflammatory, antiviral, hepatoprotective, antioxidant and anti-tussive properties (Doll, et al., 1962; Finney, et al., 1959). 18ß-glycyrrhetinic acid inhibited the growth and differentiation of mouse melanoma cells *in vitro* (Nishino, et al., 1986).

Recently we reported the antigenotoxic potential of glycyrrhetinic acid in DMBA induced genotoxicity (Kowsalya, et al., 2011). We also observed that glycyrrhetinic acid completely prevented the tumor formation during DMBA induced hamster buccal pouch carcinogenesis (unpublished data). However, there were no scientific reports on the anti-lipid peroxidative potential of glycyrrhetinic acid in DMBA-induced hamster buccal pouch carcinogenesis. The present study was therefore designed to focus the anti-lipid peroxidative effect of glycyrrhetinic acid in DMBA induced hamster buccal pouch carcinogenesis.

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MATERIALS AND METHODS

Chemicals

The carcinogen, DMBA and glycyrrhetinic acid was obtained from Sigma-Aldrich Chemical Pvt Ltd, Bangalore India. All other chemicals used were of analytical grade.

Animals

Male golden Syrian hamsters 8-10 weeks old weighing 80-120 g were purchased from National Institute of Nutrition, Hyderabad, India and were maintained in Central Animal House, Rajah Muthaiah Medical College and Hospital, Annamalai University. The animals were housed five in a polypropylene cage and provided standard pellet diet and water *ad libitum*. The animals were maintained under controlled conditions of temperature and humidity with a 12 h light/dark cycle. The local institutional animal ethics committee (Register number 160/1999/ CPCSEA), Annamalai University, Annamalai Nagar, India, approved the experimental design. The animals were maintained as per the prin-

ciples and guidelines of the ethical committee for animal care of Annamalai University in accordance with Indian National Law on animal care and use.

EXPERIMENTAL DESIGN

A total number of 40 hamsters were randomized into four groups and each group contained 10 hamsters. Group I animals served as the control and were treated with liquid paraffin (vehicle) alone three times a week for 14 weeks on their left buccal pouches. Group II animals were treated with 0.5% DMBA in liquid paraffin three times a week for 14 weeks on their left buccal pouches. Group II animals received no other treatment. Group III animals were treated with DMBA as in group II, received in addition oral administration of glycyrrhetinic acid (45 mg/kg body weight/day), starting 1 week before exposure to the carcinogen and continued on alternate days to DMBA painting until the animals were sacrificed. Group IV animals received oral administration of glycyrrhetinic acid (45 mg/kg body weight/day) alone, as in group III, throughout the experimental period. The experiment was terminated at the end of 16th week and all animals were sacrificed by cervical dislocation.

Biochemical estimations

Biochemical estimations were carried out in the plasma and buccal mucosa of control and experimental animals in each group. Lipid peroxidation was estimated as evidenced by the formation of thiobarbituric acid reactive substances (TBARS). TBARS in plasma were assayed by the method of Yagi (1987). TBARS in buccal mucosa was estimated by the method of Ohkawa *et al* (1979). Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities were determined in plasma and buccal mucosa by the methods of Kakkar *et al* (1984), Sinha (1972) and Rotruck *et al* (1973) respectively. The reduced glutathione (GSH) level in the plasma and buccal mucosa was determined by the method of Beutler and Kelley (1963). The vitamin E level in the plasma and buccal mucosa was determined by the method of Desai (1984) and Palan *et al* (1991) respectively.

Statistical analysis

The data are expressed as mean ± standard deviation (SD). Statistical comparisons were performed by One way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT). The results were considered statistically significant if the p-values were less than 0.05.

RESULTS

The status of TBARS and antioxidants [vitamin E, GSH, SOD, CAT, GPx] in the plasma of control and experimental hamsters in each group are shown in figure 1. The concentration of TBARS was increased, where as the status of antioxidants was significantly decreased in tumor-bearing animals, as compared to control animals. Oral administration of glycyrrhetinic acid to DMBA-painted animals significantly reverted the status to normal concentrations of TBARS and antioxidants. Hamsters treated with glycyrrhetinic acid alone showed no significant difference in TBARS and antioxidants status, compared to control animals.

The status of TBARS and antioxidants [vitamin E, GSH, SOD, CAT, GPx] in the buccal mucosa of control and experimental animals in each group are shown in figure 2. Decrease in TBARS level and disturbances in antioxidant status (vitamin E, GSH and GPx were increased; SOD and CAT were decreased) were noticed in oral cancer animals, compared to control animals. Oral administration of glycyrrhetinic acid to DMBA-painted animals reverted the concentration of TBARS and antioxidants to near normal range. Hamsters treated with glycyrrhetinic acid alone showed no significant difference in TBARS and antioxidants status as compared to control animals.

DISCUSSION

ROS can cause damage to proteins, lipids and nucleic acids, if they are excessively generated in the cell. However, cells have sophisticated defense mechanism to combat the deleterious effects of ROS (Suresh, et al., 2006). DMBA, a potent organ site and organ specific carcinogen, mediates carcinogenesis by inducing chronic inflammation, over production of ROS and inactivating the activities of antioxidants. Profound studies have documented over production of ROS and impaired antioxidant defense mechanism during DMBA induced experimental carcinogenesis (Senthil et al., 2007; Baskaran et al., 2010; Manoharan et al., 2010).

In the present study, elevated lipid peroxidation and decline in enzymatic [SOD, CAT, GPx] and non–enzymatic antioxidants [vitamin E, reduced glutathione (GSH)] were noticed in hamsters treated with DMBA alone. Measurement of plasma TBARS in pathological conditions could help to assess the extent of tissue damage. Increased levels of plasma TBARS could therefore be due to over production of ROS in the cancerous conditions with subsequent leakage into plasma in hamsters treated with DMBA. Vitamin E and reduced glutathione are regarded as potent scavenger of ROS as well as inhibitors of tumor formation in experimental carcinogenesis (Manoharan, et al., 2009). Lowered levels of these antioxidants could be due to utilization by tumor tissues to meet their nutrient demand or to scavenge excessively generated ROS in the system (Miyata et al., 2001). SOD, CAT and GPx play crucial role in the elimination of a wide variety of ROS and form the first line of defense against deleterious effects of ROS. Lowered activities of these enzymatic antioxidants in plasma were reported in several cancers including oral cancer (Manoharan, et al., 2009; Pugalendhi & Manoharan, 2010). Our results are in line with these findings.

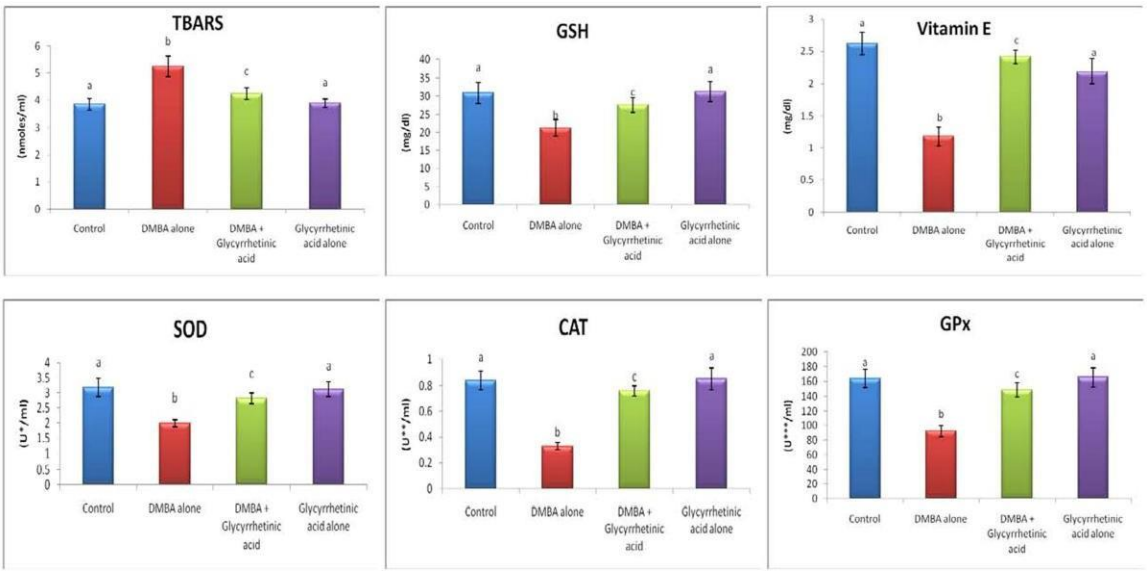

Figure 1: Status of plasma TBARS and antioxidants in control and experimental animals in each group (n=10)

Values are given as mean ± SD. Values not sharing a common superscript letter differ significantly at P<0.05 (DMRT). * The amount of enzyme required to inhibit 50% nitroblue-tetrazolium (NBT) reduction. ** Micromoles of hydrogen peroxide utilized/s. *** Micromoles of glutathione utilized/min.

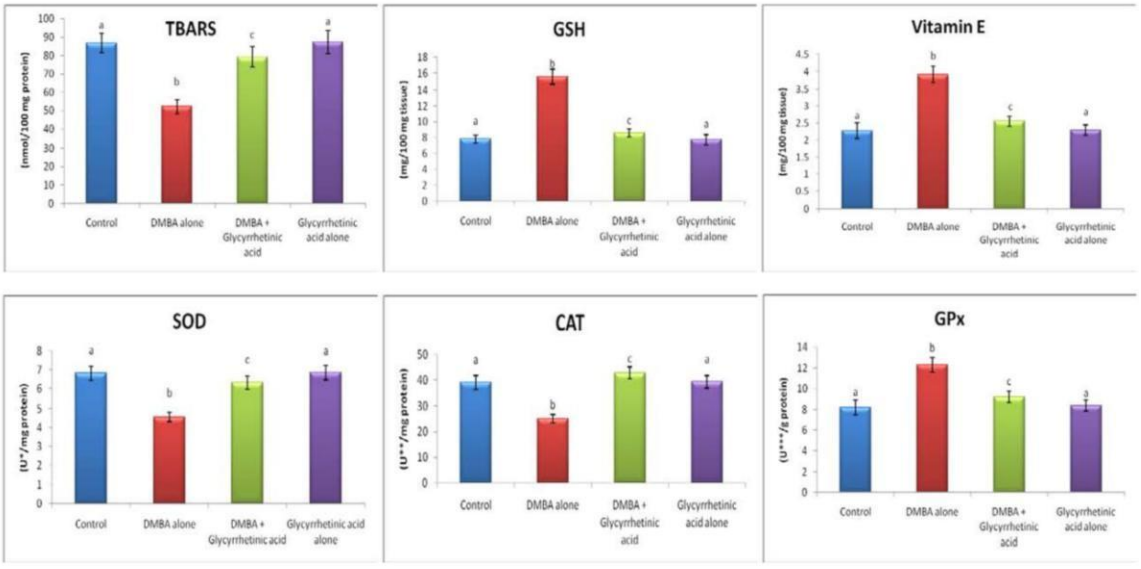

Figure 2: Status of Buccal mucosa TBARS and antioxidant status in control and experimental animals in each group (n=10)

Values are given as mean ± SD. Values not sharing a common superscript letter differ significantly at P<0.05 (DMRT). * The amount of enzyme required to inhibit 50% nitroblue-tetrazolium (NBT) reduction. ** Micromoles of hydrogen peroxide utilized/s. *** Micromoles of glutathione utilized/min.

Tumor tissues and their adjacent normal tissues showed different biochemical and molecular phenomena. Lowered TBARS levels were reported in the tumor tissues of human and experimental cancers (Kolanjiappan, et al., 2003: Rasheed, et al., 2007). Lowered levels of tumor tissue TBARS are probably due to decreased PUFA content or increased rate of cell proliferation occurring during carcinogenesis (Kolanjiappan, et al., 2003). Glutathione peroxidase and reduced glutathione play crucial role in the regulation of cell proliferation. Decreases in SOD and CAT activities were reported in the tumor tissue of several cancers including oral cancer (Manoharan, et al., 2010: Manikandan, et al., 2008). Present study corroborates these observations.

Oral administration of glycyrrhetinic acid at a dose of 45 mg/kg b.w to hamsters treated with DMBA restored the status of lipid peroxidation and antioxidants in plasma and buccal mucosa. Present results thus suggest that glycyrrhetinic acid has significant role in im-

proving the status of lipid peroxidation and antioxidants during DMBA induced hamster buccal pouch carcinogenesis.

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