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Original

Possible action mechanism for curcumin in pre-cancerous lesions based on serum and salivary markers of oxidative stress

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Abstract: Extensive research within the past half-century has indicated that curcumin (diferuloylmethane), a yellow pigment in curry powder, exhibits anti-oxidant, anti-inflammatory, and pro-apoptotic activities. We investigated whether the anti-pre-cancer activities assigned to curcumin are mediated through an anti-oxidant and DNA-protecting mechanism. Patients with oral leukoplakia, oral submucous fibrosis or lichen planus, and healthy individuals (n = 25 for each group) aged 17-50 years were selected. Salivary and serum oxidative markers such as malonaldehyde (MDA), 8-hydroxydeoxyguanosine (8-OHdG), vitamins C and E were measured just prior to the intake of curcumin, after one week of curcumin intake and following clinical cure of precancerous lesions. Serum and salivary vitamins C and E showed increases, while MDA and 8-OHdG levels showed decreases in patients with oral leukoplakia, submucous fibrosis and lichen planus after intake of curcumin for all categories of precancerous lesions. The changes in these values were observed to be statistically significant after clinical cure of the disease ($P < 0.05$). The five-point rating scale for pain, as well as lesion size in oral leukoplakia, submucous fibrosis and lichen planus, improved significantly ($P < 0.05$). In addition, in submucous fibrosis, mouth opening ($P < 0.05$) recovered significantly. In oral leukoplakia, submucous fibrosis and lichen planus, the levels of serum and salivary vitamins C and E increased significantly, while MDA and 8-OHdG levels decreased after 131(15), 211(17), and 191(18) days, respectively. Values for serum and salivary vitamins C and E showed a significant decrease in oral leukoplakia, submucous fibrosis and lichen planus, in contrast to healthy individuals, but increased significantly in all groups subsequent to curcumin administration after clinical cure of lesions. Based on these results, we can conclude that curcumin mediates its anti-pre-cancer activities by increasing levels of vitamins C and E, and preventing lipid peroxidation and DNA damage. (J Oral Sci 52, 251-256, 2010)

Keywords: curcumin; antioxidant; precancerous lesions; serum; salivary; anti-pre-cancer.

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Introduction

Oral cancer is the sixth most common form of cancer worldwide (1). Its incidence is particularly high in India, other Asian countries, and in certain places in the Western hemisphere, e.g., parts of France and Brazil, where smoking

and alcohol consumption are major risk factors. In India, the chewing and smoking of tobacco products in various forms is primarily responsible for the high incidence. The World Health Organization (WHO) has estimated that 90% of oral cancers in India among men are attributable to chewing and smoking habits (2). About 48.2% of cancers in men and 20.5% of cancers in women are related to tobacco, a major proportion of which is in the oral cavity, pharynx, larynx, esophagus (74.7%), while lung cancers account only for 15%. Control of cancers of the head and neck, lung, cervix and breast, which account for 50-55% of the cancer load in India, will thus have a measurable effect on the incidence of cancer (3).

Oral squamous cell carcinoma develops through a multi-step process of genetic, epigenetic and metabolic changes resulting from exposure to carcinogens (4). The initial presence of a precursor subsequently developing into cancer is well established in oral cancer (5). Oral leukoplakia and submucous fibrosis are two major pre-cancerous lesions, but only 8-10% of these lesions ultimately become malignant (6). The ability to clinically predict malignant transformation is limited and routine histopathological diagnosis has limited prognostic value. The presence of epithelial dysplasia is an important parameter used in the prognostication of leukoplakia. However, there are limitations in its usage; the diagnosis is essentially subjective, all lesions exhibiting dysplasia do not eventually become malignant and some may even regress, and carcinoma can develop from lesions in which epithelial dysplasia was not diagnosed in previous biopsies (5). Therefore, it is necessary to develop other methods for predicting the malignant potential of pre-malignant lesions and preventive measures (7).

Free radical-mediated lipid peroxidation is involved in various cancers, and several studies have described the role of free radicals in oral cancers. Low salivary lipid peroxidation products such as malonaldehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG) have also been reported in oral cancer (8). Increases in lipid peroxidation products and decreases in antioxidant activity in cancer have been reported in the literature (9,10), and randomized controlled trials have shown that antioxidant (vitamins C and E) supplementation may be beneficial in the prevention of cancer (11,12).

Various research over the past half-century has indicated that curcumin (also called diferuloylmethane), a yellow coloring agent present in turmeric, is an antioxidant more potent than α-tocopherol (13). Curcumin has been linked with the suppression of mutagenesis; and has been used as a chemopreventive agent in a wide variety of cancers, including those of the colon, breast, prostate, esophagus, lung and oral cavity, as well as in inhibition of atherosclerosis and inhibition of viral and bacterial growth (14). It has been demonstrated that curcumin downregulates STE (khaini) or NNK-induced NF-κB and COX-2 in oral premalignant and cancer cells in vitro (15). In the present study, we hypothesized that curcumin may suppress or prevent oral pre-cancerous and cancerous lesions and conditions by inhibiting free radicals. To test this hypothesis, we examined the effects of curcumin in patients with leukoplakia, oral submucous fibrosis and lichen planus by measuring salivary and serum levels of MDA, 8-OHdG, and vitamins C and E, before and after curcumin administration.

Materials and Methods

Twenty-five patients [male (M):female (F), 13:12] with oral leukoplakia, 25 patients (M:F, 11:14) with oral submucous fibrosis and 25 patients (M:F, 12:13) with lichen planus, as well as 25 normal healthy individuals (aged 17-50 years, attending Jain Diagnostic Centre, New Delhi, India) were selected. All diagnostic tests were evaluated for diagnosing oral pre-cancerous lesions. Each subject completed a medical and dental history questionnaire to determine the status of systemic disease, smoking, alcohol and drug history. Clinical examinations for systemic diseases, chronic diseases, oral and dental diseases were conducted, and patients were excluded if they had any history of systemic disease, chronic disease, dental disease, smoking habit, or alcohol and drug history.

Curcumin 1 g caplets (900 mg curcumin, 80 mg desmethoxycurcumin, and 20 mg bisdesmethoxycurcumin) were obtained from Sabinsa Corporation (Piscataway, NJ, USA).

Pain control and lesion healing were the 2 main clinical variables for evaluating cure of oral leukoplakia, oral lichen planus and oral submucous fibrosis. To measure pain, we used a visual scale analog ranging from 0.5 (very mild pain) to 5 (severe pain). For healing, we measured changes in lesion size, including ulcer size from baseline, while in oral submucous fibrosis, in addition to the above variables, change in mouth opening was considered. Final outcome was confirmed by clinical examination. Clinical and histopathological examinations were conducted, along with collection of serum and salivary samples prior to intake of curcumin. One week after clinical cure of disease, clinical examination was again performed along with collection of serum and salivary samples. Whole unstimulated saliva produced in a 5-min period (about 3 ml) was collected, allowed to drain into a plastic container, and was centrifuged at $3,000 \times g$ at 4°C for 5 min in order to remove bacterial and cellular debris. Saliva samples were

stored at -80°C until analysis. Blood samples were collected into vacutainer tubes. Blood was centrifuged at 1,700 × *g* for 10 min and plasma was separated. Plasma was stored at -80°C until analysis was performed. Serum and salivary levels were assessed for MDA using thiobarbituric acid according to the method of Buege and Aust et al. (16). Concentrations of both vitamins were measured using liquid chromatography (17). Quantitative measurements of the oxidative DNA adduct 8-OHdG were performed according to the method of Toyokuni et al. (18). Briefly, saliva samples were centrifuged at 10,000 × *g* for 10 min and the supernatant was used to determine 8-hydroxy-deoxyguanosine (8-OHdG) levels with a competitive ELISA kit (Japan Institute for the Control of Aging, Shizuoka, Japan). The determination range was 0.5-200 ng/ml. Serum 8-OHdG levels were measured in duplicate by competitive ELISA kit (OXIS; Portland, OR, USA) according to the manufacturer's instructions. The sensitivity of the method was 1 ng/ml. All data were statistically analyzed using the SPSS statistical package (Version 13; Chicago, IL, USA). Data are expressed as means ± standard deviation. Differences were analyzed for significance, using a one-way analysis of variance (ANOVA) test. Correlation assessment was performed by Spearman correlation analysis. Statistical significance was set at $P < 0.05$.

Results

The mean and median values of serum and salivary vitamins C and E showed increases, while MDA and 8-OHdG levels showed decreases in oral leukoplakia, submucous fibrosis, lichen planus patients one week after intake of curcumin, as compared to before treatment in all precancerous patients. Furthermore, the values were statistically significant after clinical cure of disease (Table 1; $P < 0.05$; Figs. 1-8). Pain scores and size of lesion in oral leukoplakia, submucous fibrosis and lichen planus improved significantly ($P < 0.05$). In addition, in submucous fibrosis, mouth opening recovered significantly [24.64(3.2) to 39.4(3) mm; $P < 0.05$]. Oral leukoplakia, submucous fibrosis and lichen-planus were clinically cured after 131(15), 211(17), and 191(18) days, respectively. Serum and salivary vitamin C and E levels showed significant decreases, while MDA and 8-OHdG levels showed significant increases in oral leukoplakia, submucous fibrosis and lichen planus patients, as compared to healthy controls (Table 1; $P < 0.05$). Median salivary and serum MDA, 8-OHdG, and vitamin C and E levels were significantly different in precancerous patients before intake of curcumin and after clinical cure, and after 209 days in healthy subjects, although the differences were greater in precancerous patients (Figs. 1-8). Serum and salivary correlation analysis revealed strong and highly significant correlations for MDA, vitamins C and E, and 8-OHdG in all groups (r = 0.86, r = 0.67 and r = 0.76, $P < 0.001$, and r = 0.67, r = 0.66 and r = 0.64, $P < 0.001$, respectively).

Table 1 Salivary and serum MDA, 8-OHdG, and vitamin C and E levels in oral leukoplakia, oral lichen planus and oral submucous fibrosis patients, and healthy controls prior to intake of curcumin, one week after curcumin intake, after clinical cure of oral precancerous lesions and after 209 days

Participant group	MDA		8-OhdG		Vitamin C		Vitamin E	
	Salivary (μmol/l)	Serum (μmol/l)	Salivary (ng/ml)	Serum (ng/ml)	Salivary (μg/l)	Serum (μg/l)	Salivary (μg/l)	Serum (μg/l)
Prior to intake of curcumin (A)								
Normal	0.11 (0.13)	0.98 (0.86)	0.11 (0.12)	2.17 (1.45)	1.46 (0.86)	9.05 (2.21)	0.91 (0.43)	8.97 (2.34)
Oral lichen planus	0.35 (0.26)	1.16 (0.89)	0.39 (0.23)	2.14 (1.89)	1.04 (0.69)	8.48 (2.66)	0.68 (0.31)	8.15 (2.33)
Oral leukoplakia	0.36 (0.17)	1.23 (0.56)	0.34 (0.24)	2.13 (1.12)	1.08 (0.98)	8.78 (3.12)	0.65 (0.31)	8.01 (1.23)
Oral submucous fibrosis	0.32 (0.16)	1.19 (0.37)	0.32 (0.14)	2.12 (2.24)	1.01 (0.32)	8.56 (3.56)	0.67 (0.32)	8.08 (1.13)
After 7 days								
Normal	0.09 (0.11)	0.95 (0.56)	0.09 (0.12)	2.01 (1.11)	1.67 (0.89)	9.08 (2.66)	0.98 (0.43)	8.99 (2.35)
Oral lichen planus	0.29 (0.24)	1.08 (0.67)	0.34 (0.23)	2.04 (1.96)	1.28 (0.67)	8.98 (4.67)	0.70 (0.33)	8.35 (3.14)
Oral leukoplakia	0.32 (0.23)	1.07 (0.81)	0.31 (0.23)	2.01 (1.09)	1.43 (0.65)	8.89 (3.34)	0.69 (0.44)	8.12 (3.43)
Oral submucous fibrosis	0.28 (0.21)	1.16 (0.89)	0.29 (0.13)	2.05 (2.02)	1.23 (0.54)	8.88 (3.67)	0.68 (0.34)	8.16 (2.32)
After curing of lesions and 209 days in normal healty								
Normal	0.07 (0.08)	0.53 (0.68)	0.07 (0.06)	1.71 (1.65)	1.72 (0.76)	9.65 (3.93)	1.07 (0.85)	9.15 (4.56)
Oral lichen planus	0.13 (0.12)	0.96 (0.62)	0.12 (0.13)	1.78 (0.98)	1.56 (0.81)	9.05 (2.86)	0.91 (0.45)	9.09 (3.45)
Oral leukoplakia	0.13 (0.12)	0.97 (0.56)	0.12 (0.09)	1.88 (1.67)	1.54 (0.89)	9.09 (3.86)	0.96 (0.45)	8.99 (4.67)
Oral submucous fibrosis	0.11 (0.11)	0.98 (0.67)	0.11 (0.12)	1.89 (1.78)	1.45 (0.79)	9.05 (2.86)	0.89 (0.29)	8.97 (3.43)

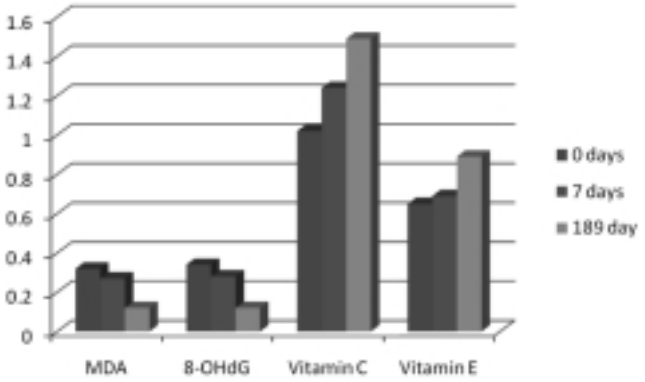

Fig. 1 Salivary MDA (µmol/l), 8-OHdG (ng/ml), and vitamin C (µg/l) and E (µg/l) levels prior to intake of curcumin, one week after curcumin intake, and after 189 days in healthy subjects.

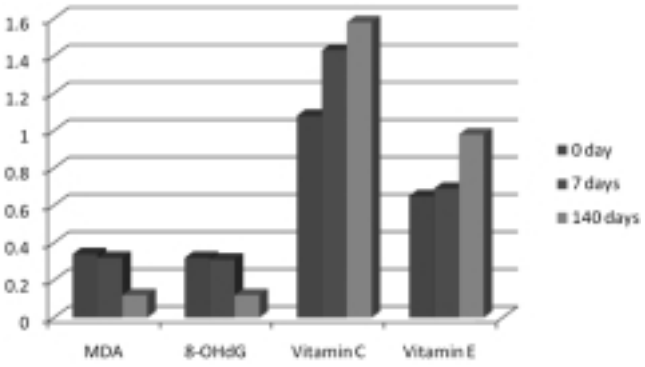

Fig. 2 Salivary MDA (µmol/l), 8-OHdG (ng/ml), and vitamin C (µg/l) and E (µg/l) levels prior to intake of curcumin, one week after curcumin, and after clinical cure of oral leukoplakia.

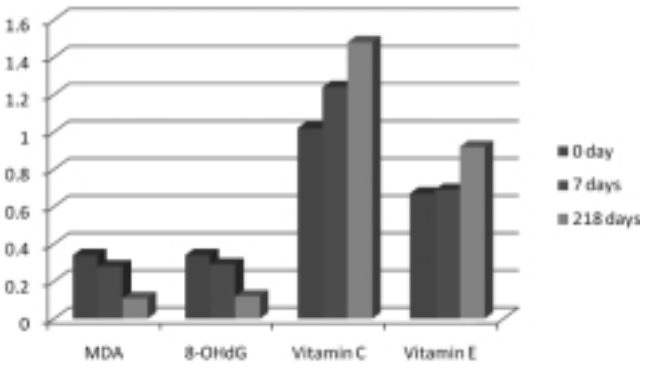

Fig. 3 Salivary MDA (µmol/l), 8-OHdG (ng/ml), and vitamin C (µg/l) and E (µg/l) levels prior to intake of curcumin, one week after curcumin intake, and after clinical cure of oral submucous fibrosis.

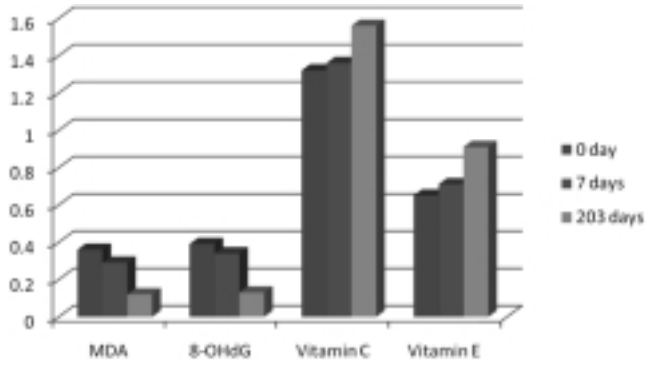

Fig. 4 Salivary MDA (µmol/l), 8-OHdG (ng/ml), and vitamin C (µg/l) and E (µg/l) levels prior to intake of curcumin, one week after curcumin intake, and after clinical cure of oral lichen planus.

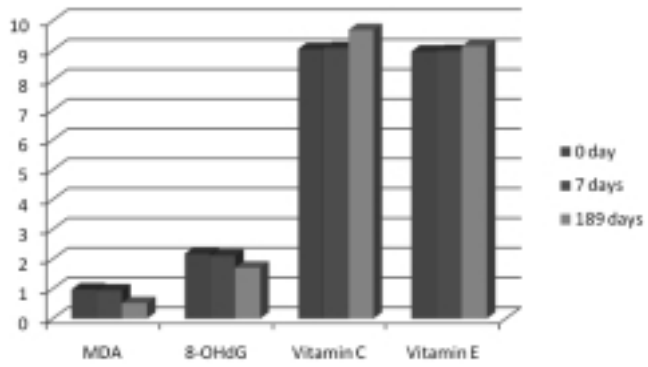

Fig. 5 Serum MDA (µmol/l), 8-OHdG (ng/ml), and vitamin C (µg/l) and E (µg/l) levels prior to intake of curcumin, one week after curcumin intake, and after 189 days in healthy controls.

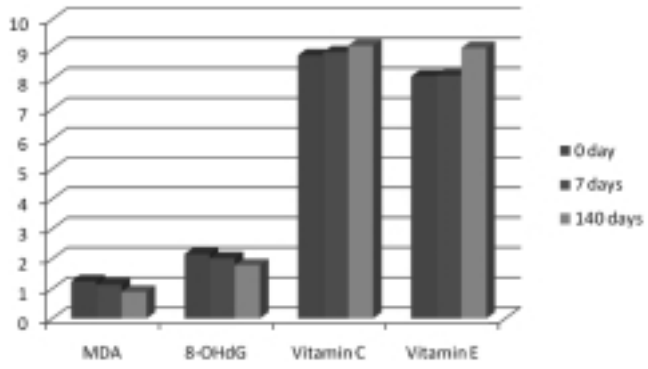

Fig. 6 Serum MDA (µmol/l), 8-OHdG (ng/ml), and vitamin C (µg/l) and E (µg/l) levels prior to intake of curcumin, one week after curcumin intake, and after clinical cure of oral leukoplakia.

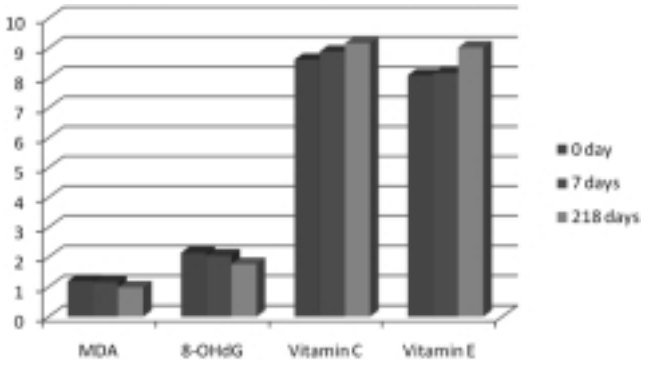

Fig. 7 Serum MDA (µmol/l), 8-OHdG (ng/ml), and vitamin C (µg/l) and E (µg/l) levels prior to intake of curcumin, one week after curcumin intake, and after clinical cure of oral submucous fibrosis.

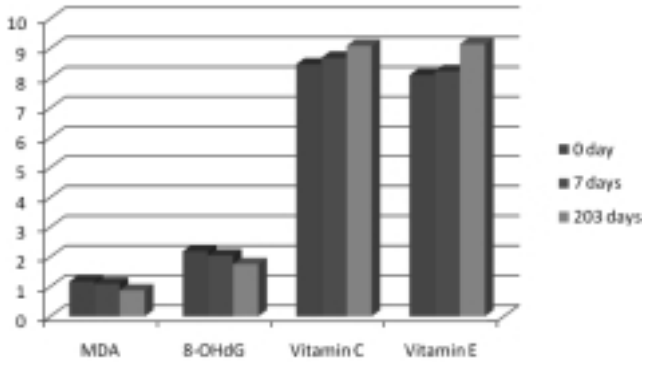

Fig. 8 Serum MDA (µmol/l), 8-OHdG (ng/ml), and vitamin C (µg/l) and E (µg/l) levels prior to intake of curcumin, one week after curcumin intake, and after clinical cure of oral lichen planus.

Discussion

The goal of this study was to determine whether the anti-precancerous effects of curcumin are mediated through an antioxidant mechanism. Serum and salivary vitamin C and E levels were found to increase, while MDA and 8-OHdG levels decreased in oral leukoplakia, submucous fibrosis and lichen-planus patients after intake of curcumin, as compared to pre-treatment levels. Furthermore, the changes in these values were found to be significant after clinical cure. Pain and lesion size diminished significantly in oral leukoplakia, submucous fibrosis and lichen-planus, while in submucous fibrosis, mouth opening recovered significantly. Our results suggest that curcumin significantly increases the local and systemic antioxidant status and the levels of vitamins C and E, while it decreases the lipid peroxidation and DNA damage of patients with pre-cancerous lesions. This could be due to curcumin-induced production of vitamins C and E, and preventive DNA damage by decreasing the oxidation stress. This suggests that the anti-precancerous effects of curcumin are mediated through pro-oxidant and anti-oxidant pathways.

The mechanisms by which curcumin mediates its pro-oxidant effects remain unclear. It has been suggested that mitochondria play a role in curcumin-induced apoptosis. It is possible that curcumin activates the mitochondrial enzymes that lead to production of reactive oxygen species (ROS) (19-20). The induction of ROS by curcumin may occur through its interaction with thioredoxin reductase, thus altering its activity to NADPH oxidase, which could then lead to the production of ROS (21). There have also been reports suggesting that curcumin quenches ROS production and thus acts as an antioxidant, while others have reported that curcumin quenches ROS production at low concentrations and induces ROS production at high concentrations (22-23). It has also been stated that micronutrients enhance the levels of vitamins A and C, as well as selenium, in the supplemented groups, with a concomittant regression of precancerous lesions present on the palate (24). We did not record any treatment-related toxic effects at doses up to 8 g/d, as reported previously (25). The oral leukoplakia, submucous fibrosis and lichen planus were cured in almost the same period of time. The antioxidant status of healthy subjects also improved with curcumin intake. Median salivary and serum MDA, 8-OHdG, vitamin C and E levels changed in precancerous patients before intake of curcumin and after clinical cure, and after 209 days in healthy subjects, although the changes were higher in precancerous patients. A significant correlation was observed in serum and salivary markers in all groups.

As saliva can be easily collected in a non-invasive manner, measurement of salivary disease biomarkers may prove vital in early detection of oral cancer risk. Moreover, salivary analysis for oral diagnosis may prove to be a cost-effective method for screening large populations. Further studies are required in larger samples in order to determine the relationship between curcumin, biomarkers and oral cancer, and to further contribute to the understanding of the mechanisms of action.

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