Proposed New possible action of Curcumin in Oral leukoplakia: Oxidative stress Serum and salivary markers

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Abstract

Extensive research within the past half-century has indicated that curcumin (diferuloylmethane), a yellow pigment in curry powder, exhibits antioxidant, anti-inflammatory, and pro-apoptotic activities. We investigated whether the anticancer activities assigned to curcumin are mediated through its pro-oxidant/antioxidant and preventive DNA damage mechanism. Twenty patient of oral leukoplakia and 50 normal healthy in age group 17-50 years were selected and measured the salivary and serum oxidative markers such as Malonaldehyde, 8-hydroxydeoxyguanosine, vitamin C and E just before the given the curcumin ,after curing of lesion. The value of serum and salivary Vitamin C &E showed statistically significant decrease in oral leukoplakia as compared to normal healthy while significantly increased in all groups after given curcumin. MDA and 8-OH dG levels showed statistically significant increase in oral leukoplakia as compared to normal healthy while decreased in all groups after given curcumin. Based on these results we conclude that curcumin mediates its anticancer activities through induced the production of Vitamin C&E, preventation of lipid perioxidation and anti DNA damage.

Key Words: Curcumin, Antioxidant, Precancerous lesions, Serum, salivary, Anticancer

Introduction

Oral cancer is the sixth commonest cancer in the world[1]. Its incidence is particularly high in India, some other countries in Asia, and in certain places in the Western hemisphere, e.g. parts of France and Brazil, where smoking and alcohol drinking are major risk factors. In India, 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 were 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, of which a major proportion is in the oral cavity, pharynx, larynx, oesophagus (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 have a maximum 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 wellestablished in oral cancer[5]. Oral leukoplakia and submucous fibrosis are two major known precursor lesions. Only 8-10% of these lesions ultimately turn into malignancy[6]. Ability to clinically predict malignant transformation is limited and routine histopathological diagnosis has limited prognostic value. The presence of epithelial dysplasia is one of the important parameters used in prognostication of leukoplakia. However, there are limitations for its use such as, 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 measured[7]. Free radical mediated lipid peroxidation have been involved in various cancers. Several studies have reported role of free radicals in oral cancers. Low salivary lipid peroxidation product malonaldehyde (MDA) and 8-OHdG levels have also been reported in

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oral cancer.[8] It has been reported that increase in lipid peroxidation products in cancer and a decrease in antioxidant activity in cancer compared with normal have been reported in literature.[9,10] Randomized controlled trials have shown that antioxidant (vitamin C and E) supplementation may be beneficial in prevention of cancer.[11,12]

Extensive research in the past half a century has indicated that curcumin (also called diferuloylmethane), a yellow coloring agent present in turmeric, is an antioxidant more potent than even a-tocopherol[13]. Curcumin has been linked with the suppression of mutagenesis; has been used as a chemopreventive agent for a wide variety of cancers, including colon, breast, prostate, esophagus, lung and oral; inhibition of atherosclerosis and inhibition of viral and bacterial growth[14]. It has been demonstrated that curcumin downregulates STE (khaini) or NNK-induced NF--B and COX-2 in oral premalignant and cancer cells in vitro[15]. In this study,we hypothesized that curcumin may suppress or prevent the oral pre-canceous and cancerous lesions and condition by inhibiting free radical .To test this hypothesis, we examined the effect of curcumin on of oral leukoplakia by measured the salivary and serum levels of Malonaldehyde, 8-hydroxydeoxyguanosine, vitamin C and E before , after given the curcumin

Materials and methods

Twenty five patient (M:F, 10:10) of oral leukoplakia in age group 17-50 years attending Jain Diagnostic Centre, New Delhi, India. All diagnostic tests were evaluated for diagnosing particular disease. 50 (M:F, 25:25) normal healthy subject with age (15-60 years) observed as controls. Each subject completed a medical and dental history questionnaire to determine the status of systemic diseases, smoking, alcoholic and drugs history as well as clinical examination for systemic diseases, chronic diseases and oral & dental diseases. Patients were excluded from study who had systemic diseases, chronic diseases, dental disease, smoking, alcoholic and drugs history.

Curcumin- 1 g caplet form Curcumin (900 mg curcumin, 80 mg desmethoxycurcumin, and 20 mg bisdesmethoxycurcumin) from Sabinsa was obtained .

Blood and saliva samples were taken just before curcumin given, after one week, after after cure disease. Whole unstimulated saliva was collected over a five-min period from subjects with directions to allow saliva to pool at the bottom of the mouth and drain into a collection tube, when necessary. Unstimulated whole saliva produced in a 5-min period (about 3 mL) was collected, allowed to drain into a plastic container, and centrifuged at 3,000 ×g, in 4°C for 5 min to remove bacterial and cellular debris. Saliva samples were stored at -80°C until analysis. Blood samples were collected into Vacutainer tubes. The blood was centrifuged at 1,700 g for 10 min and the plasma was separated. Plasma was stored at -80°C until analysis. Serum and salivary levels were assessed for MDA using thiobarbituric acid (TBA) method of Buege and Aust[16]. Concentrations of both vitamins were measured using liquid chromatography[17]. Quantitative measurement of the oxidative DNA adduct 8-OHdG was performed according to the method described by Toyokuni et al.[18] Briefly, the saliva samples were centrifuged at 10,000g for 10 minutes and the supernatant was used to determine 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 a 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 SPSS statistical package (SPSS, version13, Chicago,IL,USA). Data are expressed as mean ± standard deviation. Differences were analyzed for significant, using one-way ANOVA test. Correlation assessment was performed using the Spearman correlation analysis. Statistical significance was defined as $p< 0.05$.

Results

The value of serum and salivary Vitamin C &E showed statistically significant decrease in oral leukoplakia as compared to normal healthy while significantly increased in all groups after given curcumin(Table-1-2, $P<0.05$) . MDA and 8-OH dG levels showed statistically significant

increase in oral leukoplakia as compared to normal healthy while decreased in all groups after given curcumin (Table-1-4, P<0.05). Serum and salivary correlation analysis revealed strong and highly significant correlation for MDA, Vitamin C& E and 8 dihydro-2 deoxyguanosine (8-OHdG) in all groups (r=0.86,r=0.67,r=0.76, P<0.001) & (r=0.67,r=0.66,r=0.64, P<0.001) respectively.

Discussion

The goal of this study was to determine whether the anticancer effects of curcumin are mediated through and through an antioxidant or pro-oxidant mechanism. Our results suggest that curcumin significantly increased the local and systemic antioxidant status through increased the vitamin C & E , decreased lipid perioxidation and DNA damage of precancerous lesions patients. It may be due to curcumin induced the production of vitamin C and E and prevent DNA damage by decreased the oxidation stress. All of this evidence suggests that the anticancer effects of curcumin are mediated through the pro-oxidant and oxidant pathway. The mechanism by which curcumin mediates its prooxidant effects remains unclear. It have been suggested that role of mitochondria in curcumin-induced apoptosis . Thus, it is possible that curcumin activates mitochondrial enzymes that lead to production of ROS [19-20]. The induction of ROS by curcumin could occur through its interaction with thioredoxin reductase , thus changing its activity to NADPH oxidase, which could then lead to the production of ROS [21].There are also reports which suggest that curcumin quenches ROS production and thus acts as an antioxidant while other reported that curcumin quenches ROS production at low concentrations and induces ROS production at high concentrations[22,23]. It has been reported that micronutrients improved the vitamin A,C and selenium nutritive in the supplemented group with a concomittant regression of precancerous lesions present on the palate[24] as supported by this study. We did not record any treatment-related toxic effects up to doses of 8 g/d as in previous study[25]. The antioxidant statuses of healthy subjects were also improved with curcumin. The value of serum and salivary Vitamin C & E showed statistically significant decrease and increased MDA and 8-OH dG levels in oral leukoplakia , as compared to normal healthy as in our previous study[8]. The significant correlation was observed in serum and salivary marker in all groups. Since, saliva can be easily collected, measurement of biomarkers of diseases may prove to useful in early detection of oral cancer risks. Moreover, a salivary analysis for oral diagnosis may prove a cost effective method

Table 1. Salivary and serum MDA, Vitamin C& E and 8 dihydro-2 deoxyguanosine (8-OHdG) concentration in the plasma and saliva of leukoplakia subject in before Curcumin Given (A), one week after Curcumin Given (B), After curing of leukoplakia (C) .

Serum and saliva Markers	A	B	C
Salivary MDA (µmol/L)	0.32±0.16 a	0.28±0.21 *, a	0.11±0.11*
Serum MDA (µmol/L)	1.19 ±0.37 a	1.16 ±0.89*, a	0.98 ±0.67*
Salivary Vitamin C (µg /L)	1.01±0.32 a	1.23±0.54 *, a	1.45±0.79*
Serum Vitamin C (µg/L)	8.56±3.56 a	8.88±3.67 *, a	9.05±2.86*
Salivary Vitamin E (µg/L)	0.67±0.32 a	0.68±0.34 *, a	0.89±0.29*
Serum Vitamin E (µg/L)	8.08±1.13 a	8.16±2.32 *, a	8.97±3.43*
Salivary8-OH dG (ng/ml)	0.32±0.14 a	0.29±0.13*, a	0.11±0.12*
Serum 8-OH dG (ng/ml)	2.12 ± 2.24 a	2.05 ± 2.02*, a	1.89 ± 1.78*

for screening large populations. Further studies are required on large samples to determine the relationship between curcumin, biomarkers and oral cancer to clearly mechanism of action.*p < 0.05, as compared to after condition (C)

ap < 0.05, as compared to Before condition (A).

Table 2. Salivary and serum MDA, Vitamin C& E and 8 dihydro-2 deoxyguanosine (8-OHdG) concentration in the plasma and saliva of Normal healthy subject in before Curcumin Given (A), one week after Curcumin Given (B), After curing of Normal healthy (C).

Serum and saliva Markers	A	B	C
Salivary MDA (µmol/L)	0.11±0.13 a	0.09±0.11* , a	0.7±0.08*
Serum MDA (µmol/L)	0.98 ±0.86 a	0.95 ±0.56*, a	0.93 ±0.68*
Salivary Vitamin C (µg /L)	1.46±0.86 a	1.67±0.89 *, a	1.72±0.76*
Serum Vitamin C (µg/L)	9.05±2.21 a	9.08±2.66 *, a	9.65±3.93*
Salivary Vitamin E (µg/L)	0.91±0.43 a	0.98±0.43 *, a	1.07±0.85*
Serum Vitamin E (µg/L)	8.97±2.34 a	8.99±2.35 *, a	9.15±4.56*
Salivary8-OH dG (ng/ml)	0.11±0.12 a	0.09±0.12*, a	0.07±0.06*
Serum 8-OH dG (ng/ml)	2.17 ± 1.45 a	2.01 ± 1.11*, a	1.71 ± 1.65*

p < 0.05, as compared to after condition (C)
ap < 0.05, as compared to Before condition (A).

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