Efficacy of Lycopene in the Treatment of Gingivitis: a Randomised, Placebo-controlled Clinical Trial

Rampalli Viswa Chandra[a]/M. L. Venkatesh Prabhuji[a]/D. Adinarayana Roopa[a]/
Sandhya Ravirajan[a]/Hadal C. Kishore[a]

Purpose: The aim of the present study was to compare the effect of systemically administered lycopene (LycoRed™) as a monotherapy and as an adjunct to scaling and root planing in gingivitis patients.

Materials and Methods: Twenty systemically healthy patients showing clinical signs of gingivitis were involved in a randomised, double-blind, parallel, split-mouth study. The subjects were randomly distributed between the two treatment groups: experimental group (n = 10), 8 mg lycopene/day for 2 weeks; and controls (n = 10), placebo for 2 weeks. Quadrant allocation within each group was randomised with two quadrants treated with oral prophylaxis (OP) and two quadrants not receiving any form of treatment (non-OP). Bleeding index (SBI) and non-invasive measures of plaque (PI) and gingivitis (GI) were assessed at baseline, 1 and 2 weeks. Salivary uric acid levels were also measured.

Results: All the treatment groups demonstrated statistically significant reductions in the GI, SBI and PI. Treatment with OP-lycopene resulted in a statistically significant decrease in GI when compared with OP-placebo ($p < 0.05$) and non-OP-placebo ($p < 0.01$). Treatment with non-OP-lycopene resulted in a statistically significant decrease in GI when compared with non-OP-placebo ($p < 0.01$). The OP-lycopene group showed a statistically significant reduction in SBI values when compared with the non-OP-lycopene group ($p < 0.05$) and the non-OP-placebo group ($p < 0.001$). There was a strong negative correlation between the salivary uric acid levels and the percentage reduction in GI at 1 and 2 weeks in the OP-lycopene group ($r = -0.852$ and -0.802 respectively) and in the non-OP-lycopene group ($r = -0.640$ and -0.580 respectively).

Conclusions: The results presented in this study suggest that lycopene shows great promise as a treatment modality in gingivitis. The possibility of obtaining an additive effect by combining routine oral prophylaxis with lycopene is also an exciting possibility, which deserves further study.

Key words: antioxidant therapy, gingivitis, gingivitis therapy, lycopene

Oral Health Prev Dent 2007; 5: 327-336. *Submitted for publication: 19.08.06; accepted for publication: 22.11.06.*

Periodontal diseases are inflammatory disorders that give rise to tissue damage and loss, as a result of complex interactions between pathogenic bacteria and the host's immune response (Chapple, 1997). There is an increasing body of evidence available to implicate reactive oxygen species (ROS) in the pathogenesis of a variety of inflammatory disorders, of which periodontal disease is no exception. In recent years, the term ROS has been adopted to include molecules such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and singlet oxygen ($^1O_2$), which whilst not radicals in nature, can cause substantial tissue damage by initiating free radical chain reactions (Chapple, 1997).

The predominant inflammatory cell (96%) within the healthy connective tissues and epithelium of the gingiva is the polymorphonuclear leucocyte (PMNL). Periodontopathic bacteria in the gingivomucosal tissue may functionally activate PMNLs leading to an increased production of ROS (Chapple, 1997). Chronic inflammation subjects nearby cells to elevated levels

[a] *Krishnadevaraya College of Dental Sciences, Hunasamaranahalli, Yelahanka, Bangalore, India.*

Reprint requests: *Rampalli Viswa Chandra MDS, Senior Lecturer, Department of Periodontics, Krishnadevaraya College of Dental Sciences, Hunasamaranahalli, Yelahanka, Bangalore 562 157, India. Tel: 91-80-28467083/28477083. Fax: 91-80-28467083/28477083. Email: viswachandra@hotmail.com*

of ROS due to extracellular release from phagocytic cells. Since neutrophil-mediated tissue injury was demonstrated by Deguchi et al (1990), similar findings have been reported for neutrophil and gingival epithelial cell interactions (Altman et al, 1992). Subsequent studies demonstrated that all glycosaminoglycans of the gingival connective tissue undergo chain depolymerisation and residue modification to varying degrees, particularly in the presence of highly reactive OH^- species (Freeman and Crapo, 1982; Murrell et al, 1990). The non-sulphated glycosaminoglycan, hyaluronan, was identified as being more susceptible to degradation by ROS than sulphated glycosaminoglycans. Similar conclusions have also been made following the exposure of gingival tissue proteoglycans and collagen to ROS (Freeman and Crapo, 1982; Murrell et al, 1990). It has been demonstrated that *Fusobacterium* species, a frequent isolate from chronic gingivitis, can induce increased production of oxygen radicals, cytokines and elastase in leukocyte activated under *in vitro* conditions, which might be a possible pathogenic factor in periodontal disease (Sheikhi et al, 2000). It is likely that the role of ROS is common to both bacterial and host mediated pathways of tissue damage (Chapple, 1997).

Mammalian cells have developed elaborate antioxidant defence systems to prevent oxidative damage and to allow survival in an aerobic environment. This system includes enzymatic activities such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx) as well as non-enzymatic antioxidants including vitamins E, C, A, melatonin, uric acid and glutathione (Young and Woodside, 2001). The antioxidant systems of saliva are highly complex and rich in several antioxidants such as uric acid, glutathione, and ascorbic acid. However, there seems to be an association of periodontal diseases with reduced salivary antioxidant status and increased oxidative damage within the oral cavity (Diab-Ladki et al, 2003).

Modulation of the free radical production seems to be essential for the inhibition of tissue destruction, and treatment with drugs that block the production of free ROS or block its effects might be therapeutically valuable (Goultschin and Levy, 1986). Recent investigations on animal models suggest that antioxidant therapies, which interfere with ROS, may be of benefit in the treatment of periodontitis (Di Paola et al, 2005). Many chemotherapeutic agents used in periodontics, in addition to their antiseptic or antimicrobial effects, are known to have an antioxidative activity against spontaneous oxidation (Firatli et al, 1994). However, there is a paucity of studies that utilise potent antioxidants in the treatment of periodontal diseases.

Lycopene, the carotenoid that gives ripe tomato its bright red colour, is an effective natural antioxidant and a quencher of free radicals (Nir and Hartal, 2000). Lycopene exhibits the highest physical quenching rate with singlet oxygen (Di Mascio et al, 1989) and is at least three-fold more effective than β-carotene in preventing cell death by quenching NOO^- radicals (Bohm et al, 1995). It also reverses DNA damage induced by H_2O_2 (Singh et al, 2004). A recent study investigated the relationship between monthly tomato consumption and serum lycopene levels, and a self-reported history of congestive heart failure (CHF) in individuals with periodontitis, and concluded that a relationship exists between periodontitis and CHF risk, and high monthly tomato consumption appears to affect this relationship in a positive direction in periodontitis subjects (Wood and Johnson, 2004).

The commercially available anti-oxidant used in this study (LycoRed™, Jagsonpal Pharmaceuticals Ltd, New Delhi, India) contains 100% natural lycopene with added phytonutrients (Table 1) such as phytoene, phytofluene, β-carotene, phytosterols, and vitamin E for synergistic action (Lyc-O-Mato™, LycoRed Natural Product Industries, Beer-Sheva, Israel). The antioxidant potency of Lyc-O-Mato™ is three times greater than pure lycopene (Fuhrman et al, 1997).

The aim of the present study was to evaluate, over a two-week observation period, the effect of systemically administered lycopene (LycoRed™) as a monotherapy and as an adjunct to scaling in gingivitis patients.

MATERIALS AND METHODS

Twenty systemically healthy patients (14 males, 6 females; age: 24.8 ± 4.16 years) were involved in a randomised, double-blind, parallel, split-mouth study. The patients consulted were referred to the Department of Periodontology for the treatment of gingivitis. Test subjects who were pregnant, smokers, had systemic diseases such as diabetes and cardiovascular diseases and took medications such as antibiotics or antiseptics within the past 6 weeks or over-the-counter antioxidants such as vitamin C, vitamin E or β-carotene within the past 3 months were excluded from the study. All patients included in the study showed clinical signs of gingivitis. To exclude the possibility of an underlying periodontal disease, each patient underwent periodontal probing at six sites in all the teeth and radiographs of suspect sites were obtained at the time of examination.

Table 1 Composition of the lycopene softgels used in the present investigation (active ingredients excluding the filler)

Lycopene	2000 µg
Vitamin A concentrate as palmitate	2500 IU
β-Tocopherol acetate	10 IU
Vitamin C	50 mg
Zinc sulphate monohydrate	27.45 mg
Monohydrated selenium dioxide	70 µg

Table 2 Composition of the placebo used in the present investigation (filler material)

Soya oil	205.780 mg
Bees wax	17.178 mg
Hydrogenated castor oil	7.393 mg
Butylated hydroxy toulene	0.125 mg
Butylated hydroxy anisol	0.125 mg
Lecithin soya	17.714 mg
Citric acid	0.207 mg
Sodium citrate	0.832 mg
Simethecone	0.646 mg

Experimental design

The Ethical Committee of the Institution had approved the protocol. Lycopene used in this study was LycoRed™ 2 mg softgels. After obtaining informed consent, the subjects were randomly distributed between the two treatment groups.

Experimental group (n = 10 subjects) took 8 mg lycopene daily in two equally divided doses for 2 weeks (Table 1).

Controls (n = 10 subjects) were asked to take the placebo according to the same regimen as the experimental group. The patients were asked to take 2 placebo capsules twice a day (4 capsules a day) for 2 weeks (Table 2). Because the composition of the placebo was different from the experimental drug, the placebo was pre-tested on ten different volunteers; five patients underwent full mouth scaling with the placebo and five patients underwent scaling only, and there was no significant difference between the groups in the reduction of the sulcular bleeding index (SBI) and the gingival index (GI) scores.

In each group, a within-subject split-mouth design was followed. In five patients of each group, oral prophylaxis was performed in the first and the third quadrants, while the second and the fourth quadrants did not receive any treatment, and vice versa. Quadrant allocation was done by coin-flipping; once one arm was filled, remaining subjects would automatically go into the other. Thus, in each group, 20 quadrants received oral prophylaxis (OP) while 20 quadrants did not receive any form of local treatment. Thus, in effect, there are four treatment groups: OP-lycopene group, non-OP-lycopene group, OP-placebo group and non-OP-placebo group. OP-placebo was considered as a positive control, while non-OP-placebo was considered as a negative control. No dietary limitations were imposed during or after treatment. Normal oral hygiene procedures were permitted except for the use of chemotherapeutic mouthrinses.

Measurement parameters

All examiners and subjects were 'blinded' concerning which subjects were in groups A and B. All examinations were performed by two calibrated periodontists (KHC and SR). The following measurements were recorded on case report forms and double entered into a computer: bleeding index (SBI; modified sulcular bleeding index from Mombelli et al [1987]), non-invasive measures of plaque (PI; the Turesky et al [1970]

Table 3 Gingival Index analysis: mean values at the baseline and following visits and percentage reduction from the baseline to 1 week and 2 weeks

Design	Treatment group	Baseline	1 week	Percentage reduction from baseline to 1 week	2 weeks	Percentage reduction from baseline to 2 weeks
Lycopene	OP	1.03 ± 0.21	0.18 ± 0.23**	29.14 ± 8.4	0.12 ± 0.14**	30.02 ± 7.17†
	Non-OP	1.25 ± 0.26	0.40 ± 0.26**	21.30 ± 9.57	0.30 ± 0.14**	24.14 ± 4.81‡
Placebo	OP	1.156 ± 0.20	0.71 ± 0.17*	10.78 ± 7.9	0.42 ± 0.14**	18.85 ± 7.29
	Non-OP	1.04 ± 0.19	0.76 ± 0.18*	9.02 ± 8.0	0.56 ± 0.13**	11.94 ± 6.55

** Statistically significant within group differences ($p < 0.001$) relative to baseline
* Statistically significant within group differences ($p < 0.05$) relative to baseline
† Statistically significant relative to non-OP-placebo ($p < 0.01$) and OP-placebo ($p < 0.05$)
‡ Statistically significant relative to non-OP-placebo ($p < 0.01$)

modification of the Quigley-Hein index) and gingivitis (GI; the Lobene et al [1986] modification of the Löe and Silness index) were assessed at baseline, 1 and 2 weeks. Erythrosine dye was used to disclose plaque after gingival index and bleeding measures.

Saliva collection and preparation

Samples of submandibular saliva (1 ml) were collected from each subject directly from the mouth of the patient by an automatic pipette (Salivette®, Sarsstadt, Germany), and were collected in sterile test tubes under standardised conditions (without stimulation) between 9.00 and 9.30 am (2 hours after breakfast). After sampling, the samples were stored tightly capped at 2–8°C and the analysis of the samples was performed on the same day. Saliva samples were centrifuged at 4000 g for 10 min at +4°C; the upper parts were drawn and stored in small aliquots at 40°C.

Determination of uric acid concentration

Uric acid concentration was measured according to the method proposed by Fossati et al (1980) using a commercially available reagent kit (Autopak®, Bayer Diagnostics, India). In this assay, uric acid was transformed by uricase into allantoin and hydrogen peroxide, which, under the catalytic influence of peroxidase, oxidised the chromogen (4-aminoantipyrine) to form a red compound whose intensity of colour was proportional to the amount of uric acid present in the sample; it was read at a wavelength of 510 nm (492–550 nm). The final colour of the reaction is stable for 15 minutes. Uric acid is a major (>70%) anti-oxidant in saliva. Its levels decrease in periodontal diseases but show little variation in various population groups (Diab-Ladki et al, 2003). The present study also attempts to evaluate whether extraneous antioxidant therapy (as with lycopene in this case) improved gingival health in situations where the levels of natural antioxidants such as uric acid are reduced due to various pathological states.

Data analysis

After completion of the clinical trial the group and quadrant allocation was revealed and statistical analysis carried out. The analysis was performed with a statistical software package (SPSS, Version 10.0.5. SPSS Inc, Chicago). For each treatment arm in both groups, the mean values and standard deviation for bleeding index, gingival index and plaque index was calculated at baseline, 1 and 2 weeks. The percentage reduction in gingivitis and gingival bleeding from the baseline to the 1-week and the 2-week recording was also calculated. Statistical analysis was performed on these values. The paired *t*-test and the independent *t*-test were used to analyse the within-group and between-group differences. Correlations between salivary uric acid levels and percentage reduction in gingivitis and gingival bleeding in both the groups were evaluated using the Pearson test.

Table 4 Bleeding index analysis: mean values at the baseline and following visits and percentage reduction from the baseline to 1 week and 2 weeks

Design	Treatment group	Baseline	1 week	Percentage reduction from baseline to 1 week	2 weeks	Percentage reduction from baseline to 2 weeks
Lycopene	OP	1.74 ± 0.39	1.00 ± 0.45**	26.2 ± 9.17	0.61 ± 0.34**	38.42 ± 9.81†
	Non-OP	1.73 ± 0.46	1.29 ± 0.48**	13.05 ± 8.51	1.03 ± 0.42**	23.31 ± 7.98
Placebo	OP	1.27 ± 0.31	0.32 ± 0.15**	31.88 ± 11.55	0.16 ± 0.10**	36.94 ± 11.61
	Non-OP	1 ± 0.45	0.47 ± 0.32**	17.41 ± 6.44	0.28 ± 0.10**	23.96 ± 12.4

** Statistically significant within group differences ($p < 0.001$) relative to baseline
† Statistically significant relative to non-OP-placebo ($p < 0.01$) and non-OP-lycopene ($p < 0.05$)

RESULTS

Patient characteristics

The dental examination at the onset of the study showed comparable findings on the oral status in the patients of both the groups. The GI values at the baseline in the OP-placebo group and the non-OP-placebo group were 1.156 ± 0.20 and 1.04 ± 0.19 respectively, and in the OP-lycopene and non-OP-lycopene group were 1.03 ± 0.21 and 1.25 ± 0.26 respectively. The PI values at the baseline in the OP-placebo group and the non-OP-placebo group were 2.99 ± 1.04 and 2.93 ± 0.98 respectively, and in the OP-lycopene and non-OP-lycopene group were 2.67 ± 0.67 and 2.60 ± 0.64 respectively. The mean uric acid levels in the experimental and the placebo group were 3.31 ± 0.65 mg/dl and 3.62 ± 0.78 mg/dl respectively. The SBI values at the baseline in the OP-placebo group and the non-OP-placebo group were 1.27 ± 0.31 and 1 ± 0.45 respectively, and in the OP-lycopene and non-OP-lycopene group were 1.74 ± 0.39 and 1.73 ± 0.46 respectively.

Clinical parameters

Within-group differences
Differences within the treatment group were analysed by using the paired *t*-test. All the treatment groups demonstrated statistically significant reductions in the GI, SBI and PI. In the OP-lycopene and the non-OP-lycopene groups, statistically significant ($p < 0.001$) decreases in the GI and the SBI were observed at 1 week and 2 weeks (Tables 3 and 4; Fig 1). In the OP-lycopene group, there was also a statistically significant ($p < 0.001$) reduction of plaque from 2.67 ± 0.67 to 1.47 ± 0.58 after 1 week and to 1.26 ± 0.71 after 2 weeks, whereas in the non-OP-lycopene group there was a statistically significant ($p < 0.001$) reduction of plaque from 2.60 ± 0.64 to 2.16 ± 0.56 after 1 week and to 1.9 ± 0.53 after 2 weeks.

In the OP-placebo group, statistically significant decreases in the GI ($p < 0.05$ at 1 week; $p < 0.001$ at 2 weeks) and the SBI ($p < 0.001$ at 1 and 2 weeks) was observed (Tables 3 and 4; Fig 1). In this group, there was also a statistically significant ($p < 0.001$) reduction of plaque from 2.99 ± 1.04 to 1.86 ± 0.48 at 1 week and to 1.18 ± 0.37 at 2 weeks. In the non-OP-placebo group, statistically significant decreases in the GI ($p < 0.05$ at 1 week; $p < 0.001$ at 2 weeks) and the SBI ($p < 0.001$ at 1 and 2 weeks) were observed (Tables 3 and 4). In this group, there was also a statistically significant ($p < 0.001$) reduction of plaque from 2.93 ± 0.98 to 2.56 ± 0.81 at 1 week and to 2.28 ± 0.80 at 2 weeks.

Between-group differences
In each group, the percentage reduction in GI and SBI from the baseline to 2 weeks was calculated and differences between the treatment groups were analysed by using the independent *t*-test. Treatment with OP-lycopene resulted in a statistically significant decrease in GI when compared with OP-placebo ($p < 0.05$) and non-OP-placebo ($p < 0.01$) (Table 3, Fig 1). Although the differences in the plaque reduction between OP-lycopene and OP-placebo (39.16 ± 10.80% vs. 35.7 ±

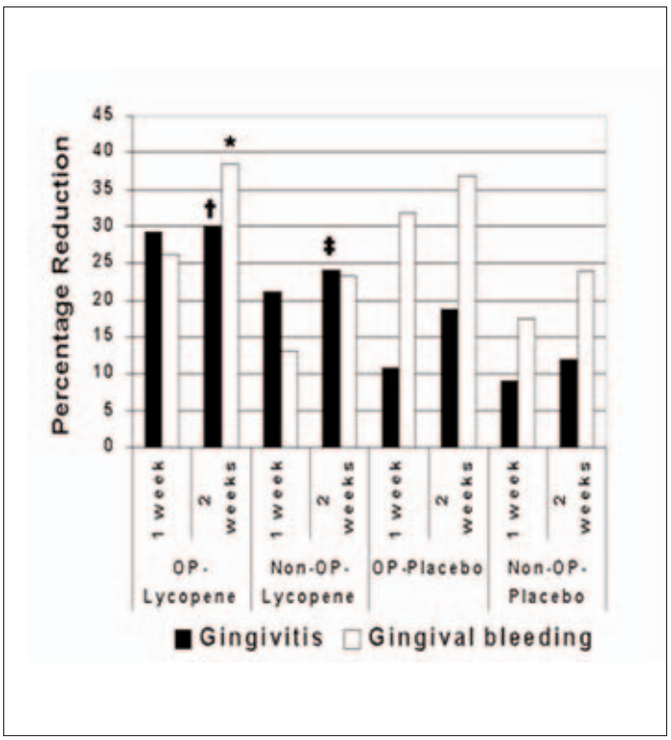

Fig 1 Percentage reduction in gingivitis and gingival bleeding at 1 and 2 weeks. († Statistically significant relative to non-OP-placebo and OP-placebo; ‡ statistically significant relative to non-OP-placebo; * statistically significant relative to non-OP-placebo ($p < 0.01$) and non-OP-lycopene).

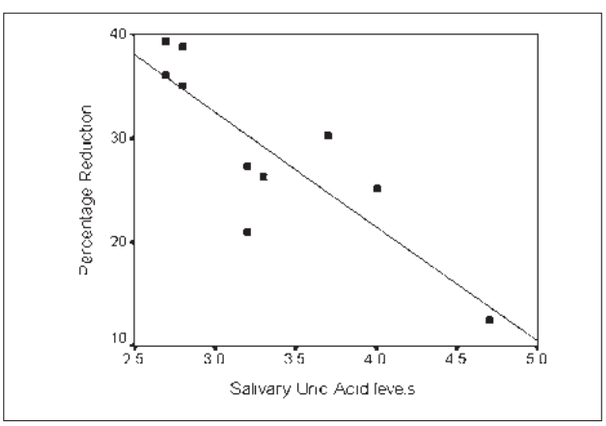

Fig 2 Scatter plot of salivary uric acid levels and percentage reduction in gingivitis at 1 week in the OP-lycopene group ($r = -0.852$).

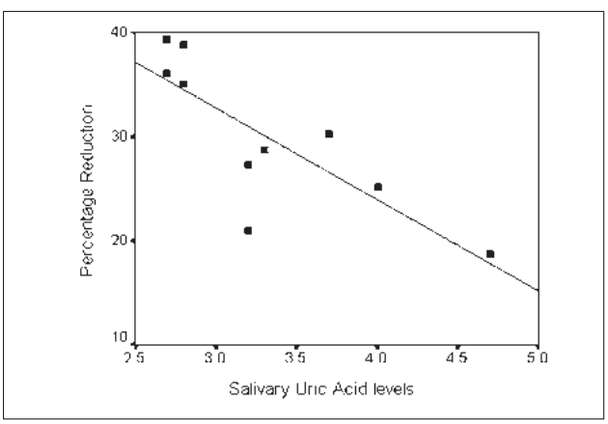

Fig 3 Scatter plot of salivary uric acid levels and percentage reduction in gingivitis at 2 weeks in the OP-lycopene group ($r = -0.802$).

16.67%) were not significant, a statistically significant decrease in the 2-week plaque scores was observed in the OP-lycopene group when compared with the non-OP-placebo group (39.16 ± 10.80% vs. 13.1 ± 3.91%). Treatment with non-OP-lycopene resulted in a statistically significant decrease in GI when compared with non-OP-placebo ($p < 0.01$), but no significant reduction could be observed when compared with the OP-placebo group (Table 3, Fig 1). The differences in the plaque reduction between the non-OP-placebo group and the non-OP-lycopene group were not significant (13.1 ± 3.91% vs. 14.14 ± 5.18%). There were no statistically significant differences in GI between OP-lycopene and non-OP-lycopene groups, although the mean percentage reduction was higher in the OP-Lycopene group. Similarly, there were no statistically significant differences in GI and SBI reduction between the OP-placebo and the non-OP-placebo groups, although the

Table 5 Relationship between salivary uric acid levels and the percentage reduction in gingivitis and gingival bleeding at the subsequent visits.

Salivary uric acid levels	vs.	Percentage reduction in gingivitis (baseline to 1 week)	Percentage reduction in gingivitis (baseline to 2 weeks)	Percentage reduction in bleeding (baseline to 1 week)	Percentage reduction in bleeding (baseline to 2 weeks)
OP-lycopene group	Pearson correlation	-0.852**	-0.802**	0.211	0.231
	Significance	0.002	0.005	0.559	0.521
	N	10	10	10	10
Non-OP-lycopene group	Pearson correlation	-0.640*	-0.580	-0.002	-0.204
	Significance	0.046	0.079	0.996	0.573
	N	10	10	10	10
OP-placebo group	Pearson correlation	-0.553	-0.478	0.348	0.283
	Significance	0.097	0.163	0.325	0.429
	N	10	10	10	10
Non-OP-placebo group	Pearson correlation	-0.463	-0.126	0.437	0.363
	Significance	0.178	0.730	0.207	0.302
	N	10	10	10	10

** Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.

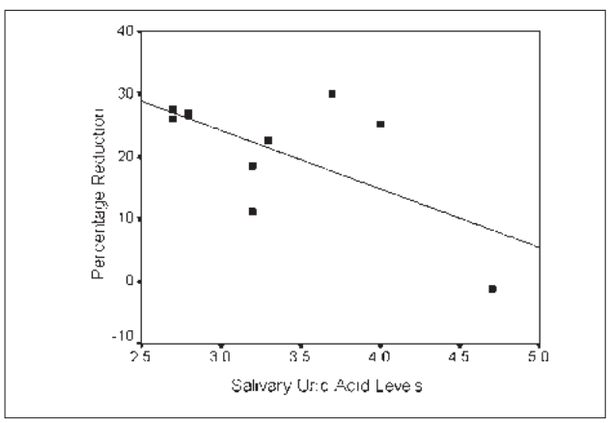

Fig 4 Scatter plot of salivary uric acid levels and percentage reduction in gingivitis at 1 week in the non-OP-lycopene group (r = -0.640).

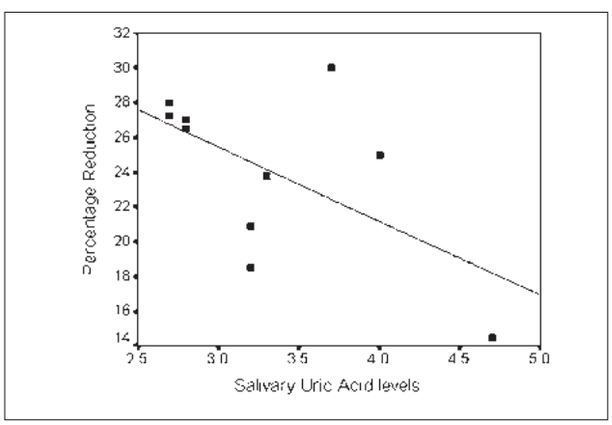

Fig 5 Scatter plot of salivary uric acid levels and percentage reduction in gingivitis at 2 weeks in the non-OP-lycopene group (r = -0.580).

mean percentage reduction was higher in the non-OP-lycopene group (Table 4, Fig 1). The OP-lycopene group showed a statistically significant reduction in SBI values when compared with the non-OP-lycopene group ($p < 0.05$) and the non-OP-placebo group ($p < 0.001$), but no significant reduction could be observed when compared with the OP-placebo group (Table 4, Fig 1).

Salivary uric acid analysis

There was a strong negative correlation between the salivary uric acid levels and the percentage reduction in GI at 1 and 2 weeks in the OP-lycopene group (r = -0.852 and -0.802 respectively). This correlation was significant at the 0.01 level (Table 5; Figs 2 and 3). However, in the non-OP-lycopene group there was a moderate negative correlation between the salivary

uric acid levels and the percentage reduction in GI (r = -0.640 and -0.580 respectively). This correlation at the first week was significant at the 0.05 level (Table 5; Figs 4 and 5). In the OP-placebo group, there was a moderate negative correlation between the salivary uric acid levels and the percentage reduction in GI at 1 week (r = -0.553), and a weak negative correlation between the salivary uric acid levels and the percentage reduction in GI at 2 weeks (-0.478). The non-OP-placebo group showed a weak negative correlation (Table 5) between the salivary uric acid levels and the percentage reduction in GI at 1 week and 2 weeks (r = -0.463 and -0.126 respectively). There was a weak correlation between the salivary uric acid levels and the percentage reduction in SBI in both the groups. The non-OP-lycopene group showed a weak negative correlation whereas the remaining groups showed a weak positive correlation (Table 5).

DISCUSSION

Whilst a myriad of possible mechanisms leading to the destruction of periodontal tissue exists, the influence of other damaging metabolic species such as ROS cannot be overlooked. This has led researchers to look to antioxidant therapy as a possible strategy for the treatment of periodontal disease. Treatment with drugs that block the production of free ROS or block its effects might be therapeutically valuable. In the present study, a lycopene preparation was tested over a period of 2 weeks in patients with mild inflammatory forms of periodontal disease (gingivitis). Because microbial deposits can induce the PMNs to release ROS (Sheikhi et al, 2000), the aim of the present study was to evaluate the effect of systemically administered lycopene (LycoRed™) both as a monotherapy in the presence of microbial deposits, and as an adjunct to scaling in gingivitis patients. The aim of this study was to evaluate the gingival changes as the drug is being taken, which was for 2 weeks since the effect of antioxidant drugs can be best observed when they are being administered to the subjects (Singh et al, 2004). The OP-lycopene group showed significant reduction in gingivitis when compared with the positive (OP-placebo) and the negative (non-OP-placebo) controls. The results seem to suggest that lycopene can be used as an adjunct to scaling in the control of gingivitis. Although the mean reduction in GI was higher in the OP-lycopene group than in the non-OP-lycopene group, there were no statistically significant differences between these two groups. Whether lycopene can be utilised as a 'stop-gap' monotherapy in the control of gingivitis, particularly during periods when oral prophylaxis needs to be deferred to a later date, requires investigation. Lycopene in itself does not seem to have an anti-plaque action with the non-OP-lycopene group showing a plaque reduction comparable to that of the non-OP-placebo group, but combined lycopene therapy and oral prophylaxis significantly reduced gingivitis, bleeding on probing and plaque accumulation.

Among the common carotenoids, lycopene stands as the most potent antioxidant, as demonstrated by *in vitro* experimental systems (Di Paola et al, 2005). The antioxidant potency of lycopene carotenoids is greater than alpha-tocopherol, α-carotene, ß-cryptoxanthin, zeaxanthin, ß-carotene and lutein (Levy and Sharoni, 2004). Mixtures of carotenoids are more effective than single compounds (Levy and Sharoni, 2004). This synergistic effect was more pronounced when lycopene or lutein was present. The superior protection of mixtures may be related to the specific positioning of different carotenoids in cell membranes (Levy and Sharoni, 2004). In the present study, 100% natural lycopene with added phytonutrients such as phytoene, phytofluene, ß-carotene, phytosterols, and vitamin E was used. A thorough safety review by an independent panel of toxicologists has resulted in a GRAS (generally recognised as safe) self-affirmation for lycopene compounds (Levy and Sharoni, 2004).

Various antioxidants have been tried in the field of periodontics. A clinical study showed that the local use of perftoran in patients with periodontal disease led to a significant reduction of lipid peroxidation (LPO) and significant increase of antioxidant activity (AOA) in saliva. The most prominent effect was observed in patients with medium or severe degree of the disease (Grudianov and Chupakhin, 2005). Previous studies did not support the use of vitamin E gel (Cohen et al, 1991) or Vitamin C supplementation (Vogel et al, 1986) for the control of gingivitis or periodontal disease. However, in one clinical study, gingival bleeding increased significantly after the period of vitamin C depletion and returned to baseline values after the period of vitamin C repletion (Leggott et al, 1991). Modest administration of lycopene may be a much more effective approach than supplementation with antioxidants such as vitamins C and E, which stoichiometrically scavenge a very small fraction of total oxidant production (Neldon et al, 2006).

Recent research suggests that mixtures of antioxidants are more effective than the single compounds (Levy and Sharoni, 2004) and the synergistic effect is more pronounced when lycopene or lutein is present.

In the present study the response to antioxidant therapy was correlated to the uric acid levels. Uric acid

is a major (>70%) antioxidant in saliva, and salivary uric acid levels are decreased in periodontal diseases but show little variation in various population groups (Moore et al, 1994). There is increasing experimental and clinical evidence showing that uric acid has an important role *in vivo* as an antioxidant (Glantzounis et al, 2005). Fewer studies have investigated individual antioxidants than measured the total antioxidant capacity (TAC) (Mashayekhi et al, 2005), trolox equivalent antioxidant capacity (TEAC) (Mashayekhi et al, 2005) or total peroxyl radical trapping parameter (TRAP) (Wayner et al, 1987), which holistically show the total antioxidant power (Mashayekhi et al, 2005); however, in the present study, uric acid levels were assessed because this analysis is easy to perform, shows little variation (Schermann et al, 1977) and is not dependent on the salivary flow rate (Mason et al, 1963). This study is in agreement with a previous study, which reported that the total antioxidant activity of saliva is significantly decreased in these patients, despite the fact that the levels of the three main antioxidants (uric acid, ascorbic acid, and albumin) are not significantly affected (Diab-Ladki et al, 2003). Uric acid levels in the present study were within the normal range but there was a better response to antioxidant therapy in subjects with lower uric acid levels. This response was further enhanced in groups where oral prophylaxis was performed than in the non-oral-prophylaxis groups. The correlation between reduction in gingivitis and uric acid in the experimental group levels was stronger than that of the placebo group. In general, the correlation between salivary uric acid levels and gingivitis reduction was stronger than the correlation between salivary uric acid levels and reduction in gingival bleeding. Whether antioxidant therapy has a 'corrective action' on the natural antioxidants needs to be investigated.

In conclusion, the results presented in the present study suggest that lycopene shows great promise as a treatment modality in gingivitis. The possibility of obtaining an additive effect by combining routine oral prophylaxis with lycopene is also an exciting possibility, which deserves further study.

ACKNOWLEDGEMENT

This study was supported by Jagsonpal Pharmaceuticals Limited, Shahpur Jat, New Delhi, India. No financial relationships exist between any of the authors and Jagsonpal Pharmaceuticals Limited.

REFERENCES

1. Altman LC, Baker C, Fleckman P, Luchtel D, Oda D. Neutrophil mediated damage to human gingival epithelial cells. J Periodontal Res 1992;27:70-79.
2. Bohm F, Tinkler JH, Truscott TG. Carotenoids protect against cell membrane damage by the nitrogen dioxide radical. Nat Med 1995;1:98-99.
3. Chapple ILC. Reactive oxygen species and antioxidants in inflammatory diseases. J Clin Periodontol 1997;24:287-296.
4. Cohen RE, Ciancio SG, Mather ML, Curro FA. Effect of vitamin E gel, placebo gel and chlorhexidine on periodontal disease. Clin Prev Dent 1991;13:20-24.
5. Deguchi S, Hori T, Creamer H, Gabler W. Neutrophil mediated damage to periodontal ligament fibroblasts: role of lipopolysaccharide. J Periodontal Res 1990;25:293-299.
6. Diab-Ladki R, Pellat B, Chahine R. Decrease in the total antioxidant activity of saliva in patients with periodontal diseases. Clin Oral Investig 2003;7:103-107.
7. Di Mascio P, Kaiser S, Sies H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 1989;274:532-538.
8. Di Paola R, Mazzon E, Zito D, Maiere D, Britti D, Genovese T, Cuzzocrea S. Effects of Tempol, a membrane-permeable radical scavenger, in a rodent model periodontitis. J Clin Periodontol 2005;32:1062-1068.
9. Firatli E, Unal T, Onan U, Sandalli P. Antioxidative activities of some chemotherapeutics. A possible mechanism in reducing gingival inflammation. J Clin Periodontol 1994;21:680-683.
10. Fossati P, Prencipe L, Berti G. Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin Chem 1980;26:227-231.
11. Freeman BA, Crapo JD. Free radicals and tissue injury. Lab Investig 1982;47:412-424.
12. Fuhrman B, Ben-Yaish L, Attias J, Hayek T, Aviram M. Tomato lycopene and b-carotene inhibit low density lipoprotein oxidation and this effect depends on the lipoprotein vitamin E content. Nutr Metab Cardiovasc Dis 1997;7:433-443.
13. Glantzounis GK, Tsimoyiannis EC, Kappas AM, Galaris DA. Uric acid and oxidative stress. Curr Pharm Des 2005;11:4145-4151.
14. Goultschin J, Levy H. Inhibition of superoxide generation by human polymorphonuclear leukocytes with chlorhexidine. Its possible relation to periodontal disease. J Periodontol 1986;57:422-425.
15. Grudianov AI, Chupakhin PV. Perfluorine (perftoran) influence on lipid peroxidation and salivary antioxidant activity in patients with periodontal disease [in Russian]. Stomatologiia (Mosk) 2005;84:16-19.
16. Leggott PJ, Robertson PB, Jacob RA, Zambon JJ, Walsh M, Armitage GC. Effects of ascorbic acid depletion and supplementation on periodontal health and subgingival microflora in humans. J Dent Res 1991;70:1531-1536.
17. Levy J, Sharoni, Y. The functions of tomato lycopene and its role in human health. Herbal Gram 2004;62:49-56.
18. Lobene RR, Weatherford T, Ross NM, Lamm RA, Menaker L. A modified gingival index for use in clinical trials. Clin Prev Dent 1986;8:3-6.
19. Mashayekhi F, Aghahoseini F, Rezaie A, Zamani MJ, Khorasani R, Abdollahi M. Alteration of cyclic nucleotides levels and oxidative stress in saliva of human subjects with periodontitis. J Contemp Dent Pract [Serial Online] 2005;4:46-53. Available from: Procter & Gamble Company, Cincinnati, OH. Accessed March 15, 2006.

20. Mason DK, Boyle R M, Duncan AM, Greig WR. The influence of flow rate on the concentration of uric acid in human parotid and submandibular saliva. J Dent Res 1963;42:1015-1022.
21. Mombelli A, van Oosten MAC, Schürch E, Lang NP. The microbiota associated with successful or failing implants. Oral Microbiol Immunol 1987;2:145-151.
22. Moore S, Calder KA, Miller NJ, Rice-Evans A. Antioxidant activity of saliva and periodontal disease. Free Radic Res 1994;21:417-425.
23. Murrell G, Francis N. Bromley L. Modulation of fibroblast proliferation by oxygen free radicals. Biochemistry 1990;265:659-665.
24. Nelson SK, Bose SK, Grunwald GK, Myhill P, McCord JM. The induction of human superoxide dismutase and catalase in vivo: a fundamentally new approach to antioxidant therapy. Free Radic Biol Med 2006;40:341-347.
25. Nir Z, Hartal D. Tomato lycopene: the phytonutrient of the new millennium. Food Ind J 2000;3:208-219.
26. Schermann JM, Meunier J, Ricordel I, Masbernard A, Giudicelli C. Comparative study of uric acid concentration in serum and saliva of healthy or hyperuricemic subjects. Ann Biol Clin (Paris) 1977;35:467-472.
27. Sheikhi M, Gustaffson A, Jarstrand C. Cytokine, elastase and oxygen radical release by Fusobacterium nucleatum activated leucocytes: a possible factor for periodontitis. J Clin Periodontol 2000;27:758-762.
28. Singh M, Krishnappa R, Bagewadi A, Keluskar V. Efficacy of oral lycopene in the treatment of oral leucoplakia. Oral Oncol 2004;40:591-596.
29. Turesky S, Gilmore ND, Glickman I. Reduced plaque formation by the chloromethyl analogue of victamine C. J Periodontol 1970;41:41-43.
30. Vogel RI, Lamster IB, Wechsler SA, Macedo B, Hartley LJ, Macedo JA. The effects of megadoses of ascorbic acid on PMN chemotaxis and experimental gingivitis. J Periodontol 1986;57:472-479.
31. Wayner DD, Burton GW, Ingold KU, Barclay LR, Locke SJ. The relative contributions of vitamin E, urate, ascorbate and proteins to total peroxyl radical trapping parameter activity of the human plasma. Biochim Biophys Acta 1987;924:408-419.
32. Wood N, Johnson RB. The relationship between tomato intake and congestive heart failure risk in periodontitis subjects. J Clin Periodontol 2004;31:574-580.
33. Young IS, Woodside JV. Antioxidants in health and disease. J Clin Pathol 2001;54:176-186.