Anti-caries effect of CPP-ACP in irradiated nasopharyngeal carcinoma patients

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ABSTRACT

Objective: Determine the effect of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) on caries progression in irradiated nasopharyngeal carcinoma (NPC) patients.
Methods: 21 males and 3 females (median age: 50 years) were randomized into two groups before radiotherapy. Subjects had at least eight teeth after oral health clearance. The test group used 0.4% stannous fluoride gel and a crème containing 10% CPP-ACP daily; the control group used a similar crème without CPP-ACP and otherwise identical care. Subjects applied the crème three-times-daily and fluoride gel once daily. Caries status, saliva and plaque parameters were measured pre-radiotherapy, at 2-weeks and 3-months post-radiotherapy.
Results: Baseline ICDAS scores were: **0**-1126 surfaces (93.9%); **1**-28 surfaces (2.3%); **2**-40 surfaces (3.3%); **3**-6 surfaces (0.5%) for the control; and **0**-1186 surfaces (95.6%); **1**-31 surfaces (2.5%); **2**-15 surfaces (1.2%); **3**-8 surfaces (0.7%) for the test group. 22 subjects returned at 3-month post-radiotherapy with reduced plaque pH, salivary flow, pH and buffering capacity. 9 test and 8 control subjects developed 32 and 59 new caries lesions respectively. Test subjects showed lower caries progression than the controls: all surfaces (OR: 0.51, 95% CI: 0.17~1.59), occlusal (OR: 0.20, 95% CI: 0.03~1.29) and smooth surfaces (OR: 0.61, 95% CI: 0.16~2.38). The difference was not statistically significant.
Conclusion: Application of CPP-ACP did not significantly reduce caries progression in NPC patients in the first three months after radiotherapy as compared to controls.
Clinical relevance: Adjunct use of CPP-ACP with stannous fluoride gel in irradiated NPC patients gave comparable results compared to stannous fluoride gel alone in reducing caries progression.

Keywords

Nasopharyngeal carcinoma, Head and neck radiotherapy, Xerostomia, Dental caries, Remineralizing agent, CPP-ACP

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is the 8th most common cancer affecting Singaporean males with an age-standardized rate of 9.5 per 100,000 per year[38]. The majority is referred by the National Cancer Centre Singapore (NCCS) to the National Dental Centre Singapore (NDCS) for pre-radiotherapy oral health assessment and clearance. As NPC is highly radiosensitive, the standard treatment modality at the NCCS is intensity-modulated radiotherapy (IMRT) with or without chemotherapy [1, 43]. The 3-year disease-free survival rate of NPC patients treated at the NCCS is 82.1% [41]. However, salivary dysfunction due to radiation effects on salivary glands still persists, resulting in chronic oral complications [24, 36, 42] and a deterioration on their quality of life (QOL) [16].

The major post-irradiation oral complication is dry mouth (xerostomia) [22, 29], with the risk of development of radiation caries, a highly destructive form of dental caries [11, 35]. Radiotherapy changes the quantity, quality and composition of saliva, resulting in decreases in salivary flow, buffering capacity, electrolyte and immunoprotein levels, oral clearance and an increase in acidity and cariogenic bacteria [6, 7, 10, 11, 21, 23]. The reported weighted prevalence of dental caries was 24% in post-radiotherapy patients and 21.4% in post-chemo-and-radiotherapy patients, with a mean DMFT (decayed, missing, filled teeth) of 17.01 for post-radiotherapy patients compared to a mean DMFT of 4.4 for healthy controls [18]. In long-term head-and-neck (H&N) cancer survivors, higher DMFT scores were associated with worse scores for various QOL domains [12]. Hence, caries disease sequelae adversely affect the patient's QOL.

The major effect of fluorides as a caries preventive measure lie in the remineralization potential of saliva. Remineralization cannot occur if the degree of saturation of calcium in saliva with respect to tooth mineral is low [2]. It can be enhanced by the provision of bioavailable calcium and phosphate ions, in conjunction with the correct ionic ratio of fluoride. The remineralizing system with the most extensively researched technology uses casein phosphopeptide (CPP) from bovine milk to stabilize calcium and phosphate ions forming casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) complexes [8]. CPP-ACP acts as a delivery vehicle to co-localise and stabilize bioavailable calcium, phosphate and fluoride ions at the tooth surface in a form that drives diffusion down activity gradients into the subsurface caries lesion [30, 32]. CPP-ACP also increases the plaque calcium and phosphate levels [31] and binds to the surface of *Streptococcus mutans* [34], forming a reservoir of bioavailable calcium and phosphate ions [-25]. Saliva is important as it allows the calcium and phosphate stabilized by CPP-ACP to become ionic [31]. In H&N RT patients where there is significant reduction in salivary flow, this ionic conversion may be severely challenged. CPP-ACP is now available commercially as Tooth Mousse® (also available as MI Paste® in different markets) (GC Corp, Tokyo, Japan). In situ studies have shown the synergistic effect of CPP-ACP with fluoride in promoting remineralization in healthy people [37].

Randomized controlled clinical trials have shown the effectiveness of CPP-ACP in reducing caries progression [26] and promoting caries regression [5] in healthy subjects.

However, research findings derived from the healthy population cannot be readily generalized to NPC patients treated with radiotherapy. Currently, there are no published reports on the effectiveness of remineralization applying CPP-ACP as an adjunct to fluoride therapy in patients with impaired salivary function. The aim of this study was to assess the effect of CPP-ACP on caries progression in NPC patients treated with IMRT.

MATERIALS and METHODS

Study subjects were identified from NPC patients referred by the NCCS radiation oncologists for pre-radiotherapy oral health clearance. The eligible subjects selected based on the inclusion and exclusion criteria were given a plain language statement by the NDCS study research coordinator and informed consent was obtained. Subjects were 21 years of age or older; had at least eight remaining teeth after oral health clearance; had no known milk allergy and were able to give informed consent. Individuals who had undergone head and neck radiotherapy previously or were receiving palliative care were excluded. Informed consent was signed before randomization but after the subject had completed the oral health clearance and met all eligibility criteria. 24 subjects were recruited for this study. The Institutional Review Boards of NCCS and NDCS granted approval.

This study was a randomized double-blind placebo-controlled study to assess the effectiveness of a crème containing 10% CPP-ACP versus a placebo crème on caries progression in NPC patients treated with IMRT. Test (10% CPP-ACP w/w) and placebo crèmes were prepared and packed in pre-weighed coded packages by the manufacturer, GC Corp (Tokyo, Japan). The placebo crème had exactly the same constituents as the test crème except that it did not contain any CPP-ACP.

All subjects were provided with toothpaste containing 0.32% sodium fluoride (Colgate Total® Professional Clean, Colgate, NY, USA), a soft-textured toothbrush and 0.4% stannous fluoride gel (Gel-Kam®, Colgate, NY, USA) to be applied in a custom tray. Subjects were instructed to use the fluoride gel once-daily for five min at night according to the manufacturer's instructions, after normal oral hygiene procedures; and to use the crème three-times-daily, once in the morning, afternoon and night. They were advised to use a pea-sized amount of crème for each dental arch placed on the tooth surfaces using a clean, dry finger and left undisturbed for five min; following which they would then use their tongue to spread the remaining crème throughout the mouth for a further two min before expectorating the excess. In the event that there is pooling of saliva, the subjects were informed to swallow the pooled saliva. The nightly application of the crème was carried out 10 mins after the use of the fluoride gel. Subjects were advised not to rinse, eat or drink for 30 min after the application of both the crème and fluoride gel.

Subjects were assigned to the intervention group (fluoride gel + CPP-ACP crème) and placebo group (fluoride gel + placebo crème) based on the randomization list prepared by

the Singapore Clinical Research Institute. The clinical examiners, subjects and other research personnel were blinded to the crème type as they were packed in pre-weighed coded packages identical in size and color. Unblinding for an individual was allowed due to medical emergency. An emergency envelope with the corresponding randomization code was kept in a secure location accessible only to limited study personnel. Unblinding after study completion was allowed after the database was locked.

Subjects were instructed not to use any other salivary substitutes, fluoride or calcium phosphate-containing home care products. Each subject was provided with a diary to record the use of any such product and the reason for its use. All products were dispensed at the pre-radiotherapy visit and replenished at the study review visits by the study coordinator. At every visit all tubes of fluoride gel and crème provided in the previous examination visit were returned and weighed to determine the amount of product used. The diaries were collected by the study coordinator at every examination visit and reviewed by the study team.

Subjects were given a dental prophylaxis at the pre-radiotherapy visit, before the start of a 7-week treatment of intensity-modulated radiotherapy. They were assessed at the following visits: pre-radiotherapy (baseline) before intervention (pre-RT), 2-weeks post-radiotherapy (post-RT 1) and 3-months post-radiotherapy (post-RT 2). The study coordinator telephoned subjects once-per-week to monitor compliance and provide help and advice where needed. To minimize loss to follow-up, reminder letters and phone calls were carried out. Safety was assessed through routine adverse event monitoring and reporting protocol of NDCS.

Subjects were asked not to brush their teeth 24 hours prior to the study visits and to refrain from eating and drinking one hour prior to the study visits. Resting and stimulated saliva flow rate measurements were carried out according to established protocols. Subjects sat in a relaxed position with the head tilt slightly forward and spat repeatedly into a pre-weighed container for five min. Resting saliva volume was measured gravimetrically, assuming a specific gravity of 1.0 and the flow rate (ml/min) recorded. Stimulated saliva was obtained by asking the subject to chew on a piece of non-flavored paraffin wax for five min, spitting regularly into a pre-weighed container and the stimulated saliva volume measured gravimetrically. Saliva pH was determined using a pH meter (Orion model 9810BN, Thermo Scientific, MA, USA). The buffering capacity of both resting and stimulated saliva was determined according to manufacturer's instructions (Saliva-Check Buffer, GC Corp, Tokyo, Japan). Plaque was collected from all tooth surfaces using a dental explorer except from the lower anterior lingual surfaces and its pH determined using the pH meter.

The caries status of the coronal tooth surfaces was scored according to the International Caries Detection and Assessment System (ICDAS II) visual criteria [20], which consists of a seven-point ordinal scale to describe the structural integrity of each tooth surface and ranges from 'sound' to 'non-cavitated' to 'extensive distinct cavity' (ICDAS score 0 to 6). Each subject was evaluated by one of four trained examiners using a blunt-ended probe.Each cleaned coronal (occlusal and smooth) surface was examined whilst wet, then air-dried for 5 sec and re-examined. For ease of convenience, follow-up visits were

arranged together with their oncologic visits, with the same examiner or another trained examiner depending on the examiner's availability. Following assessment of the coronal caries status at the three study visits, the surface level transition was scored accordingly. Lesion progression was accorded whereby the appropriate positive scores were given when transitions to more severe grades of lesions occurred. Similarly, lesion regression was accorded whereby the appropriate negative scores were given when transitions to less severe grades of lesions occurred.

Statistical Analysis

Non-parametric Mann-Whitney U test was used to compare the change in saliva and plaque parameters from baseline to 3-month post-radiotherapy between the two study groups. Logistic regression was used to evaluate the effect of the intervention on caries progression on coronal surfaces, with robust variance estimation to adjust for the within-subject correlation. Odds ratio, 95% confidence intervals as well as p-values were calculated. P-value (two-sided) <0.05 was considered statistically significant. Statistical analyses were done using Stata version 12.1 (Stata Corporation, College Station, Texas, USA).

RESULTS

The demographic and clinical characteristics for the two study groups are shown in Table 1. The age difference of subjects in the two groups was not statistically significantly different. Of the 24 randomized subjects, 22 returned for the 3-month post-radiotherapy review, with one drop-out in each group.

The plaque and saliva characteristics at 3-months post-radiotherapy are shown in Table 2. The changes from baseline to 3-months post-radiotherapy were not statistically significant between the two study groups for all the parameters considered.

All restorative work was carried out before recruitment. At baseline, in the placebo group, the numbers (%) of tooth surfaces with ICDAS 0, 1, 2 and 3 were, espectively, 1126 (93.9%), 28 (2.3%), 40 (3.3%) and 6 (0.5%); whereas in the intervention group, the respective numbers (%) were 1186 (95.6%), 31 (2.5%), 15 (1.2%) and 8 (0.7%). At 2-weeks post-radiotherapy, there was a small number of caries lesions developing but due to the small numbers involved, the data was not tabulated or analyzed. At 3-months post-radiotherapy, 9 subjects in the intervention group and 8 subjects in the placebo group developed 32 and 59 new caries lesions respectively: 38 smooth surfaces and 10 occlusal surfaces had ICDAS score 1; 27 smooth surfaces and 7 occlusal surfaces had ICDAS score 2; 8 smooth surfaces had ICDAS score 3 and one smooth surface had ICDAS score 5 (not tabulated).

In the intervention group 93.4% (n=1158) of the tooth surfaces compared to 89.5% (n=1074) in the placebo group remained stable at 3-months post-radiotherapy (Table 3). Caries regression (transition matrix scores –1 to –3) was seen on 4.1% and 5.6% of the

surfaces in the intervention and placebo groups respectively whilst caries progression (transition matrix scores 1 to 5) was seen on 2.6% and 5.0% of the surfaces in the intervention and placebo groups respectively. When the transition matrix was collapsed to a dichotomous score, ie progression (transition matrix scores 1 to 4) versus no progression (transition matrix scores -3 to 0), there was a trend for CPP-ACP to decrease the rate of progression of caries lesions compared to the placebo group and was more efficacious with time (2-weeks post-radiotherapy: OR: 0.76, 95% CI: 0.3~1.92; 3-months post-radiotherapy: OR: 0.51, 95% CI: 0.17~1.59) (Table 4), but none reached statistical significance.

At 3-months post-radiotherapy, the intervention group showed a lower rate of caries progression for all tooth surfaces (OR: 0.51, 95% CI: 0.17~1.59), with the occlusal surfaces showing lower caries progression (OR: 0.20, 95% CI: 0.03~1.29) as compared to smooth surfaces (OR: 0.61, 95% CI: 0.16~2.38), but it was not statistically significant.

DISCUSSION

Saliva plays a major role in protecting the teeth from acid challenge [39]. Early studies on H&N RT patients have shown that hyposalivation-related caries formation is characterized by rapid onset and progression, and can be initiated at any part of the tooth surface, even on sites that are normally resistant to the caries [11, 15]. Frank cavities can appear as early as three months after completion of RT. The advantage of newer targeted RT techniques such as IMRT, allows one or both of the parotid glands to be spared and placed outside the target coverage if there was no secondary metastasis or if the tumor did not affect the parotid gland itself. However, despite parotid gland sparing through improved RT techniques such as IMRT, hyposalivation conditions remain as the submandibular, sublingual and minor salivary glands are still included in the treatment portal.

In the present study, despite the use of IMRT, both resting and stimulated saliva flow rates decreased significantly, declining to less than 10% of the pre-radiotherapy flow rates after 3 months post-radiotherapy. The saliva pH and buffering capacity and plaque pH were also affected negatively. Caries lesions started to develop as early as 2 weeks post-radiotherapy and at 3 months post-radiotherapy, the placebo group showed a greater proportion of new caries lesions as compared to the intervention group.

The recommended mode of application for caries control in H&N RT patients was the use of custom tray-applied fluoride gel, however, with limited success in non-compliant patients [3, 11, 13, 19]. Control of post-radiation caries disease requires constant lifelong compliance, which is difficult for this group of patients. Fluoride is effective at preventing caries when present at low concentration at the tooth surface [14, 27,40]. Remineralization is limited by the amount of bioavailable calcium and phosphate [32] and the simultaneous provision of ionic calcium, phosphate and fluoride can reduce caries risk [4, 33] especially in those with salivary hypofunction [17, 28].

The use of fluoridated toothpaste containing calcium and phosphate in a group of H&N RT patients produced lower net root surface caries increment compared to a conventional fluoridated toothpaste group [28]. In two groups of patients with salivary gland dysfunction: those having H&N RT and others with Sjögren's syndrome; a mouthrinse containing casein derivatives complexed with calcium phosphate was tested [17]. The authors reported that the coronal caries incidence and the coronal caries increment were lower for the mouthrinse group in the H&N RT patients compared to those using a 0.5% sodium fluoride mouthrinse, but the difference was not statistically significant.

CPP-ACP stabilizes calcium, phosphate and fluoride ions allowing for super-saturation of and maintenance of high concentration gradients at the tooth surface, driving calcium and phosphate ions into the carious lesion, promoting remineralization. In the present study, both the intervention and placebo groups used toothpaste containing 0.32% sodium fluoride and trays containing 0.4% stannous fluoride gel. In addition, the intervention group used a crème containing 10% CPP-ACP whilst the placebo group used an identically similar crème without CPP-ACP. The intervention group showed a trend of a lower rate of caries progression for all tooth surface types although the effect was not statistically significant: likely to be due to the small sample size of the study. The increased residual saliva volume on occlusal surfaces compared to smooth surfaces [9] which allows for greater availability of calcium and phosphate stabilized by CPP-ACP, could be a possible reason for the lower rate of caries progression observed on occlusal surfaces.

H&N RT survivors might find it difficult to apply both the fluoride gel in custom tray carriers and CPP-ACP crème daily. A product containing calcium, phosphate and fluoride ions in the ratio present in apatite crystals in tooth structure is now available as Tooth Mousse Plus® (also available as MI Paste Plus® in different markets) (GC Corp, Tokyo, Japan). Further research is required to determine the effectiveness of Tooth Mousse Plus® as compared to custom tray-applied fluoride gel. This product has probably some potential to provide the mineral ions required for optimal effective remineralization in a single delivery vehicle, facilitating ease of use and compliance.

CONCLUSION

Reduction in saliva flow rates, pH and buffering capacity and plaque pH were observed in NPC patients treated with IMRT. New caries lesions were detected within three months after completion of radiotherapy. Within the limitations of the present study, a crème containing CPP-ACP did not statistically significantly reduce the rate of progression of caries lesions on coronal tooth surfaces.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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Table 1: Baseline characteristics

Demographics		CPP-ACP (n=12)	Placebo (n=12)
Sex	Male	11(91.7%)	10(83.3%)
	Female	1 (8.3%)	2 (16.7%)
Age (years)		47.5(44.9-52.8)	56.6(45.3-61.1)
Ethnicity	Chinese	12 (100%)	12 (100%)
Smoking history	No	2(16.7%)	8(66.7%)
	Yes	4(33.3%)	1(8.3%)
	Quitted	5(41.7%)	3(25%)
Alcohol consumption		6(50%)	7(58.3%)
Betel nut chewing habit	No	12(100%)	12(100%)
Tumour/treatment characteristics			
Tumour Stage Grouping (AJCC[a])	I	1(8.3%)	0(0%)
	II	2(16.7%)	5(41.7%)
	III	7(58.3%)	4(33.3%)
	IV	2(16.7%)	3(25%)
Treatment	Radiotherapy only	3(25%)	4(33.3%)
	Radiotherapy and chemotherapy	9(75%)	8(66.7%)
Radiation dose (Gy)		70(70~70)	70(70~70)
Baseline ICDAS scores	0	1186 (95.6%)	1126 (93.9%)
	1	31 (2.5%)	28 (2.3%)
	2	15 (1.2%)	40 (3.3%)
	3	8 (0.7%)	6 (0.5%)

Data are number of subjects (%) or median (inter-quartile range).
[a]AJCC = American Joint Committee on Cancer

Table 2: Change in saliva and plaque characteristics

	Visit	CPP-ACP		Placebo		P value
		n	Median (IQR)	n	Median (IQR)	
Resting saliva flow rate	Baseline (Pre-RT)	12	0.69(0.24, 0.9)	12	0.34(0.18, 0.47)	
	3-month Post-RT (Post-RT 2)	11	0.1(0, 0.15)	11	0(0, 0.04)	
	Change from baseline	11	-0.55(-0.88, -0.15)	11	-0.31(-0.44, -0.13)	0.278
Resting saliva pH	Baseline (Pre-RT)	12	7.09(6.88, 7.35)	12	7.39(7.13, 7.61)	
	3-month Post-RT (Post-RT 2)	8	5.87(5.6, 5.98)	6	6.24(5.65, 6.81)	
	Change from baseline	8	-1.15(-1.63, -0.89)	6	-1.19(-2.19, -0.46)	0.897
Resting saliva buffering capacity	Baseline (Pre-RT)	12	7.5(5, 8.5)	12	8(5.5, 9.5)	
	3-month Post-RT (Post-RT 2)	8	2.5(2, 4.5)	6	2.5(1, 5)	
	Change from baseline	8	-1.5(-6, -0.5)	6	-6(-8, -4)	0.219
Stimulated saliva flow rate	Baseline (Pre-RT)	12	1.4(0.96, 2.75)	12	1.26(0.86, 1.63)	
	3-month Post-RT (Post-RT 2)	11	0.1(0, 0.21)	11	0.01(0, 0.08)	
	Change from baseline	11	-1.05(-2.64, -0.92)	11	-1.27(-1.51, -0.85)	0.412
Stimulated saliva pH	Baseline (Pre-RT)	12	7.79(7.25, 7.86)	12	7.86(7.74, 8.03)	
	3-month Post-RT (Post-RT 2)	8	6.5(5.86, 6.77)	6	6.31(5.91, 7.32)	
	Change from baseline	8	-0.92(-1.82, -0.48)	6	-1.34(-1.71, -0.68)	0.439
Stimulated saliva buffering capacity	Baseline (Pre-RT)	12	10.5(9, 12)	12	10(8, 10.5)	
	3-month Post-RT (Post-RT 2)	8	5(4, 5)	6	5(3, 8)	
	Change from baseline	8	-6(-8, -1)	6	-6(-7, -2)	0.745
Plaque pH	Baseline (Pre-RT)	11	6(5.56, 6.75)	10	5.96(5.63, 6.64)	
	3-month Post-RT (Post-RT 2)	9	5.12(4.88, 5.33)	9	5.45(5.21, 6.11)	
	Change from baseline	8	-0.89(-1.61, -0.43)	8	-0.26(-1.31, 0.17)	0.208

Table 3: Frequency distribution of transition matrix scores (number of lesions) at 3-months post-radiotherapy

Transition scores (baseline to post-RT 2)	CPP-ACP n (%)	Placebo n (%)
-3	6 (0.5)	5 (0.4)
-2	11 (0.9)	31 (2.6)
-1	33 (2.7)	31 (2.6)
0	1158 (93.4)	1074 (89.5)
1	18 (1.5)	32 (2.7)
2	10 (0.8)	24 (2)
3	4 (0.3)	2 (0.2)
4	0 (0)	0 (0)
5	0 (0)	1 (0.1)
Total	1240 (100)	1200 (100)

Table 4: Effect of CPP-ACP in caries progression

		Category	CPP-ACP n(%)	Placebo n(%)	Odds Ratio (95% CI)
All surfaces	Post-RT 1	0 (no progression)	1202 (96.9%)	1051 (96.0%)	0.76 (0.3 ~ 1.92)
		1 (progression)	38 (3.1%)	44 (4.0%)	
	Post-RT 2	0 (no progression)	1208 (97.4%)	1141 (95.1%)	0.51 (0.17 ~ 1.59)
		1 (progression)	32 (2.6%)	59 (4.9%)	
Occlusal surfaces	Post-RT 1	0 (no progression)	241(97.2%)	217(99.1%)	3.15 (0.33 ~ 30.45)
		1 (progression)	7 (2.8%)	2 (0.9%)	
	Post-RT 2	0 (no progression)	245(98.8%)	226(94.2%)	0.20(0.03~1.29)
		1 (progression)	3(1.2%)	14(5.8%)	
Smooth surfaces	Post-RT 1	0 (no progression)	961(96.9%)	834(95.2%)	0.64(0.24~1.68)
		1 (progression)	31(3.1%)	42(4.8%)	
	Post-RT 2	0 (no progression)	963(97.1%)	915(95.3%)	0.61(0.16~2.38)
		1 (progression)	29(2.9%)	45(4.7%)	