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Curcumin Gum Formulation for Prevention of Oral Cavity Head and Neck Squamous Cell Carcinoma

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Objectives/Hypothesis: Head and neck squamous cell carcinoma represents the sixth most common cancer. As a result of field cancerization, second primaries and recurrences are high. Hence, research has focused on chemoprevention. Curcumin, a polyphenol compound with anticarcinogenic properties, is one such promising nutraceutical. As poor bioavailability limits curcumin’s use, a novel gum formulation was tested allowing for direct mucosal absorption into the bloodstream. This preliminary study validates curcumin gum efficacy by assessing release and transmucosal absorption, along with measuring its effects on serum cytokine levels.

Study Design: Clinical trial.

Methods: Protocols consisting of initial chew (chewing gum for 30 minutes) and revised chew (alternating chewing and parking gum against buccal mucosa for 30 minutes) were tested in healthy volunteers. High-performance liquid chromatography measured remnant curcumin in chewed gum, serum, and saliva. Serum levels were assayed for 15 proinflammatory cytokines via multiplex analysis.

Results: Revised chew samples demonstrated significantly higher curcumin release and absorption ($P = .0078$). Curcumin serum levels were significantly higher at 4 hours in samples > 2.0 g of curcumin release ($P = .01$). As saliva levels decreased, a concurrent increase in serum levels was observed, with no significance in the inverse relationship ($P = .1423$). When evaluating differences between gender, race, and age, the Asian population showed significantly lower curcumin release and serum levels ($P = .009$). CXCL1 (GR0-α) and TNF-α were significantly decreased in serum after chewing the gum ($P = .036, P < .001$, respectively).

Conclusions: Enhanced mucosal contact appears critical in improving curcumin release and absorption. CXCL1 and TNF-α both represent potential biomarkers for the future study of curcumin chemoprevention.

Key Words: Curcumin, gum, bioavailability, release, chemoprevention, head and neck squamous cell carcinoma, high-performance liquid chromatography, CXCL1, TNF-α, premalignant.

Level of Evidence: 2b

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common type of cancer in the world with roughly 600,000 cases diagnosed annually. Most tumors arise from the oral cavity and oropharynx.$^{1,2}$ HNSCC development is a multistep process preceded by premalignant lesions caused by carcinogens present in alcohol and tobacco. Recurrences and second primaries are frequent as a result of “field cancerization, in which the entire mucosa exposed to the carcinogen undergoes precancerous changes.” $^{3}$ As a result of field cancerization, there is a need for safe and effective chemopreventatives. To date, studies have looked at chemopreventives such as retinoids, beta-carotene, alpha-tocopherol, and ascorbic acid. Retinoids significantly reduce the development of second primaries; however, their mucocutaneous side effects have limited their use.$^{4}$ Beta-carotene, alpha-tocopherol, and ascorbic acid showed high relapse rates with serious side effects.$^{5}$ Hence, there is a need for safer agents with long-term use. Research has focused on studying nutraceuticals, which may be less toxic and safer for prolonged use.

Curcumin is the primary component of turmeric spice and is derived from the East Indian plant Curcuma longa. Turmeric contains curcuminoids, comprised of curcumin, demethoxycurcumin, and bisdemethoxycurcumin, with curcumin being the main curcuminoid and responsible for the yellow color and its antioxidant, anti-inflammatory, analgesic, antimalarial, and anti-inflammatory properties.$^{6,7}$ Curcumin is known to upregulate carcinogen...
detoxifying enzymes and antioxidants while suppressing cyclooxygenase-2 expression and nuclear factor-κB (NF-κB) release, ultimately leading to the downregulation of various proinflammatory cytokines (e.g., tumor necrosis factor [TNF], interleukins [ILs]).8–11 in vitro studies using curcumin have shown suppression of cell proliferation in breast cancer, colon cancer, and HNSCC.12–14

In both human and animal studies, curcumin has been found to undergo metabolic reduction and conjugation through first pass hepatic metabolism, resulting in poor systemic bioavailability when orally administered. Curcumin conjugation, through glucuronidation and sulfation, may explain low concentrations of free curcumin when administered orally as noted in previous studies for chemoprevention.13 Curcumin's development is limited by poor bioavailability. Oral mucosal exposure allows for direct mucosal absorption into the bloodstream, also bypassing this hepatic first pass metabolism. Prior studies in HNSCC using curcumin capsule formulations found it to have anti-inflammatory, antioxidative, and anticarcinogenic properties; however, these effects were hampered by poor bioavailability. Our laboratory has conducted prior studies of curcumin microgranules, which can be pouched in the sublingual area of the mouth to allow for improved mucosal absorption. Higher curcumin in the serum was found with microgranule administration compared to capsular administration in healthy and HNSCC patients. Although systemic levels of curcumin improved with the microgranules, the serum concentration largely failed to reach levels necessary to affect certain serum biomarkers.16 Studies in colon cancer have shown promising chemopreventive effects, where curcumin is able to remain on the intestinal mucosa allowing for prolonged contact and mucosal absorption.17

Applying this same idea of direct, sustained tissue contact to the oral cavity, a novel gum formulation of curcumin was created. This preliminary study aims to validate curcumin gum efficacy by assessing release and transmucosal absorption. We can assess the efficacy of the proposed curcumin gum by assessing curcumin levels in serum and saliva after chewing. We also measured the levels of curcumin in the chewed gum samples to assess release of curcumin from this formulation. Assessing the release and transmucosal absorption of curcumin will help validate the proposed formula for potential chemopreventive studies in the oral cavity.

**MATERIALS AND METHODS**

Healthy adults between the ages of 30 and 68 were recruited at Louisiana State University Health Shreveport. All studies were approved by the Louisiana State University Health Sciences Center Shreveport Institutional Review Board, with written informed consent obtained from each volunteer after the study objectives, risks, and benefits were explained. Inclusion criteria consisted of no consumption of curcumin-rich foods or vitamins to the subject’s knowledge within the previous 48 hours and age ≥ 18 years to ≤ 80 years. Exclusion criteria consisted of known hypersensitivity to turmeric, severely immunocompromised subjects, subjects known to be human immunodeficiency virus (HIV) positive, any major illness that in the investigator's judgment would substantially increase the risk associated with the subject’s participation in the study, unwillingness or inability to comply with required study procedures in this protocol, consumption of aspirin in the previous 48 hours, and subjects on coagulation therapy. Ten normal volunteers were enrolled, with one excluded from the study due to gum disintegration (Table I). No medications were used by volunteers that could influence curcumin absorption. Demographic information including age, gender, and race were obtained by subject interview. Participants in the study were labeled as follows: 1, 1R, 2, 2R, 3, 3R, 5R, 6R, 8R, 11R, 12R, 13R; the R corresponds to healthy volunteers who underwent additional revised chew testing at a later date.

The current gum formulation involves the use of claims approved with US Patent No. 9,700,525 entailing curcumin powder mixed with gum peppermint base and liquid corn syrup resulting in 10 g of curcumin added to make each batch. A 4-g per administration dose was used because 3.6 to 4 g has been shown to be safe in humans and to provide levels adequate to elicit antioxidative effects.18 Two chew methods were used to assess curcumin release. The initial chew method consisted of chewing 4 g of curcumin gum for 30 minutes using the volunteer’s personal discretion. The revised chew method consisted of first priming the mouth by chewing one 250-mg piece of gum. Next, eight pieces (250 mg each for a total of 2 g) were chewed 6 to 8 times, followed by parking the gum against the buccal mucosa for 4 minutes. This chew/park cycle was repeated for 30 minutes. The 2 g of gum were then discarded and the procedure was again repeated with a remaining 2 g of gum, for a total dose of 4 g administered over 60 minutes. Among nine volunteers, three were administered both chewing methods: an initial chew and revised chew. The six subsequent volunteers were administered only the revised method, emphasizing prolonged mucosal contact.

**Curcumin Analysis**

Four distinct areas from each chewed sample of gum were taken from each participant and measured for percent curcumin remaining per section of gum. Based on an average of this percentage, along with the overall weight of chewed gum remaining, an estimate of curcumin release per subject sample was calculated.

**High-Performance Liquid Chromatography Detection**

Preparation of extracted curcumin from chewed gum was as follows. Yellow filtered light conditions were used for...
extraction to prevent curcumin degradation. Between 10 and 50 mg of curcumin gum was used per extraction slice. Samples were homogenized in phosphate-buffered saline using the sonication probe, and 1 mL of 90% ethyl acetate/10% MeOH solvent was added for extraction, followed by 10-second vortex and 30-minute cold room centrifugation (repeated once). Curcumin pooled extracts were then concentrated using a SpeedVac (Thermo Fisher Scientific, Waltham, MA). Preparation of serum and saliva samples were processed using our previous high-performance liquid chromatography (HPLC) method, with liquid chromatography (LC) conditions concerning instrument configuration, analytical column, and the same sample gradient throughout.16

**Measurement of Serum Cytokines**

Cytokine expression levels were measured using a magnetic bead–based multiplex immunoassay kit (HCYTOMAG-60K; Millipore Sigma, Burlington, MA). The protocol was followed according to the manufacturer’s instructions. A panel of 15 cytokines was selected based on our previous results6 and included: EGF, PGE-2, Fli-3, GM-CSF, IFNγ, CXCL1 (aka GRO-α), IL-13, IL-17, IL-1β, IL-6, IL-8, IP-10, MIP-1β, TNF-α, and VEGF. The Bio-Plex 200 (Bio-Rad Laboratories, Hercules, CA) and Luminex Platform Array System (Luminex Corp., Austin, TX) were used. Briefly, a standard curve was prepared. Subsequently, magnetic beads with capture antibodies covering their surfaces for 15 cytokines were added to each well. Once all standards and samples were added, the microplate was placed on a titer plate shaker at 600 rpm for 1 hour at room temperature (RT). A Bio-Plex Pro Wash Station (Bio-Rad Laboratories) was used. Subsequently, detection antibodies were added, followed by streptavidin-PE, and then incubated in a shaker at RT for 1 hour. Unknown concentrations for each sample were calculated from the standard curve with Luminx xPonent Software (Luminex Corp.). All samples were assayed in duplicate and results were averaged.

**Statistical Analysis**

Statistical analyses were performed with SPSS software (IBM, Armonk, NY). Based on our prior data of peak serum curcumin (Cmax) levels from a healthy volunteer trial using microgranular curcumin, we aimed to match the average Cmax of 170.6 ng/mL using the novel gum formulation. Power analysis performed liquid chromatography (HPLC) method, with liquid chromatography (LC) conditions concerning instrument configuration, analytical column, and the same sample gradient throughout.16

**RESULTS**

The amount of curcumin released ranged from 0.1 g to 1.4 g (SD ± 0.6) in the initial chew (protocol A) method compared to 0.5 g to 3.2 g (SD ± 1.0) in revised chew(s) (protocol B). In the initial chew, a mean of 0.67 g of curcumin was released per participant compared to 1.67 g (P = .0078) released per participant in the revised chew method (Fig. 1). Curcumin release varied widely between test subjects (Fig. 2).

Serum levels for the revised chew gum formulation increased with time after chew (Tmax), with 1 hour exhibiting peak levels. Saliva curcumin levels decreased over time, with peak levels at the immediate 0 hour postchew time point. As saliva levels decreased, a concurrent increase in serum levels was observed as expected (Fig. 3). A Pearson correlation test showed no significance in the inverse relationship between serum and salivary curcumin levels (P = .1423). Levels were sustained throughout the time course, and were particularly higher at 4 hours in samples with >2.00 g of curcumin release (P = .01). At 4 hours, serum levels ranged from 15 to 263 ng/mL in samples with ~2.00 g of curcumin release and 0 to 72 ng/mL in samples with <2.00 g released (Fig. 4). Average Cmax serum concentration was at 166 ± 65 ng/mL for the gum formulation with Tmax and area under the curve (AUC) values at 1.89 ± 0.54 hours and 233.84 ± 71.22 (ng × hr/mL), respectively (Table II). Paired t tests were used to evaluate differences in grams of curcumin released between gender (male vs. female), race (Asian vs. Caucasian), and age (<50 years old and >50 years old) of the participants. The P values were .8, .009, and .06, respectively.

Cytokine levels were measured at specific time points after curcumin chewing. Our results suggest that curcumin chewing gum induced a statistically significant decrease in serum CXCL1 levels at 30 minutes (P = .049) and at 4 hours (P = .036) postchew compared to baseline. Similarly, curcumin chewing gum administration was found to significantly decrease levels of TNF-α at 4 hours after consumption compared to baseline (P < .001). Figure 5A and B demonstrate the progression of serum levels for CXCL1 and TNF-α after gum administration, where statistically significant decreases are noted with an asterisk at the 4-hour time point. Figure 5C represents the results of the multiplex analysis, where 13 other
cytokines in the panel were compared and did not show a significant change after curcumin gum administration.

DISCUSSION

This study provides insight into the efficacy of a curcumin gum formulation with the overall goal of potential utilization for chemoprevention of oral cavity squamous cell carcinoma. Our study focuses on healthy volunteers, as this gum would primarily target the at-risk population that smokes and chews tobacco. The chew method appears to be crucial to the bioavailability of the gum. Revised chew method sets had significantly higher curcumin release as well as lower average percent curcumin remaining in chewed gum samples compared to initial chew sets \((P = .0078)\). This indicates that a combination of chewing and parking the gum against the buccal mucosa for prolonged mucosal contact could lead to better release and absorption. This is consistent with studies showing prolonged mucosal contact of curcumin on the intestines to be effective at increasing bioavailability in colon cancer chemoprevention.\(^{17}\) Additionally, the Asian population showed significantly lower curcumin release and serum levels \((P = .009)\); however, the limitation of only two Asian subjects in our cohort does not allow for further conjecture.

In comparing all revised chew samples, about 44% of our samples had greater than 2 g of curcumin release, with a maximum amount of 3.2 g released. One explanation for why the other 56% did not achieve greater than 2 g release, with three samples having under 1 g released, may be related to the amount of curcumin in the saliva. When initial saliva curcumin levels were high, curcumin release was decreased, which may indicate that saliva can become saturated with curcumin, leading to less release over the course of chewing. Additionally, this difference may be due to the fact that there are variations in how participants chew. Jaw/chewing fatigue may account for less effective chewing in these subjects as well as poorer dentition and saliva pH, all of which were not screened for at the beginning of this study.

Our data suggest that formulated curcumin chewing gum has the ability to significantly decrease the levels of CXCL1 in patient serum. Although it is well established in mouse models and cell lines, there are no reports of curcumin inducing a decrease in CXCL1 in human serum in vivo prior to this study. Increased CXCL1 promotes a carcinogenic microenvironment via leukocyte recruitment, angiogenesis, invasion, and metastasis.\(^{19}\) It has been shown that neutrophils are recruited by CXCL1 to the site of

![Fig. 2. The percent of curcumin remaining in chewed gum samples. Bars represent the average percent of curcumin remaining in chewed gum samples. The average was calculated by converting measured levels in each of the four sections to percent curcumin per piece, taking the average of these percentages, converting the average to grams via multiplication with the overall weight of the chewed specimen, then dividing by 4 g (the initial amount of drug per prechewed piece) to get percentage remaining.](image1)

![Fig. 3. The serum versus saliva levels of curcumin. Serum and saliva samples were collected at 0, 0.5, 1, 2, and 4 hours after gum administration. Mean ± standard error of the mean is shown. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.](image2)

![Fig. 4. Curcumin serum levels. Bar 1 represents mean curcumin serum levels for sets with <2.00 g curcumin released from gum, whereas bar 2 is for those sets >2.00 g. *P ≤ .01 for the two-tailed t test.](image3)
chronic inflammation and/or tumor and once present, the neutrophils begin to secrete VEGF-A, which stimulates neovascularization. CXCL1 has been found to be overexpressed in approximately 20 HNSCC cell lines as well as melanoma cell lines in recent years. Furthermore, CXCR2 receptors found on the luminal surface of endothelial cells promote CXCL1-induced arteriogenesis in the tumor microenvironment. Interestingly, CXCR2 is highly expressed on the surface of laryngeal cancer specimens, demonstrating the need for further investigation into CXCL1 not only as a screening tool for high-risk HNSCC patients responsiveness to curcumin, but also as a marker for laryngeal cancer immunopathogenesis.

Chronic inflammation induces a metastasis-prone microenvironment by promoting a positive feedback loop between NF-κB and CXCL1/2. NF-κB dysfunction leads to constitutive expression of cytokines and chemokines involved in cell growth, oncogenesis, and escape from apoptosis. Curcumin disrupts this feedback loop by inhibiting the nuclear translocation of NF-κB, thus preventing the transcription of its gene products, which decreases cancer-related inflammation as well as the chances of metastases.

We also demonstrated that our curcumin gum has the ability to significantly decrease the levels of TNF-α in human serum. TNF-α activity in head and neck cancer is often a result of a chronic reactive inflammatory state in response to a constant irritant such as cigarette smoke, tobacco, or alcohol. Eventually, TNF-α overactivity can promote transformation to a malignancy if not mitigated. Chronic inflammation leads to increased TNF-α, which in turn increases aberrant NF-κB signaling and the overexpression of a multitude of proinflammatory cytokines, chemokines, and growth factors. Interestingly, TNF-α levels are reportedly high in the saliva of patients with hairy oral leukoplakia and even higher in patients with oropharyngeal squamous cell carcinoma. It has been previously shown that curcumin decreases TNF-α in vitro and in mouse models; however, studies on TNF-α in patients have been lacking.

Not only does curcumin chewing gum hold promise for HNSCC chemoprevention, but we also propose future trials focusing on radiation xerostomia. It is the most frequently reported long-term complication after external beam radiation therapy (XRT) or chemoradiotherapy for HNSCC, and the symptomatology is dose dependent. Patients can experience decreased functionality after having received as little as 10 Gy. Doses at 50 Gy higher reduce the vast majority of parotid function in most cases. The pathogenesis of this condition begins with the vascular inflammatory phase that starts directly after a patient receives a dose of XRT. During this phase, exposed endothelial and epithelial cells release reactive oxygen species and proinflammatory factors, including IL-1β and TNF-α, which causes further damage by increasing vascular permeability and allowing more inflammatory components to extravasate into peripheral tissues. Additionally, if the patient is receiving chemotherapy, this increased vascular permeability can contribute

| Table II. Curcumin Serum and Saliva Levels After Gum Administration. |
|--------------------------|-----------------|-----------------|
|                          | Tmax, hr         | AUC (ng h/mL)   | Cmax (ng/mL)   |
| Serum                    | 1.89 ± 0.54     | 233.84 ± 71.22  | 166.74 ± 65.31 |
| Saliva                   | 0               | 587.589 ± 310.350 | 1,743,423 ± 924,005 |

*Serum and saliva levels measured by high-performance liquid chromatography. Data represent revised method subjects mean + standard error of the mean (n = 9). AUC = area under the curve; Cmax = peak serum curcumin; Tmax = time after chew.

**Fig. 5.** Effect of curcumin gum on serum levels of cytokines. Healthy volunteers were allowed to chew curcumin gum for 60 minutes, followed by parking in the buccal cavity. Blood specimens were collected at time 0 and 4 hours following chew and subjected to analysis of cytokine levels. Data represent mean ± standard error of the mean (SEM) (ng/mL) (n = 10 in each group). (A) TNF-α. (B) CXCL1. (C) Thirteen additional cytokines were tested, and no significant changes were observed (mean ± standard deviation). *Significant effect of treatment on postchew cytokine levels.
toward allowing chemotherapeutics to accumulate in the salivary gland tissues as well. A previous mouse model study reported that curcumin inhibits 5-fluourouracil induced expression of CXCL1 and 2 in mice, thus highlighting the ability of curcumin to decrease the proinflammatory microenvironment.28,29

Nonetheless, local release of CXCL1 and CXCL2 by immune cells is destructive to surrounding tissues because the CXCR2 receptor on the surface of local neutrophils becomes activated, leading to massive protease release that degrades tissue architecture at a time when it is most needed for healing.18 CXCL1 and TNF-α have been proven to recruit neutrophils to sites of chronic inflammation in the head and neck.30 Amifostine is a radioprotectant that scavenges free radicals to protect subcellular structures from damage, and we propose that curcumin chewing gum may be beneficial as adjunctive or alternative therapy in those whom amifostine is not effective because it is also a well-known free radical scavenger, and it decreases proinflammatory cytokines. If patients were taking daily curcumin weeks before and/or during their course of radiation, this may have the potential to help mitigate the reactive free radical tissue damage and inflammatory reaction that ensues in response to an insult such as radiotherapy. Curcumin decreases the unfavorable tumor microenvironment by decreasing levels of TNF-α, CXCL1, and NF-κB mediated transcription pathways, while also decreasing free radical exposure to promote tissue healing and recovery.18 We propose that a future small-scale randomized, controlled clinical trial should focus on comparing mucosal and systemic curcumin therapies for radiation xerostomia.

Future directions for this study include validating the gum formula with a larger population size and enrolling more male volunteers to look at gender differences, as well as expanding our ethnic participants to include not only more South Asian volunteers but also to incorporate several more ethnicities to look for differences in curcumin release and absorption. Another area of focus may be to consider the body mass index of participants to further explain the differences observed in absorption. It would also be important to explore amendments to our formulation that could aid in increasing homogeneity and/or amount of curcumin released per administration. One final variation to consider would be adding a midcheek saliva and serum collection point, possibly between 2-g dosages, to see if variability exists within the process of chewing. It may be that jaw fatigue comes into play, or the mouth becomes dry with longer chewing. It may be that jaw fatigue comes into play, or the mouth becomes dry with longer chewing. This may add further insight into the suspected negative correlation between postcheek saliva levels and drug release.

CONCLUSION

This pilot study of curcumin gum in healthy volunteers provides valuable information that can help optimize the design of future drug development and clinical evaluation of curcumin for HNSCC chemoprevention. Prolonged mucosal contact in our revised chew method appears critical in improving curcumin release. Our gum formulation also demonstrated the ability to significantly decrease the levels of CXCL1 and TNF-α in the serum of healthy volunteers. Although previous studies have demonstrated this in vitro and in small animal models, there are no reports demonstrating a decrease in serum levels of CXCL1 after oral curcumin administration in healthy volunteers. These findings lay the foundation for future prospective trials using curcumin gum as a chemopreventive agent in patients with premalignant lesions.

Acknowledgments

The authors thank Douglas Boudreau, PD, and Patricea “Patsy” Angelle, PD, FACD, FAACP, for their expertise and assistance in the production of the curcumin gum compound. The information in this article involves the use of claims approved with US Patent No. 9,700,525.

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