Drug Development and Industrial Pharmacy, 2011; 37(10): 1192–1199
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ISSN 0363-9045 print/ISSN 1520-5762 online
DOI: 10.3109/03639045.2011.563783

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RESEARCH ARTICLE

Buccal tablets containing cysteine and chlorhexidine for the reduction of acetaldehyde levels in the oral cavity

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Abstract

There is growing evidence that a large proportion of upper digestive tract tumors are ascribable to heavy alcohol drinking and tobacco consumption. The cancer-promoting action of ethanol is mediated by acetaldehyde, its first metabolite, also derived from the bacterial oxidation of alcohol by the oral microflora, classified by the International Agency for the Research on Cancer as a carcinogen. Acetaldehyde is also one of the major components of tobacco smoke. These findings suggest two different strategies to decrease the risk of alcohol-related oral cancers: the reduction of the levels of alcohol-derived acetaldehyde in saliva and the reduction of oral bacterial flora. Therefore, the aim of our study was to develop and characterize some buccal tablet formulations containing both 20 mg L-cysteine hydrochloride (able to chemically neutralize acetaldehyde) and 10 mg chlorhexidine diacetate (well-known antiseptic compound active against a large spectrum of oral microbes). One of these formulations, chosen on the basis of its favourable delivery kinetics of the active principles, was demonstrated to be able to reduce acetaldehyde concentration in an *in vitro* system and to lower its salivary levels in volunteers after ethanol contact. Our findings support the hypothesis that the application of buccal devices containing cysteine and chlorhexidine could reduce salivary acetaldehyde levels and thus the incidence of upper gastrointestinal cancer in drinkers and smokers.

Keywords: Buccal tablets, acetaldehyde, cysteine, chlorhexidine, alcohol-induced cancer

Introduction

Excessive alcoholic beverages consumption is a well-known risk factor for the development of oropharyngolaryngeal and digestive tract cancer (Blot et al., 2001). The mechanism of the tumor-promoting effect of ethanol is so far not completely understood because ethanol *per se* is not mutagenic; however, different epidemiologic, genetic, and biological data would suggest that acetaldehyde, the first ethanol metabolite, can play a main role in alcohol-induced carcinogenesis (Pöschl and Seitz, 2004). According to the International Agency for the Research on Cancer (IARC, 1999), acetaldehyde is carcinogenic in experimental animal models and is a possible carcinogen for humans. After ingestion of ethanol, acetaldehyde is produced in the oral cavity both by mucosal and microbial alcohol dehydrogenases (ADHs), and it is further oxidised to acetic acid by aldehyde dehydrogenases (ALDHs) (Bosron and Li, 1986); its salivary levels vary depending on several factors, especially the dose of ethanol, dental status, oral hygiene, oral microflora, and some environmental and nutritional factors (for example, the consumption of some type of fruits and some fermented beverages or foodstuffs; Salaspuro, 2009). Recent evidences strongly support the role of locally produced acetaldehyde in the pathogenesis of upper aerodigestive cancer. Epidemiologic studies have shown that the risk of alcohol-related oral cancer is particularly high among Asian populations, who show high frequencies of mutation in *ALDH2* gene and therefore are not able to properly detoxify acetaldehyde (Murata et al., 1999); as a consequence, Asian ALDH2-deficient heavy drinkers, after alcohol ingestion, show salivary acetaldehyde concentrations two to three times higher than those with the normal ALDH2 enzyme. Moreover, the risk of digestive

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(Received 07 January 2011; revised 08 February 2011; accepted 14 February 2011)

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tract cancer is increased in subjects with the rapidly metabolizing ADH3 genotype (Harty et al., 1997). The role of alcohol in oral cancer seems to be also supported by epidemiologic data demonstrating that the risk of oropharyngeal cancer is increased in both men and women who regularly use mouthwashes with a high alcohol content (McCullough and Farah, 2008). Oral microbial flora also plays a role in determining the carcinogenic potential of ethanol: many microorganisms representing the oral microbiota, such as Gram-positive bacteria and yeasts of *Candida* genus, possess the enzyme ADH and are able to produce *in vitro* significant amounts of acetaldehyde from ethanol (Muto et al., 2000; Kurkivuori et al., 2007), and this observation may support the epidemiologic finding that poor oral hygiene is an independent risk factor for oral cavity cancer (Homann et al., 1997). The ability of *Candida* yeasts to produce acetaldehyde not only from alcohol, but also from glucose, could partly explain the development of oral cancer in nondrinker people or in patients previously treated with chemotherapy for another oral cavity tumor (Nieminen et al., 2009), who often present heavy oral mucosal colonization with these microorganisms.

Besides alcohol consumption, smoking is an independent, well-known cause of cancer; smoking increases salivary acetaldehyde concentration not only because tobacco has a high content of this compound (Homann et al., 2000) but also because it induces alterations in composition of oral microflora and consequently increases microbial acetaldehyde production in the saliva of smokers. In fact, cigarette use predisposes individuals to oral *Candida* infections (Arendorf et al., 1983; Holmstrup and Besserman, 1983; for other references see Soysa and Ellepola, 2005) and switches local bacterial flora from Gram-negative to Gram-positive strains (Colman et al., 1976).

Because smoking and heavy alcohol consumption significantly increase salivary acetaldehyde production with different mechanisms, it would be important to find a system to reduce the concentration of this metabolite. A first approach implies its chemical neutralization and is based on the ability of some thiol compounds to react with acetaldehyde, forming nontoxic compounds (Sprince, 1985); in particular, cysteine, a nonessential amino acid, binds it covalently forming 2-methylthiazolidine-4-carboxylic acid (Sprince et al., 1974), and chewing gums and buccal tablets containing cysteine were proven to be able to significantly decrease acetaldehyde salivary levels after alcohol intake (Salaspuro et al., 2002; Kartal et al., 2007).

On the other hand, considering the role of oral microflora in producing acetaldehyde from alcohol, another strategy to reduce the salivary concentration of this metabolite might be the control of microorganisms in the oral cavity by using antiseptic mouthwashes. This approach has been demonstrated correct by Homann et al. (1997), and some authors have hypothesized that the application into the oral cavity of drinkers or smokers of a polymeric dental device for the controlled delivery of the antiseptic drug chlorhexidine may represent an alternative to the chemical detoxification of acetaldehyde (Rota and Poggi, 2003).

A combination of both these approaches—chemical inactivation of acetaldehyde and reduction of oral microorganisms responsible for its production—might be proposed as a rational strategy to reduce the risk of oral cancer for smokers and drinkers. Therefore, the aim of this study was to design buccal formulations (tablets) containing suitable amounts of both chlorhexidine diacetate and cysteine hydrochloride, and to test the ability of a selected one to neutralize both *in vitro* and *in vivo* acetaldehyde. On the basis of our previous investigations on buccal mucoadhesive tablets (Giunchedi et al., 2002; Juliano et al., 2004), we chose alginate and chitosan as excipients because they are nonirritating vehicles characterized by mucoadhesive properties; in addition, chitosan possesses anticandidal activity (Juliano et al., 2008) and inhibits the adhesion of *Candida albicans* cells to human buccal epithelium (Knapczyk et al., 1992).

Materials and methods

Materials

Sodium alginate (high viscosity, HV: viscosity of 2.0% w/v aqueous solution at 25°C approximately 14,000 cPs, manufacturer value; medium viscosity, MV: viscosity of 2.0% w/v aqueous solution at 25°C approximately 3500 cPs, manufacturer value) and chlorhexidine diacetate were supplied by Sigma (Milan, Italy). Chitosan base (medium molecular weight, deacetylation degree 75–85%; viscosity of 1.0% w/v solution in acetic acid 200–800 cPs) was from Aldrich (Milwaukee, WI). Chitosan chloride (Protasan UP CL 113; deacetylation degree >75–90%) was supplied by ProNova (Oslo, Norway). L-Cysteine hydrochloride (biochemistry grade) was purchased from Merck (Milan, Italy) and acetaldehyde from Carlo Erba (Milan, Italy). All other reagents were obtained from Sigma (Milan, Italy); water from MilliQ R4 Millipore system (Milan, Italy) was used, and all other solvents used were of analytical grade.

Analytical methods

Determination of cysteine

For the determination of cysteine, the spectrophotometric method by Tutem and Apak (1991) has been used. This method is based on the color reaction with the copper (II)-neocuproine reagent. In brief, 200 μL of 0.1 M $CuCl_2$ water solution, 500 μL of 3 mM neocuproine (2,9-dimethyl-1,10-phenantroline) in 96% ethanol and 200 μL of 1.0 M ammonium acetate solution were mixed with 100 μL of sample or standard. After centrifugation at 13,000 rpm for 5 min, the absorbance of the resulting chelate was recorded at 450 nm against the reagent blank. The concentration of cysteine was determined by comparing the values obtained with calibration curves prepared using cysteine concentrations ranging from 10.0 to 100.0 μg/mL

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($y = 0.005\,x - 0.0086$; $r^2 = 0.999$). The results were expressed as micrograms of cysteine per millilitre.

Determination of acetaldehyde

Acetaldehyde determination was performed with a spectrophotometric method based on the Schiff reagent (Papaefstathiou et al., 1997; modified): acetaldehyde reacts with fuchsin-sulphurous acid to yield a colored product, which is monitored spectrophotometrically at 567 nm. In brief, 500 µL of the sample or standards were mixed with 50 µL of 6 mM fuchsin solution (prepared by dissolving 20.3 mg of fuchsin in 3% of ethanol and 0.5 M sulfuric acid), and the mixture was vortex mixed for 30 min; then, 50 µL of 18 mM Na_2SO_3 aqueous solution was added, and after 30 s of vortex mixing, the resulting mixture was centrifuged at 13,000 rpm for 5 min. The absorbance was recorded after 20 min at 567 nm against a blank prepared by using water instead of acetaldehyde-containing sample. The concentration of acetaldehyde was determined by comparing the values obtained with calibration curves prepared using acetaldehyde concentrations ranging from 0.5 to 12.5 µg/mL ($y = 0.1357x - 0.0117$; $r^2 = 0.999$). The results were expressed as micrograms of acetaldehyde per millilitre of dissolution medium or saliva.

Determination of chlorhexidine

The concentration of chlorhexidine, in the samples or standards, was evaluated spectrophotometrically at 262 nm by comparing the values obtained with calibration curves prepared using chlorhexidine concentrations ranging from 1.0 to 20.0 µg/mL ($y = 0.0413x - 0.0066$; $r^2 = 0.999$). The results were expressed as micrograms of chlorhexidine per millilitre of dissolution medium.

None of these three compounds—chlorhexidine diacetate, L-cysteine hydrochloride, and acetaldehyde—interferes with the dosage of the others (data not shown).

All spectrophotometric determinations were performed using UV/Vis Spectrophotometer, Perkin-Elmer Lambda 3 (Uberlingen, Germany).

Preparation and characterization of chitosan microspheres loaded with chlorhexidine diacetate by spray-drying

Chlorhexidine-loaded chitosan microparticles were prepared by spray-drying with a drug-to-polymer weight ratio of 1:4. Chitosan base (2 g) was dissolved in 200 mL of hydrochloric acid (0.1 M at 1% w/v), and the solution was evaporated to dryness at reduced pressure in a Rotavapor R110 (Buchi, Switzerland) at 50°C. The obtained residual and 0.5 g of chlorhexidine diacetate were dissolved in 200 mL Milli-Q water, and the resulting solution was sprayed through the nozzle of a spray-dryer (model Büchi Mini Spray Dryer B-191, co-current flow type), equipped with a standard 0.7-mm nozzle. The process parameters were inlet air temperature 99–100°C; outlet air temperature 74–75°C; spray flow rate about 5 mL/min.

Particles size of microspheres was determined by laser diffractometry using a Coulter LS 100Q (Beckman Coulter Particle Characterization, Miami, FL). Particle size analysis was performed by suspending microparticles in light silicon oil (fluid silicon 344, Dow Corning). The average particle size was expressed as the volume-surface diameter, d_{vs} (Edmundson, 1967).

To determine their drug content, chlorhexidine-loaded microparticles were dissolved in 95% ethanol (2.5 mg of microspheres in 100 mL alcohol). Drug concentration was evaluated spectrophotometrically in the resulting solution at 262 nm; the test was performed in triplicate.

Finally, a dissolution test was performed on microparticles to investigate their drug release, as explained further.

Preparation of buccal tablets

Seven different tablet formulations (A–G) were prepared, each containing 10 mg of chlorhexidine diacetate and 20 mg of L-cysteine hydrochloride. Formulations A–E were obtained by mixing chlorhexidine diacetate (10 mg per tablet), L-cysteine hydrochloride (20 mg per tablet), and suitable amounts of chitosan hydrochloride and/or sodium alginate (MV) and saccharine in a Turbula apparatus (W.A. Bachofen, Basel, Switzerland) at 90 rpm for 10 min; tablets were prepared by direct compression of the blends obtained at 1000 kg of compression force (hardness 6–8 kP), by using a hydraulic press (Perkin Elmer, Bucks, United Kingdom) equipped with 13 mm flat punches. Formulation F was prepared by mixing 20 mg of L-cysteine hydrochloride with 69 mg of chlorhexidine-loaded microspheres (corresponding to 10 mg of drug), 30 mg of sodium alginate (HV), and 1 mg saccharine. Finally, formulation G was designed as bi-layered tablets made of chitosan hydrochloride and prepared by a direct compression procedure involving two consecutive steps; the double-layered structure—the inner layer, intended to adhere to the buccal surface, containing cysteine and the outer one containing chlorhexidine—was expected to provide cysteine delivery in an unidirectional fashion to the mucosa, avoiding the drug loss due to washout with saliva, whereas chlorhexidine, delivered from the outer layer, should have a good oral retention because of its substantivity.

Table 1 shows the theoretical compositions (% w/w) of the formulations prepared. The weight of the tablets was 120 mg; their diameter was 12.8 mm and their thickness was 0.75 mm. All tablets prepared in this study meet the United States Pharmacopoeia (USP) 32 requirements for weight variation tolerance; coefficient of variation of all formulations was less than 1%.

In vitro drug release tests (dissolution tests)

In vitro tests were performed to characterize both chlorhexidine-loaded chitosan microparticles and buccal tablets with respect to their drug release behaviour.

Suitable amounts of microspheres, corresponding to about 3 mg of chlorhexidine diacetate, were suspended in 300 mL of USP 24 phosphate buffer (pH 7.0), chosen

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Table 1. Composition of tablet formulations.

	Chlorhexidine diacetate	Cysteine hydrochloride	Chitosan base	Chitosan hydrochloride	Alginate medium viscosity	Alginate high viscosity	Saccharine
Formulations				milligrams			
A	10.0	20.0	89.0				1.0
B	10.0	20.0			89.0		1.0
C	10.0	20.0	44.5		44.5		1.0
D	10.0	20.0		89.0			1.0
E	10.0	20.0		44.5	44.5		1.0
F	10.0	20.0		59.0		30.0	1.0
G*	10.0	20.0		89.0			1.0

Composition of Formulation G in details: layer 1 (55 mg): chlorhexidine diacetate 10 mg, chitosan hydrochloride 44.5 mg, saccharine 0.5 mg; layer 2 (65 mg): cysteine hydrochloride 20 mg, chitosan hydrochloride 44.5 mg, saccharine 0.5 mg

as a representative medium for saliva (Giunchedi et al., 2002), at 37°C. Dissolutions (six replicates) were performed by using the USP apparatus no. 2 (paddle; 50 rpm). Chlorhexidine diacetate as a powder was used in comparison. Drug was dosed as previously reported; results are reported in Figure 1.

In vitro release tests of the tablets were performed separately to verify the release profile of chlorhexidine diacetate and cysteine hydrochloride. In both cases, dissolutions were performed in the USP rotating basket apparatus (operating speed 50 rpm); dissolution medium (1000 mL) was USP phosphate buffer (pH 7.0) for chlorhexidine diacetate and MilliQ water for cysteine (in buffer, phosphate anions make copper cations of the copper(II)-neocuproine reagent precipitate). Tests were performed in triplicate. Results are shown in Figures 2 and 3.

On the basis of the results obtained from the dissolution tests in terms of chlorhexidine and cysteine release, formulation F (tablets containing chlorhexidine-loaded chitosan microspheres) was the most suitable for our purposes. Therefore, the other assays were performed on these tablets.

In vitro neutralization of acetaldehyde

Considering the high volatility of acetaldehyde, some preliminary experiments were performed to verify whether acetaldehyde leakage occurred from the dissolution vessel to the atmosphere, resulting in a loss of the product and a poor reproducibility of dosages. For this purpose, different volumes of 0.36 M acetaldehyde aqueous solution were added to 1000 mL of phosphate buffer contained in the vessel of the abovementioned dissolution apparatus (obtaining acetaldehyde final concentrations ranging from 40 and 200 μmol/L, comparable to those ones present in human saliva after moderate alcohol consumption according to Homann et al., 1997, and Salaspuro et al., 2002), and after carefully wrapping the top with parafilm, the solution was warmed at 37°C and stirred with the paddle. At predetermined intervals of time, samples of the solution were removed and acetaldehyde dosed as previously described.

By using the same apparatus and the same experimental conditions, some tests were performed to verify

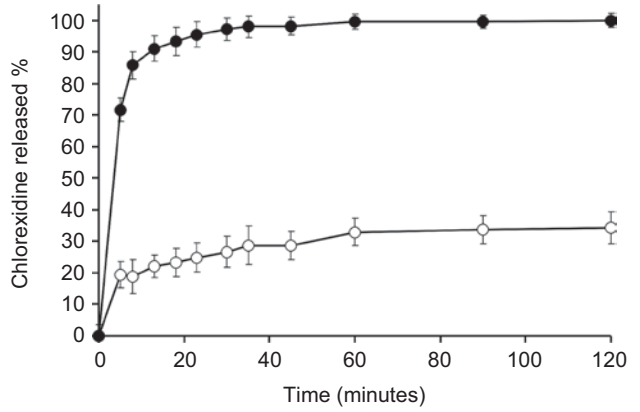

Figure 1. *In vitro* release profiles (USP apparatus no. 2, paddle, 50 rev/min; USP phosphate buffer pH 7.0) of chlorhexidine (drug as powder) (○) and microspheres with a chlorhexidine-to-chitosan ratio of 1:4 (•). Each point represents the mean ± SD ($n = 6$).

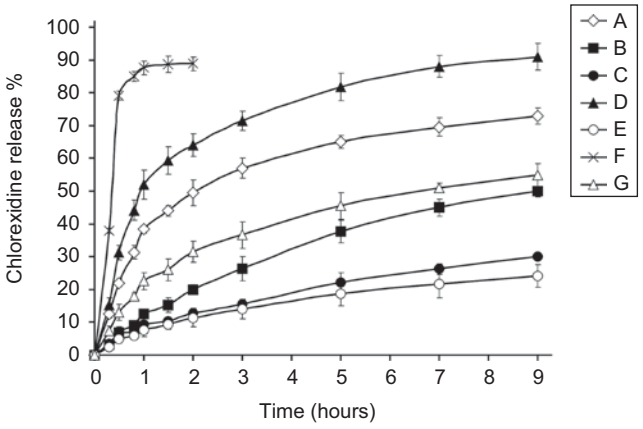

Figure 2. *In vitro* release profiles of chlorhexidine diacetate from tablet formulations (USP apparatus no. 1, basket, 50 rev/min; USP phosphate buffer pH 7.0). Each point represents the mean ± SD.

whether acetaldehyde was neutralized by the addition of cysteine as a powder or by tablets of formulation F. For this purpose, 100 mg of L-cysteine hydrochloride or one tablet was added to 1000 mL of buffer at 37°C containing suitable concentrations of acetaldehyde (227 and 114 μmol/L, respectively); at various time points, samples of this solution were collected and acetaldehyde concentrations were determined as previously described.

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All tests were performed in triplicate, and the results of this test are shown in Figures 5 and 6.

In vivo neutralization of acetaldehyde

In vivo preliminary tests were performed on tablet formulation F after approval of the protocol by the Ethical Committee of the University of Sassari. Three healthy female volunteers (aged 27–30, nondrinkers and nonsmokers, in good oral and dental status, and following a proper daily dental hygiene) participated in the study.

Preliminary *in vivo* tests confirmed that both alcohol ingestion and alcohol application on oral mucosa significantly increased salivary acetaldehyde production; subjects participating in our investigation were required to rinse their mouths with an alcoholic solution to avoid an undesired ethanol ingestion. Therefore, after baseline saliva collection (following standard oral hygiene), volunteers repeatedly rinsed for 15 min their oral cavities with a total of 100 mL of a solution of ethanol in water (20% v/v). After this time, another sample of saliva was collected to perform acetaldehyde dosage; then, volunteers applied the tablet on the buccal mucosa between cheek and gingiva, in front of the first lower molar; saliva samples were collected immediately and after 15 and 30 min, and acetaldehyde levels were evaluated. Tests were performed in triplicate, and the results are reported in Figure 7. Acetaldehyde level variations were compared with those obtained in similar experiments where ethanol washings were not followed by tablet application.

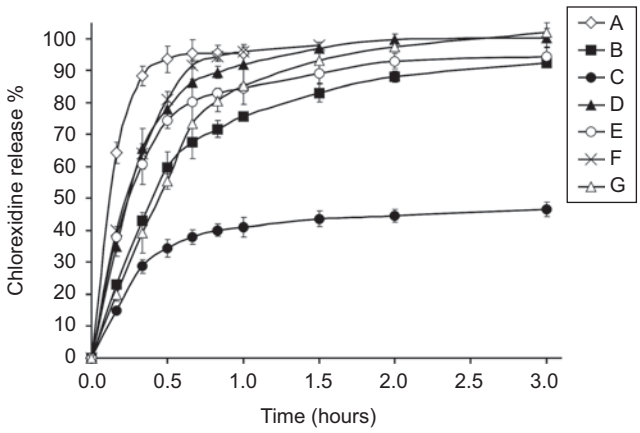

Figure 3. *In vitro* release profiles of L-cysteine hydrochloride from tablet formulations (USP apparatus no. 1, basket, 50 rev/min; MilliQ water). Each point represents the mean ± SD ($n = 6$).

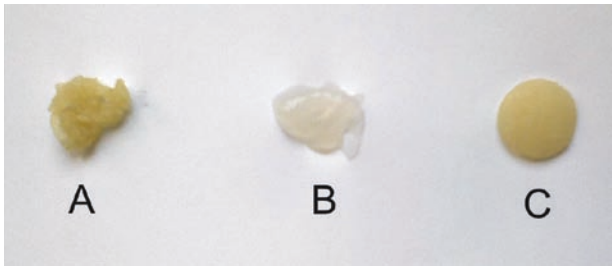

Figure 4. Appearance of formulations A, B and C after 3 hours of dissolution in USP 24 phosphate buffer (pH 7.0); the same behavior was exhibited in distilled water.

Results

Chlorhexidine-loaded microspheres characterization

The actual drug content of chlorhexidine-loaded chitosan microspheres was 14.5%, corresponding to an encapsulation efficiency of 77.6%. As shown by particle size analysis, loaded microspheres had a d_{vs} of 6.1 μm.

Figure 1 shows the *in vitro* release profile obtained from the drug-loaded microparticles compared with the dissolution profile of chlorhexidine diacetate as a powder. Although the rate of dissolution of chlorhexidine was slow, loading of the drug into chitosan microspheres resulted in a remarkable improvement of its dissolution rate.

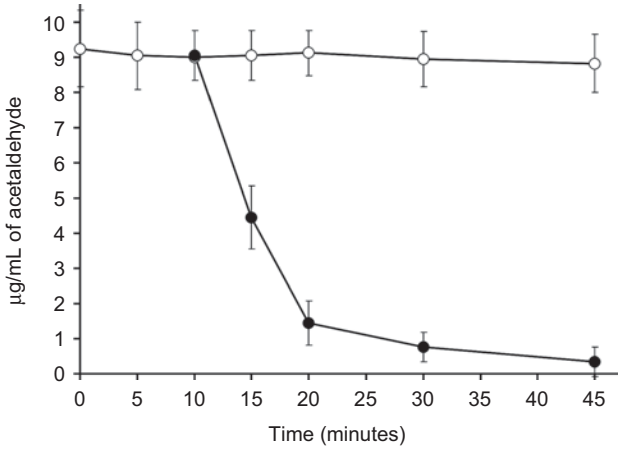

Figure 5. Neutralizing effect of cysteine against acetaldehyde: *in vitro* acetaldehyde concentrations in absence (○) and in presence (•) of cysteine hydrochloride. Cysteine powder was added at 10 minutes. Medium: phosphate buffer pH 7.0. Each point represents the mean ± SD ($n = 6$).

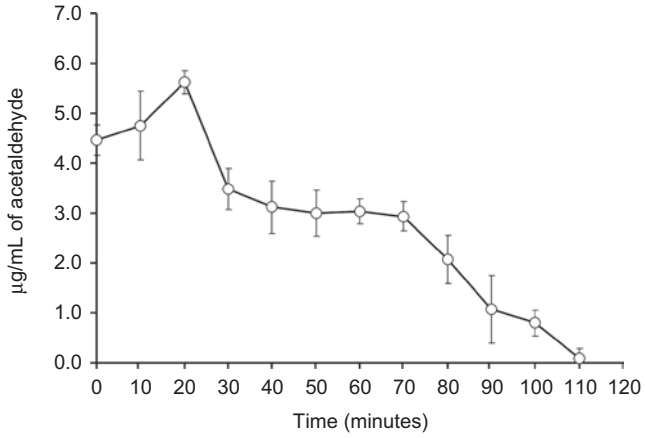

Figure 6. *In vitro* neutralization of acetaldehyde by tablet formulation F (USP apparatus 1, basket, phosphate buffer pH 7.0, 37°C, 50 rev/min). The tablet was added to the vessel at 10 minutes.

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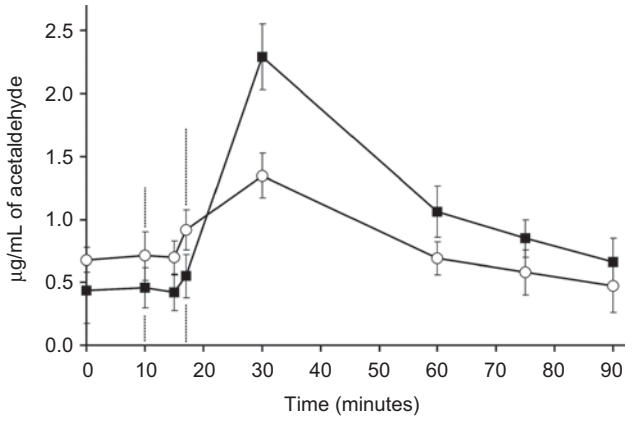

Figure 7. Modification of *in vivo* salivary concentrations of acetaldehyde produced by ethanol washings by application of cysteine-containing tablet (Formulation F). ■ = baseline (no tablet); ○ = application of tablet; T1 = ethanol washings; T2 = tablet application.

In vitro drug release tests

As far as the appearance of tablets after dissolution is concerned, some of them (C, E, and F) simply swelled, remaining intact and maintaining their shape, whereas the other formulations (A, B, D, and G) partly eroded (Figure 4). The dissolution profiles in phosphate buffer (pH 7.0) of chlorhexidine diacetate from the tablet formulations are shown in Figure 2. The kinetics of drug delivery are different; chlorhexidine was released rapidly from the tablets F, characterized by the presence of drug-loaded chitosan microspheres and alginate HV, reaching 50% in 20 min. Formulation D, containing as an excipient chitosan hydrochloride, also released chlorhexidine quickly (50% of drug delivered after about 50 min). In the case of the tablets C and E (whose excipients were chitosan base/alginate MV 1:1 and chitosan hydrochloride/alginate MV 1:1, respectively), a more sustained drug release was observed (about 10% of chlorhexidine in 3 h); that sustained drug release could be ascribable to the formation of an ionic complex between sodium alginate and chitosan after water absorption from the dissolution medium. Other formulations exhibited intermediate drug release kinetics.

In comparison to cysteine hydrochloride, all the formulations (especially A, F, and D) showed a fast drug delivery, with the exception of formulation C, characterized by a cysteine release particularly slow (only about 40% after 3 h; Figure 3).

On the basis of these release profiles, formulation F was judged the most suitable for our purposes and was then chosen for further experiments of acetaldehyde neutralization.

In vitro neutralization of acetaldehyde

Preliminary tests demonstrated that our dissolution apparatus prevented acetaldehyde leakage and was then suitable for its dosage. As expected, on the basis of the literature, cysteine hydrochloride as a powder neutralizes quickly acetaldehyde in solution, as shown in Figure 5. Cysteine is also able to reduce *in vitro* acetaldehyde concentration when included in a tablet. Figure 6 shows the acetaldehyde decrease in phosphate buffer solution after addition of a tablet of formulation F; the aldehyde is completely neutralized in 110 min.

In vivo neutralization of acetaldehyde

Figure 7 shows the variations in the salivary acetaldehyde levels in three volunteers after ethanol oral washings and subsequent application of a cysteine–chlorhexidine-containing tablet into the oral cavity, compared with the acetaldehyde levels dosed in absence of the tablet (control). At the end of the test, the tablet appeared swelled but was intact and maintained its shape. After repeated alcohol washings, acetaldehyde concentration increased: the increase can be observed throughout 15 min after tablet application because cysteine needs some time to be delivered from polymeric matrix. The presence of the tablet in the oral cavity for 30 min resulted in the progressive reduction of salivary acetaldehyde, whose concentration finally came back to the basal level; after the same period of time, in absence of the tablet, the final salivary concentration of acetaldehyde did not lower to the initial one. Moreover, the maximum acetaldehyde concentration reached in the control experiment was higher than the peak reached in presence of the tablet.

Discussion

A large body of evidence from epidemiologic studies supports the fact that alcohol consumption and tobacco smoking are strongly associated with an increase in the risk of cancers of the upper aerodigestive tract, that constitute approximately 4% of all malignancies (Muir and Weiland, 1995).

Acetaldehyde, the first metabolite of ethanol, has been shown to be mutagenic and carcinogenic in animal models and is considered responsible for the cancer-promoting action of alcohol. Because acetaldehyde is also one of the major components in the smoke of tobacco cigarettes (Risner and Martin, 1994), it has been suggested that a common mechanism can justify alcohol intake and tobacco smoking as principal known causes of aerodigestive tract tumors (Tuyns et al., 1988). In addition, poor dental status and inadequate oral hygiene also result in an increased salivary acetaldehyde formation from ethanol (Homann et al., 2001) because of the alcohol metabolism by oral microflora.

These observations suggest that all factors having an effect on salivary acetaldehyde concentrations can be important in preventing upper gastrointestinal cancer; in particular, it has been demonstrated that orally administered L-cysteine, slowly released from simple buccal formulations (tablets, chewing gums), is able to bind salivary acetaldehyde originating from ingested alcohol (Salaspuro et al., 2002) or during smoking (Salaspuro et al., 2006). On the other hand, Homann et al. (1997) demonstrated that salivary acetaldehyde levels can be

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significantly reduced by using an antiseptic mouthwash, and some authors have proposed the application of a controlled-release chlorhexidine chip to reduce oral acetaldehyde levels and meet patient compliance (Rota and Poggi, 2003).

A joined strategy to reduce acetaldehyde and then prevent upper digestive tract cancer may combine the chemical inactivation of this compound by L-cysteine and the reduction the oral microflora by antiseptic drugs. The formulations we prepared are inspired by this double concept; buccal tablets containing L-cysteine hydrochloride and chlorhexidine diacetate were prepared by direct compression of powder mixtures (in one case microspheres) containing mucoadhesive excipients. The release of each of these active principles was not affected by the other; the study of their dissolution profiles allowed us to choose the formulation containing chlorhexidine-loaded chitosan microspheres (formulation F) for further experiments. Chlorhexidine is a drug poorly soluble in water and, as already reported (Giunchedi et al., 2002), its loading into chitosan microparticles leads to a remarkable improvement of its dissolution rate; on the other hand, spray drying technique, used to obtain the microparticles, is a simple and rapid one-step process. Formulation F releases 90% of chlorhexidine and 95% cysteine in about 60 min (Figures 2 and 3); this kinetics is suitable for a possible *in vivo* application because chlorhexidine is released quickly, delivering therapeutically significant concentrations of drug (minimum inhibitory concentration against *C. albicans* 7.8 µg/mL; Giunchedi et al., 2002); moreover, thanks to its substantivity, chlorhexidine binds to soft and hard surfaces into the oral cavity, thereby establishing a drug reservoir (Bonesvoll, 1978) Moreover, formulation F was able to neutralize acetaldehyde *in vitro*.

The use of our formulations is safe. L-Cysteine is a non-essential amino acid without adverse effects, regularly consumed with food; in humans, cysteine supplemented diets (6.5 mg/ kg/day) have been used without any evidence of possible toxicity (Raguso et al., 2000) and therefore its application could be possible in the prevention of upper gastrointestinal tract cancers among smokers and heavy drinkers. In this connection, in 2010, a Finn company marketed a new over-the-counter product in capsules containing L-cysteine, intended for patients suffering from *Helicobacter pylori* infection or achlorhydria or undergoing long-term therapy with drugs reducing acid gastric secretion, and designed to reduce microbial overgrowth-related acetaldehyde production in the stomach. On the other hand, the biocompatibility of chlorhexidine, at the clinically used concentrations, is acceptable, although in rare cases it may cause allergic reactions; moreover, chewing gums and lozenges containing chlorhexidine are available in many countries worldwide for the treatment of gingivitis, periodontitis, and infections of oral cavity or throat (Imfeld, 2006). Because the use of these compounds is safe, our study offers a new strategy for prevention of upper gastrointestinal tract tumors in smokers and heavy drinkers.

Conclusions

Numerous studies have shown the determinant role of acetaldehyde from different sources (especially bacterial and mucosal metabolism of ethanol and tobacco consumption) in the carcinogenesis of the upper gastrointestinal tract. The aim of this article was to present a novel experimental approach to decreasing local acetaldehyde exposure of the upper digestive tract with L-cysteine. We demonstrated that acetaldehyde produced in the oral cavity by ethanol oxidation is reduced by the local application of buccal tablets containing L-cysteine and chlorhexidine diacetate. Because the use of these compounds is safe, our study offers a new strategy for prevention of upper gastrointestinal tract tumors in smokers and heavy drinkers.

Declaration of interest

The authors declared no conflict of interest.

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