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ORIGINAL ARTICLE

Computational prediction of local drug effect on carcinogenic acetaldehyde in the mouth based on in vitro/in vivo results of freely soluble L-cysteine

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Abstract

Background: The computational models for predicting oral drug absorption in humans using in vitro and in vivo data have been published. However, only a limited number of studies are available on the prediction of local drug efficacy in the mouth using computational models. *Aim*: The goal of this study was to develop a simulation model for prediction of drug amount and effect on carcinogenic acetaldehyde in the mouth. *Methods*: The model was based partly on our previous studies in which we showed in vivo that L-cysteine-containing tablets can eliminate carcinogenic salivary acetaldehyde in the mouth during smoking. To develop as informative a model as possible, we also investigated whether a lower saliva pH (4.7) can affect the freely soluble L-cysteine dissolution rate and cysteine stability profile in the mouth, compared to the normal saliva pH of 7.4. *Results*: Stability of the active drug is not pH dependent and thus users with normal, healthy saliva pH and those with lower pH can benefit from cysteine-containing products. The simulated saliva profiles of L-cysteine and acetaldehyde corresponded to the in vivo results. *Conclusions*: The model developed can be used as an alternative tool to obtain faster and cheaper answers on how freely soluble drugs affect local conditions in the mouth. Because tobacco smoke contains more than 60 carcinogenic compounds, the model developed can offer a new view in eliminating or reducing not only one toxic compound from smoke but also many others compounds using only one formulation containing various active compounds.

Key words: *Acetaldehyde; amino acid; computational modeling; cysteine; drug local mouth effect; oral cancer; smoking*

Introduction

The reason for increasing interest in the local treatment of oral cavity diseases is in the fact that these diseases are among the most prevalent in humankind[1,2]. Almost everyone is affected by some type of oral disease, such as dental caries. Tobacco smoking significantly increases the risks of some oral diseases, and in many studies multivariate analyses suggested smoking as an independent risk factor for periodontal diseases[3]. The World Health Organization (WHO) stated that oral diseases are major public health problem[4]. For example, treatment of oral cancer can be not only difficult to take mentally and physically, but also very economically demanding for the community (as the fourth most expensive disease to treat in the industrialized countries). Localized drug delivery systems to the mouth have been used for a long time, generally for the therapy of diseases affecting the oral cavity (e.g., aphthous ulcers, bacterial and fungal infections, lichen planus, inflammation, and dental stomatitis)[2]. They offer several advantages and, because no systemic drug effect is needed, probably the most important advantage is the ability to avoid unnecessary side effects caused by the peroral route and thus lower drug doses are needed. Patient compliance is also generally very good.

Localized drug delivery to the mouth has in some cases proved unsatisfactory because of the changing

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nature of the oral cavity[5]. Therefore, it is desirable to predict the local effect of drug candidates before promoting them to human trials. This is not an easy task because many different factors can affect therapeutic efficacy. For example, speaking and mastication, as well as dilution and rapid elimination of the drugs from the oral cavity due to continuous saliva production, can have unwanted effects on local drug efficacy in the oral cavity[6,7]. Also saliva pH changes should be considered in preformulation studies. Oral cavity normal, healthy saliva has a pH between 6.7 and 7.4[8], but it can temporarily drop below 5 when sweets, carbonated fruit drinks, and other dietary acids are consumed[9–11]. Some drugs, such as beta blocking agents, nitrates, and diuretics, as well as tobacco smoking can also reduce salivary pH[12]. In addition, drug physicochemical properties are very crucial factors, for example, low-molecular-weight and water-soluble compounds may penetrate through the buccal mucosa. As a result, therapy efficacy is low and the compounds can cause systemic side effects.

Computational models can be an alternative tool for obtaining answers on drug efficacy and safety more rapidly, with more certainty and at lower cost[13]. Lately, computational modeling and simulation have provided researchers with useful information on selecting proper drug candidates into early preformulation studies and on running successful clinical program. This may include decisions on compound selection, amount of dose, or study design. Models can be used to predict the effect of the drug in different patient populations, and thus be of great help in finding the population that benefits from the drug[14]. To build as useful a model as possible, it is important to utilize all available data and information and to determine what additional data should be obtained. Furthermore, developed models can be rebuilt and refined, depending on the results.

Computational models for predicting oral drug absorption in humans, using in vitro and in vivo data, have been published[15,16]. However, only a limited number of studies are available on predicting local drug efficacy in the mouth, using computational models.

Previous studies by our research group have shown that L-cysteine-containing tablets bind carcinogenic salivary acetaldehyde in the mouth during smoking[17]. L-Cysteine, a nonessential amino acid, reacts covalently with acetaldehyde to form nontoxic 2-methylthiazolidine-4-carboxylic acid. Besides its carcinogenicity, there is also the hypothesis that tobacco smoke acetaldehyde acts synergistically with nicotine in the brain of the rodents[18]. According to the studies, in which the rodents self-administered either saline, acetaldehyde, or nicotine alone and acetaldehyde/nicotine mixture, acetaldehyde increased nicotine self-administration. Thus, elimination of acetaldehyde, one of the major toxic components of tobacco smoke, does not only mean the elimination of the carcinogenic compound, but also the removal of the possible agent which may increase the addictive potential of tobacco. In addition, while most smokers from industrialized countries would agree that their habit is dangerous, and the majority would want to quit, they often fail. Even after stopping, most relapse within 6 months, which is attributable to nicotine dependence[19]. Cysteine may well play a significant role not only in preventing cancer but also in fighting against tobacco addiction. However, cysteine-containing products do not make smoking safe, and we should keep in mind that tobacco smoke contains other carcinogens and addictives, and more studies are needed to support those results.

Based on previous results of our group, the main goal of this study was to develop a simulation model predicting drug amount and local drug effects on carcinogenic acetaldehyde in the mouth. The effect was measured as acetaldehyde concentration in saliva during the period of different L-cysteine amount exposures. To develop a model, as much as possible informative, we also investigated in vitro and in vivo whether lower saliva pH (4.7) can affect the freely soluble L-cysteine dissolution rate and cysteine stability profile in the mouth, compared to normal saliva pH of 7.4.

Methods

Chemicals

Ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F), L-cysteine, and ferric sulfate were obtained from Fluka Chemicals, Buchs, Switzerland. Dimethylformamide, ferrozine, and sodium perchlorate from Sigma-Aldrich, Steinheim, Germany; and calcium dichloride ($CaCl_2$), 37% hydrochlorid acid (HCl), potassium dihydrogen phosphate (KH_2PO_4), sodium bicarbonate ($NaHCO_3$), sodium hydrochloride (NaCl), and sodium hydroxide (NaOH) were from Sigma-Aldrich, Seelze, Germany. Ethylenediaminetetraacetic acid disodium salt dehydrate (Na_2EDTA), tri-*n*-buthylphosphine, and 10% trichloroacetic acid were from Sigma, St. Louis, MO, USA. Black currant flavoring was from Quest International, Naarden, the Netherlands. Magnesium stearate, mannitol, potassium chloride (KCl), and 85% orthophosphoric acid were from Merck, Darmstadt, Germany. Methanol was obtained from Rathburn, Walkenburg, Scotland.

Preparation of tablet

The formulation theoretically contained 5 mg L-cysteine. Because cysteine is very sensitive to high pressure and

increases in temperature induced by compression[20], the amount of cysteine found in the formulation after preparation was 4 mg.

The L-cysteine powder was ground and the fraction with sizes at 90–315 μm was used in the study. Mannitol (725 mg) was used as a filler and 20 mg of black currant as a flavoring agent to disguise the unappealing taste of L-cysteine. The components of each formulation, except for magnesium stearate, were mixed for 20 minutes in a Turbula shaker mixer (T2C Willy A. Bachofen A6 Maschinenfabrik, Basel, Switzerland). Magnesium stearate (2% of total weight) was then added and the formulation mixed for a further 2 minutes. The tablets were compressed with an instrumented eccentric tablet machine (Korsch EK-0; Erweka Apparatebau, Frankfurt am Main, Germany), using flat-faced punches with a diameter of 13 mm. The compression force applied was 7–8 kN. Tablets were prepared immediately before the in vitro and in vivo studies.

In vitro dissolution studies

Dissolution tests of pure cysteine were carried out with 20 mg of cysteine powder, using the basket method described in USP 24 in 500 mL artificial saliva, pH 7.4 and 4.7, at 37 ± 0.5°C. The artificial saliva was prepared as described by Duffó and Castillo[21]: 4.201 g NaCl, 0.151 g KCl, 0.149 g $CaCl_2$, and 0.104 g $NaHCO_3$ were dissolved in pure water (500 mL), pH adjusted with a few drops of HCl. The speed of rotation was 50 rpm. Samples (2 mL) were taken every 2.5 minutes over the 10-minute period using a pump (Marlow 503S; Smith and Nephew, Falmonth, UK) and the volume replaced with 2 mL artificial saliva at 37 ± 0.5°C. The drug concentrations were determined by spectrophotometry as described by Eid[22].

To mimic each tablet's behavior in the mouth, this study was carried out in artificial saliva at pH 7.4 as described above, using a paddle dissolution method at 150 rpm.

In vivo studies

The study was approved by the Coordinating Ethics Committee, Hospital District of Helsinki and Uusimaa (Finland). Six volunteers took part in the studies (three males and three females, mean age 31 ± 2.8 years; range 28–36). Four of the volunteers (three males and one female) were active smokers (between 5 and 10 cigarettes per day), and two (two females) were habitual smokers (<10 cigarettes per week). The volunteers sucked 4 mg of pure L-cysteine or a tablet containing 4 mg of L-cysteine for 5 minutes. Saliva was collected continuously for 2.5 minutes before the test, during the 5 minutes of the test, and for 5 minutes after the test. Saliva for each 2.5-minute interval was collected into separate collection tubes, yielding five samples altogether. During saliva collection, volunteers were told not to swallow the secreted saliva, but to spit it into test tubes. All volunteers had normal, healthy saliva pH (mean pH 7.4 ± 0.3). To lower the saliva pH to a mean of 4.7 ± 0.4, the volunteers sucked a small amount of citric acid for 1 minute before the test started. All subjects refrained from drinking, eating, and smoking for 30 minutes before saliva collection. Volunteers rinsed their mouth 5 minutes before study and after the study. No smoking was involved during the study. Each study has been done on separate days. The remaining amount of L-cysteine was measured with high-performance liquid chromatography (HPLC) method.

HPLC analysis

The method was adapted from Zappacosta et al.[23] and Frick et al.[24] In brief, 60 μL of saliva, 30 μL of Dulbecco's phosphate-buffered saline (D-PBS) (Ph.Eur.), pH 7.4, and 30 μL 20% tri-*n*-butylphosphine in dimethylformamide were added in tubes and stored for 30 minutes at 4°C. The solution was then mixed with 90 μL of cold 10% trichloroacetic acid solution containing 1 mM Na_2EDTA. The samples were immediately vortexed for 5 minutes and centrifuged for 10 minutes at 1000 × *g*, after which 50 μL of the clear supernatant was added to tubes containing 10 μL 1.55 M NaOH, 50 μL of SBD-F solution (2 mg/mL) in 0.125 M borate buffer, and 125 μL of 0.125 M borate buffer (pH 9.5) containing 4 mM Na_2EDTA. The mixture was incubated for 60 minutes at 60°C in the dark. After cooling, 150 μL of the samples were transferred into HPLC vials. The samples were done in triplicate and analyzed immediately. The standard curve was linear over the concentration range used (50–600 μg/mL). QC samples were run in duplicate at three concentrations at 50, 300, and 600 μg/mL in all analytical runs before and after samples.

The samples were analyzed with a Waters HPLC apparatus (Waters Millennium, USA), with Waters 486 Fluoroscence Detector, 717 Autosampler, and the 510 pump. The mobile phase was 0.1 M KH_2PO_4 with 5% methanol, adjusted to pH 2.7 with 85% orthophosphoric acid. A SunFire™ C-18 column was used (150 × 4.6 mm, Waters, Dublin, Ireland) with flow rate of 1.5 mL/min. The injection volume was 10 μL and total analysis time 7 minutes. The fluorescence intensities were measured with excitation at 385 nm and emission at 515 nm.

PAEM model

The computational model presented here was built using Stella™ modeling software (Stella v9.0.2, isee systems, Inc., Lebanon, NH, USA). The Stella program has

a graphical user interface by which computational models can be built by drawing. The models are represented by 'stocks' and 'flows' to which describing parameters are attached. Model was based on results described in sections 'In vitro dissolution studies' and 'In vivo studies' as well as previous results from our research group[17]. In brief, salivary acetaldehyde increased rapidly during active smoking to 228 ± 115 μM from the basal level (0) and declined rapidly after 5 minutes of smoking with a placebo tablet (Figure 1). In the study, the developed cysteine-containing tablet released cysteine into the oral cavity during the 5-minute smoking period and thus eliminated all of the carcinogenic acetaldehyde from the mouth formed during smoking. Based on these results, the main goal of this study was to develop a simulation model predicting drug amount and local drug effects on carcinogenic acetaldehyde in the mouth. The effect was measured as acetaldehyde concentration in saliva during the period of different L-cysteine amount exposures. Acetaldehyde production to the saliva during smoking was modeled as a zero-order process of 5-minute duration. Release of cysteine from the formulation to the saliva was modeled to mimic in vitro data. The reaction kinetics of acetaldehyde binding and swallowing of the saliva were also mechanistically modeled. Simulation model for the prediction of drug amount and the effect on carcinogenic acetaldehyde in the mouth was named as PAEM (prediction of drug amount and effect in the mouth).

Statistical analyses

In vitro dissolution test for L-cysteine was statistically analyzed using one-way ANOVA. In vivo studies were statistically analyzed using two-way ANOVA. A level of $P < 0.05$ was considered statistically significant.

Results and discussions

In vitro dissolution studies

The results of the dissolution tests at two different pH levels (4.7 and 7.4) are presented in Figure 2. In both cases the dissolution profiles of cysteine were very similar, with no significant difference between pH 4.7 and 7.4 ($P > 0.05$). In both cases more than 90% of cysteine was dissolved (at pH 4.7 95.4% and at pH 7.4 91%) after 5 minutes. This observation was expected, because cysteine is freely soluble in water.

Because there were no differences between dissolution profiles of pure cysteine at different pH levels, we tested the dissolution profiles of tablets only at a normal, healthy saliva pH of 7.4. At 5 minutes approximately, 83% of the cysteine was released and the tablet was completely dissolved at 10 minutes (Figure 2).

The dissolution profiles of pure cysteine and cysteine tablets suggest that cysteine could also behave in the same way in vivo and thus have the same efficacy in patients with normal and lower saliva pH. This is an important finding because consumption of acidic drinks and some diseases can reduce saliva pH whereas over longer time periods tobacco smokers can also have a lower saliva pH[25].

In vivo studies

The in vivo results are in good accordance with the above in vitro results. Changes in saliva pH did not affect the remaining amount of pure cysteine or cysteine released from tablets (Figure 3). There was also no

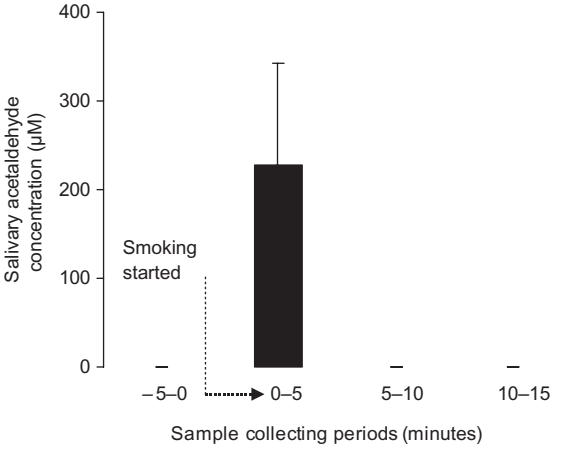

Figure 1. Salivary acetaldehyde levels collected in 5-minute periods before, during, and after smoking a cigarette with placebo tablet. Smoking was stopped after 5 minutes (Modified from Salaspuro et al.[17]).

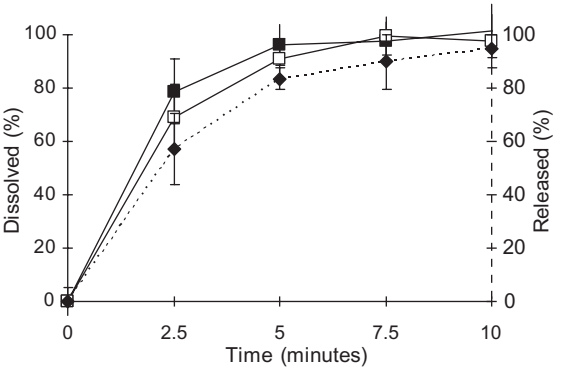

Figure 2. In vitro dissolution profiles in artificial saliva of pure L-cysteine (left side as dissolved, %) at pH 4.7 (—■—) and at 7.4 (—□—) at 50 rpm and dissolution profile of L-cysteine-containing tablet (right side as released, %) at pH 7.4 (---◆---) at 150 rpm. Data are mean ± S.D., $n = 6$.

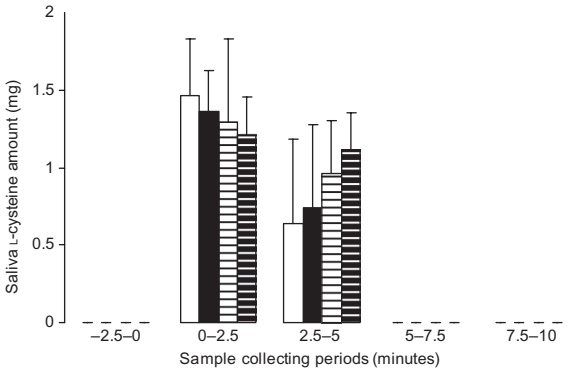

Figure 3. Remaining amount (mg) of cysteine in saliva when pure cysteine was sucked for 5 minutes at saliva pH 7.4 (□) and 4.7 (■) and cysteine-containing tablet was sucked for 5 minutes at saliva pH 7.4 (▤) and 4.7 (▬). Saliva was collected continuously for 2.5 minutes before the test, during the 5 minutes of the test, and for the 5 minutes after the test. Results are normalized in proportion to saliva volume. No significant differences were seen between remaining amounts of saliva cysteine during 5 minutes of sucking pure cysteine or cysteine-containing tablet ($P > 0.05$). Data are mean ± S.D., $n = 6$. (Variables tested were formulation and pH. In a case of formulation the test pairs were pure cysteine versus cysteine-containing tablet. In a case of pH, the test pairs were pH 7.4 versus pH 4.7.)

difference between the remaining amounts of saliva cysteine during 5 minutes of sucking pure cysteine or cysteine-containing tablets ($P > 0.05$). After 5 minutes, the cysteine amounts were 0. In considering that acetaldehyde levels are high only during smoking (about 5 minutes)[17] and cysteine effect is needed during that time, it is important to note that it is a desired effect. The reason for not finding entirely the whole tablet's cysteine amount (53% and 60% of the total amount from pure cysteine and tablet, respectively) can be explained by elimination of the drug from the oral cavity due to the swallowing reflex and by some degradation of active drug. It should also be considered that the drug may be able to penetrate through the buccal mucosa[26]. Our preliminary studies, which will be published separately, suggest that cysteine is not absorbed through the paracellular pathway or by active transport through Caco-2 cell lines and thus probably does not penetrate through the buccal mucosa. Neither Caco-2 cells nor the oral buccal mucosa express L-type amino acid transporter (LAT2), which is responsible for absorption of cysteine in the human small intestine[27].

PAEM model

The physiologically based simulation model, PAEM, for local oral effect was built, using the Stella program (Figure 4). The PAEM is more of a mechanistic model of ongoing processes during treatment and not a typical parametric pharmacokinetic model. It is based on the in vivo results of six volunteers and was not validated for a larger population during the work presented here. Parameters used in the Stella model and their in vitro, in vivo, or literature-derived values are defined in Table 1. On the left in the figure, release of cysteine from the tablet to the saliva is modeled. The release is described by the release rate constants k_1, k_2, and k_3, each for their corresponding time interval. The values for these constants were derived from the in vitro dissolution experiments (in Figure 2 the data for tablets at 150 rpm). The release rate (Cysteine release in Figure 4) is mathematically described in the following equation:

$$\frac{\mathrm{d}M_{\text{cysteine in tablet}}}{\mathrm{d}t} = k_n \cdot M_{\text{cysteine in tablet}} \qquad (1)$$

where $M_{\text{cysteine in tablet}}$ is the amount (mass) of cysteine remaining in the formulation and k_n is k_1, k_2, or k_3 for the corresponding time interval. Acetaldehyde input to the saliva produced by tobacco smoking is modeled on the right-hand side of the model, taking into account the duration of smoking and the total amount of acetaldehyde produced by a single tobacco 'Acetaldehyde release':

$$\frac{\mathrm{d}M_{\text{acetaldehyde in tobacco}}}{\mathrm{d}t} = \frac{M_{a0}}{t_s} \qquad (2)$$

where $M_{\text{acetaldehyde in tobacco}}$ is remaining acetaldehyde in the tobacco, M_{a0} is the total amount of acetaldehyde produced while smoking one tobacco, and t_s is the total duration of smoking. The binding reaction for acetaldehyde is represented in the middle combining flows of both cysteine and acetaldehyde. Similar molar amounts of cysteine and acetaldehyde are consumed in the reaction described by the reaction rate parameter in the model 'Binding':

$$\frac{\mathrm{d}M_{\text{saliva cysteine}}}{\mathrm{d}t} = M_{\text{saliva cysteine}} \cdot k_{\text{rate of reaction}}(44/121) \qquad (3)$$

where (44/121) is the correcting for different molar masses of acetaldehyde and cysteine; 'Binding 2':

$$\frac{\mathrm{d}M_{\text{saliva acetaldehyde}}}{\mathrm{d}t} = M_{\text{saliva cysteine}} k_{\text{rate of reaction}} \qquad (4)$$

The reaction rate is set so that the binding reaction takes place fully in 1 minute 40 seconds in accordance with previous findings. The effects of natural saliva flow from the mouth to the esophagus caused by swallowing

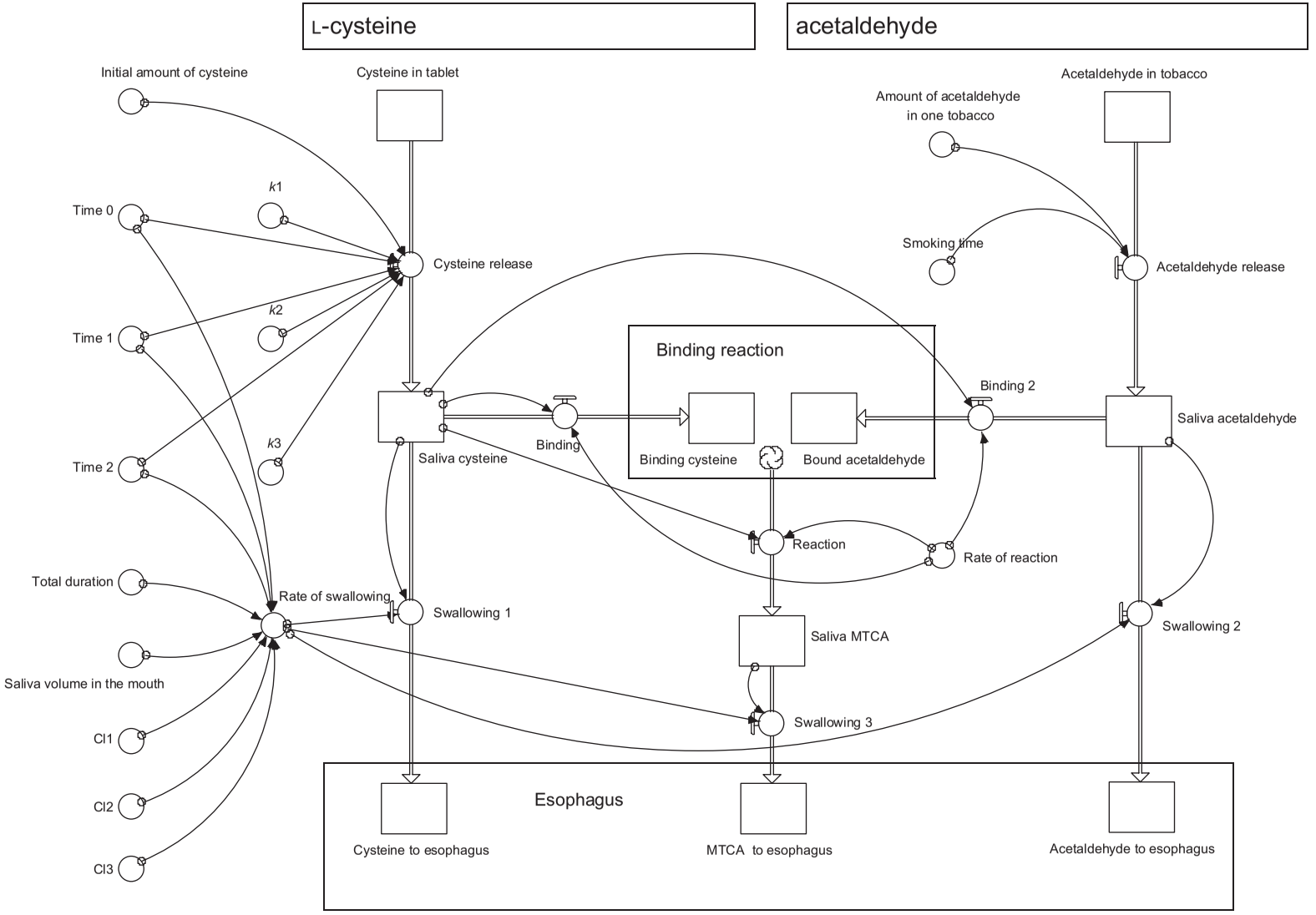

Figure 4. PAEM (Prediction of drug Amount and Effect in the Mouth) simulation model for prediction of drug amount and effect on carcinogenic acetaldehyde in the mouth. On the left side release of cysteine from the tablet to the saliva is modeled and acetaldehyde input to the saliva produced by tobacco smoking is modeled on the right-hand side of the model. The binding reaction for acetaldehyde is represented in the middle combining flows of both cysteine and acetaldehyde. Model was based on results described in sections 'In vitro dissolution studies' and 'In vivo studies' as well as previous results from our research group[17].

Table 1. Parameters used in the Stella model and their in vitro, in vivo, or literature-derived values.

Parameter	In vitro value	In vivo value	Literature value
Time 0		0 minute	
Time 1		2.5 minutes	
Time 2		5 minutes	
Total duration		5 minutes	
Saliva volume in the mouth			2 mL
Cl_1		1.8 mL/min	1–3 mL
Cl_2		2.0 mL/min	1–3 mL
Cl_3		1.3 mL/min	1–3 mL
k_1	0.00381		
k_2	0.00175		
k_3	0.00043		
Cysteine in tablet	4 mg		
Initial amount of cysteine	4 mg		
Acetaldehyde in tobacco			1000 mg
Amount of acetaldehyde in one tobacco			1000 mg

are also modeled for each of the three compounds. The amount of saliva in the mouth was approximately 2 mL. The flows are described by clearance values in this case, again for the three time intervals (Cl_1, Cl_2, and Cl_3) 'Swallowing 1':

$$\frac{dM_{\text{saliva cysteine}}}{dt} = M_{\text{saliva cysteine}} k_{\text{rate of swallowing}} \quad \text{and} \quad k_{\text{rate of swallowing}} = \frac{Cl_n}{V_{\text{saliva volume in the mouth}}} \tag{5}$$

where Cl_n is Cl_1, Cl_2, or Cl_3. 'Swallowing 2 and 3' are similarly defined. The values are derived from the actual measured saliva volumes per time interval during the in vivo studies conducted with healthy volunteers. Taking into account that cysteine is not absorbed in the mouth and the surface area of the oral mucosa is relatively small, approximately 100 cm^2, it is satisfactory to model the two main routes of cysteine flow explained earlier.

The model was designed so that different smoking times, varying amounts of cysteine in the formulation, and biological variation in the saliva excretion rates can easily be simulated. At the first stage, the values of all parameters were adjusted so that the previously obtained results were mimicked as closely as possible in silico. A good correlation was observed between computational acetaldehyde and cysteine levels and levels detected in in vivo samples (Figures 3 and 5). In the in vivo study, where acetaldehyde from oral cavity was eliminated during smoking close to 100%, concentration of acetaldehyde after administration of tablet

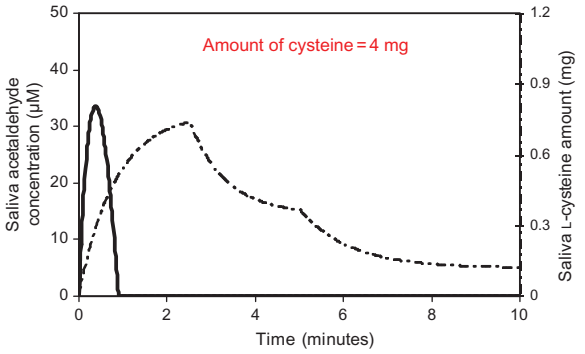

Figure 5. Simulated saliva L-cysteine amount (mg) (---) and simulated saliva acetaldehyde concentration (μM) (—) for a simulation period of 10 minutes when selected L-cysteine amount was same as in in vivo studies (4 mg).

containing 2.5 or 5 mg of cysteine were 9 ± 7 and 0.09 ± 0.2 μM, correspondingly[17]. Thus, it can be seen that the amount of cysteine selected is sufficient to rapidly drive acetaldehyde levels to below the harmful threshold of 50 μM[28–30].

Effect of cysteine amount

The effects of varying the release rate of cysteine were studied after the initial verification of the model. A drop in the cysteine amount from 4 to 3 mg results in a slightly higher acetaldehyde level in the first peak and the appearance of a second peak between 4 and 5 minutes (Figure 6a), which would be undesired in vivo behavior. Compared to Figure 5, where the cysteine amount used was 4 mg, an increase in cysteine to 5 mg would diminish the peak value of the acetaldehyde level from 33 to 27 μM (Figures 5 and 6b). Although this is a favorable level in vivo, it may be considered an unnecessary improvement because the levels are already well below the harmful level. In conclusion, the model suggests that the amount of cysteine selected, 4 mg per formulation, is well justified.

Effect of saliva excretion rate

The potential effects of biological variance in saliva excretion rate were also studied. The widely accepted normal values for stimulated flow rates range from 1.0 to 3.0 mL/min[31]. When the initial in vivo-derived values (around 2 mL/min) were replaced by a flow rate of 1.0 mL/min, the peak in acetaldehyde level was slightly lowered (Figure 7a). Importantly, a much more dramatic effect was observed when a rapid excretion rate of 3 mL/min was simulated: a second peak appeared again after 3 minutes having a larger AUC than the first peak (Figure 7b). Based on the simulation, it suggests

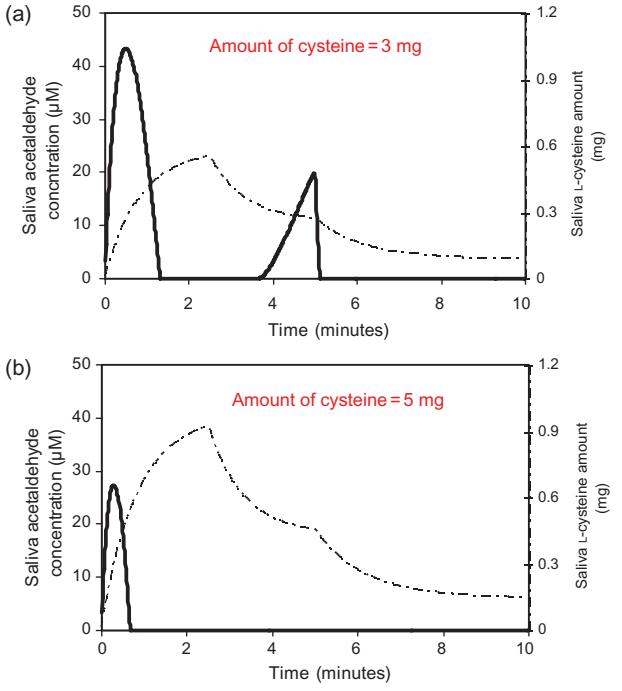

Figure 6. Effect of lower (3 mg) (a) and higher (5 mg) (b) amount of L-cysteine on simulated saliva L-cysteine amount (mg) (---) and simulated saliva acetaldehyde concentration (µM) (—) for a simulation period of 10 minutes.

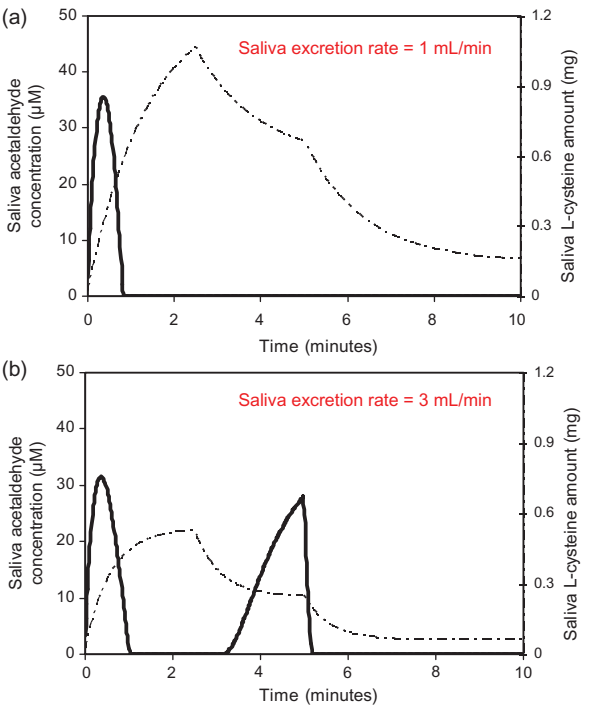

Figure 7. Effect of saliva excretion rate (1 mL/min (a) and 3 mL/min (b)) on simulated saliva L-cysteine amount (mg) (---) and simulated saliva acetaldehyde concentration (µM) (—) for a simulation period of 10 minutes when selected L-cysteine amount was same as in in vivo studies (4 mg).

that in this extreme case more cysteine would be needed for effective binding of acetaldehyde. When necessary, this level could be reached by adding more cysteine in the formulation or by developing a slower releasing tablet formulation. This would be expected, because smoking lasts longer in the in vivo experiments than cysteine release from the tablet.

Conclusions

In conclusion, in comparing the results obtained from the in vitro and in vivo studies, we conclude that the stability of cysteine is not pH dependent. This is a very important finding, because it is well known that some diseases, consumption of acidic drinks, and tobacco smoking can reduce saliva pH. Thus, the cysteine product developed is effective for users with normal, healthy saliva pH and also for those with lower saliva pH.

Because cysteine is a freely water-soluble substance that does not penetrate through the buccal mucosa, the PAEM model can be used to predict the drug amount and local effect in the mouth of similar substances or tablet formulations. Furthermore, the model can be developed and refined based on the in vivo results. In addition, because tobacco smoke contains more than 60 carcinogenic compounds, PAEM model can offer a new view in eliminating or reducing not only one toxic compound from tobacco smoke but also many other compounds using only one formulation containing various active compounds. Validation of the model needs further simulation runs based on larger in vivo data sets for different formulations, test setups, or subject groups. Theoretically, there are no limitations in studying different doses and individual variation in modeled parameters with the PAEM model.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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