Short Communication

Eliminating Carcinogenic Acetaldehyde By Cysteine From Saliva During Smoking

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Abstract

Tobacco smoking is one of the strongest risk factors not only for lung cancer but also for cancers of the upper gastrointestinal tract. Acetaldehyde has been shown to dissolve into the saliva during smoking and to be a local carcinogen in the human upper digestive tract. Cysteine can bind to acetaldehyde and eliminate its toxicity. We developed a tablet that releases cysteine into the oral cavity during smoking and could therefore be a potential chemopreventive agent against toxicity of tobacco smoke. In this study, the efficacy of L-cysteine–containing tablets to reduce the carcinogenic acetaldehyde in the saliva during tobacco smoking was examined. Seven volunteers smoked five cigarettes. During every smoking period, each volunteer sucked a blinded tablet containing 0, 1.25, 2.5, 5, or 10 mg of L-cysteine. Acetaldehyde was analyzed from salivary samples gas chromatographically at 0, 5, and 10 minutes from the beginning of the smoking. All tablets containing L-cysteine reduced highly significantly the salivary acetaldehyde; 5 mg of L-cysteine was the minimum concentration to totally eliminate the acetaldehyde from saliva. The mean salivary acetaldehyde concentrations in samples collected immediately after smoking with 0, 1.25, 2.5, 5, or 10 mg of L-cysteine were 228 ± 115 μmol/L, 85 ± 42 μmol/L (P = 0.007), 9 ± 7 μmol/L, 0.09 ± 0.2 μmol/L, 0 ± 0 μmol/L (P < 0.001), respectively. In conclusion, carcinogenic acetaldehyde could be totally inactivated in the saliva during smoking by sucking tablet containing 5 mg of L-cysteine. Even a small reduction of the carcinogenicity of cigarette smoke could gain benefit at the population level. Hence, this finding warrants for further clinical trials for L-cysteine tablet in the prevention of upper digestive tract cancers in smokers. (Cancer Epidemiol Biomarkers Prev 2006; 15(1):146–9)

Introduction

Tobacco smoking is currently the main known cause of cancer-related death worldwide (1). According to the IARC working group, tobacco smoke is a multipotent carcinogenic mixture that can cause cancer in many different organs. Tobacco smoking alone is a strong and independent risk factor for the cancers of oral cavity, pharynx, larynx, esophagus, and stomach (2, 3). It has been estimated that the proportions of cancers of the oral cavity, larynx, and esophagus attributable to tobacco smoking are between 43% and 60% (3). In addition to alcohol use, tobacco smoking is the principal known cause of aerodigestive tract cancers (2, 4). Accordingly, it is estimated that 80% of these cancers could be avoided by abstaining from smoking and alcohol drinking (5).

There is substantial experimental evidence for the carcinogenicity of tobacco smoke. Multiple animal and cell studies show that tobacco smoke initiates and promotes tumor development especially concerning upper gastrointestinal tract (6). Tobacco smoke contains several classes of carcinogens that include among others polycyclic aromatic hydrocarbons, aromatic amines, and nitrosamines. Tobacco smoke contains also high concentrations of toxic aldehydes (7). The most abundant aldehyde in tobacco smoke is acetaldehyde, and its concentration in tobacco smoke is >1,000 times greater than those of polycyclic aromatic hydrocarbons and tobacco-specific nitrosamines (8).

Acetaldehyde is a known mutagen and carcinogen according to numerous *in vitro* cell culture studies and *in vivo* animal models (9). Acetaldehyde formed during ethanol metabolism has also been shown to be behind alcohol-related gastrointestinal tract carcinogenesis (10). Very recent epidemiologic studies on Asian heavy drinkers with impaired acetaldehyde metabolism due to aldehyde dehydrogenase-2 deficiency strongly suggest that acetaldehyde is a topical carcinogen in man (11). This deficiency results in the accumulation of acetaldehyde locally into the saliva during ethanol metabolism (12) and also in markedly increased risk for many upper gastrointestinal tract cancers (13-15). This human ''knock-out model'' provides strong evidence for the topical carcinogenic role of acetaldehyde in the upper gastrointestinal tract in humans. We have previously showed that acetaldehyde from the tobacco smoke is easily dissolved into the saliva during smoking (16). Thus, toxic aldehydes could mediate the carcinogenic effect of tobacco smoke through saliva to oral cavity and from there further on to the larynx, the esophagus, and even to the stomach.

Cysteine, a nonessential amino acid, is able to eliminate the toxicity of acetaldehyde by reacting covalently with acetaldehyde to form stable 2-methylthiazolidine-4-carboxylic acid (17). In our previous studies, we have been able to bind acetaldehyde originated from ethanol metabolism with orally administered L-cysteine (18). Thereby, cysteine could also be used to eliminate the acetaldehyde dissolved into the saliva

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Note: Patent application is under evaluation for binding salivary aldehydes with L-cysteine during smoking and the patent has been sold.

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during smoking and reduce the carcinogenic potential of tobacco smoke against the upper digestive tract. Thus, the aim of the study was to examine the ability of orally administered L-cysteine containing sucking tablet (compressed lozenge) to bind acetaldehyde from the saliva during smoking.

Materials and Methods

Subjects. Seven healthy volunteers (5 males and 2 females), mean age 28 ± 1.9 years (range, 21-37 years) took part in the study. Information about the smoking status, alcohol consumption, and possible medications was obtained by a self-administered questionnaire. Five of the volunteers were active smokers (>10 cigarettes/d, >5 years) and two were habitual smokers (<10 cigarettes/wk). All of the volunteers were moderate alcohol consumers, with a weekly average consumption of ≤70 g of alcohol. Exclusion criteria were as follows: treatment with antibiotics in the past month, use of antiseptic mouthwash in the past week, smoking during the previous 60 minutes. All participants were told to refrain from ethanol for at least 24 hours before the study.

Study Design. Our study was approved by the ethical committee of the Helsinki University Central Hospital. An informed consent to participate was obtained from the subjects. A placebo-controlled, single-blinded study design, in which each subject served as his/her own control, was used.

All volunteers smoked altogether five cigarettes (Marlboro, Philip Morris Finland oy, Helsinki) with at least a 30-minute washout period in between, to test placebo tablet and four tablets with different concentrations of L-cysteine. Before smoking, volunteers started to suck in randomized order, single-blinded tablets, which contained 0 mg (placebo), 1.25, 2.5, 5, or 10 mg of L-cysteine. The tablet was designed to dissolve during the cigarette smoking. The composition of the tablet was, in addition to L-cysteine (Fluka Biochemika, Buchs, Switzerland): 725 mg Mannitole (Parteck M 200, Merck KgaA, Darmstadt, Germany; in placebo tablet, 745 mg), 20 mg blackcurrant-flavor (Quest International, Naarden, the Netherlands), and 2% magnesium stearate (Ph.Eur., Merck).

To measure the acetaldehyde *in vivo* levels during smoking, salivary samples were collected from each volunteer during smoking periods with each tablet: (*a*) before smoking to measure the baseline (−5 to 0 minutes), (*b*) immediately after the smoking (0-5 minutes of collection), (*c*) 5 minutes after the smoking (5-10 minutes of collection), (*d*) 10 minutes after the smoking (10-15 minutes of collection). To obtain the saliva samples, volunteers were told not to swallow the secreted saliva but to store it into their mouths. After a 5-minute collection period, saliva was spit in a sample tube, and 500 μL were transferred into a vial and analyzed immediately. Acetaldehyde levels were analyzed by headspace gas chromatography as previously described (19). Each *in vivo* measurement was done in duplicate.

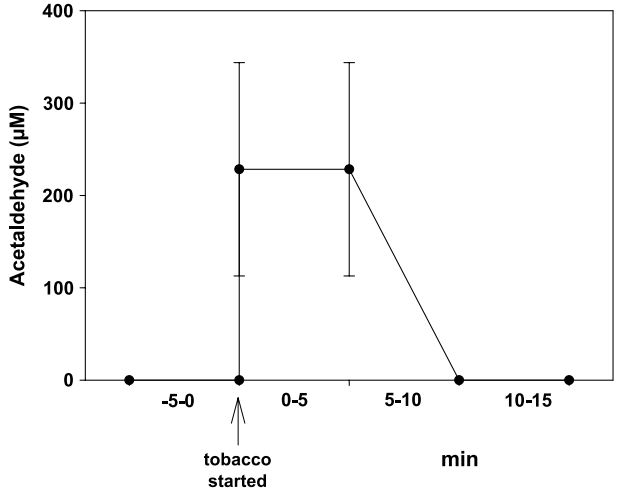

Figure 1. Salivary acetaldehyde concentration *in vivo* after smoking a cigarette with placebo tablet.

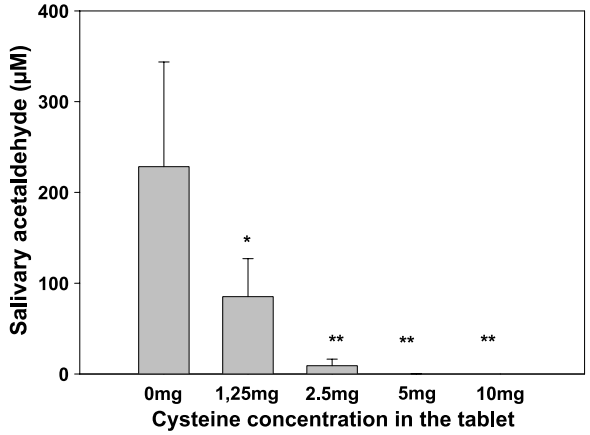

Figure 2. Salivary acetaldehyde concentrations immediately after tobacco smoking (0-5 minutes of collection period) with placebo or L-cysteine containing tablet. *, $P = 0.007$; **, $P < 0.001$.

To determine how rapidly the salivary acetaldehyde increases again after the tablet had dissolved, three volunteers smoked one cigar (Hofnar, Swedish Match Cigars B.V. Holland) to make the smoking time longer than 6.3 minutes (dissolving time of the tablet). Just before smoking, they started to suck tablet containing 5 mg of L-cysteine. Thereafter, salivary samples were taken for 3-minute intervals for 20 minutes. The sample procedure was the same as described above.

Statistical Analysis. All values are expressed as means ± SE. Statistical significance of the difference between the values obtained from study tablets was analyzed by the Mann-Whitney rank sum test. $P < 0.05$ was regarded as significant.

Results

There was highly statistically significant difference in the *in vivo* salivary acetaldehyde levels among all of the tested L-cysteine containing tablets compared with placebo tablet.

During active smoking, salivary acetaldehyde increased rapidly to 228 ± 115 μmol/L (range, 91-352 μmol/L) from the basal level (0 μmol/L) and declined rapidly after cessation of smoking with placebo tablet, which is consistent with our earlier studies (ref. 16; Fig. 1). However, all of the tested L-cysteine concentrations reduced significantly or totally eliminated the *in vivo* salivary acetaldehyde during smoking compared with placebo. The minimal L-cysteine concentration that was able to totally eliminate acetaldehyde from the saliva was 5 mg. Accordingly, the salivary acetaldehyde concentrations with placebo (0 mg) or 1.25, 2.5, 5, 10 mg of L-cysteine containing tablets immediately after the smoking (0-5 minutes of collection) were 228 ± 115 μmol/L, 85 ± 42 μmol/L ($P = 0.007$), 9 ± 7 μmol/L ($P < 0.001$), 0.09 ± 0.2 μmol/L ($P < 0.001$), and 0 ± 0 μmol/L ($P < 0.001$; Fig. 2).

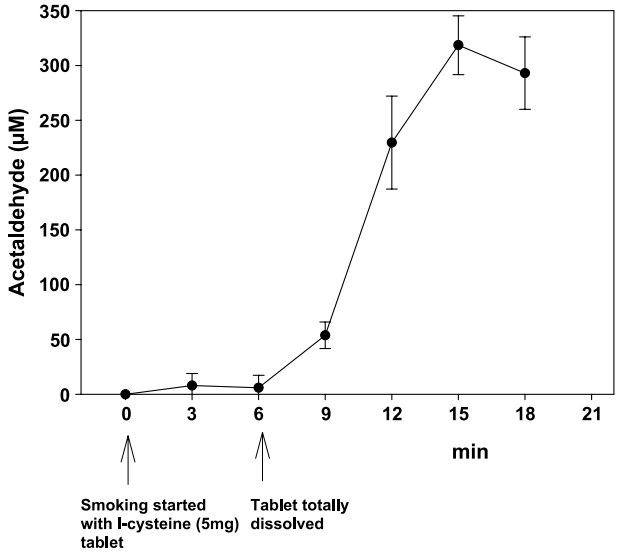

Figure 3. Salivary acetaldehyde concentrations *in vivo* during cigar smoking with tablet containing 5 mg of L-cysteine.

All of the tablets lasted longer than the tobacco smoking time. The mean resolving time of the sucking tablets were 6.3 ± 0.8 minutes (range, 5.2-8.3 minutes). After the tablet has dissolved, the salivary acetaldehyde increases after 3 minutes again to the level that is comparable with that of the placebo tablet (Fig. 3).

Discussion

Acetaldehyde is a major volatile constituent of the main stream tobacco smoke (8). We have previously shown that orally administered L-cysteine released slowly from buccal (between gum and cheek) tablet can bind two thirds of salivary acetaldehyde originated from alcohol drinking (18). This could be an essential chemoprevention strategy for alcohol associated upper gastrointestinal tract cancers because acetaldehyde has been shown to be the major factor behind ethanol induced carcinogenesis (10). In this study, we have showed that orally administered L-cysteine can totally eliminate the tobacco smoke-derived acetaldehyde from the oral cavity during smoking. Thus, cysteine-sucking tablet could be used as a chemopreventive agent also against toxicity of tobacco smoke.

Aldehydes, especially acetaldehyde, are present in the tobacco smoke in relatively high concentrations. As mentioned, acetaldehyde concentration in tobacco smoke is >1,000 times greater than those of polycyclic aromatic hydrocarbons and tobacco-specific nitrosamines (8). It has also been speculated that the concentrations of the polycyclic aromatic hydrocarbons and *N*-nitrosamines are too low to explain the carcinogenicity of the tobacco smoke or tobacco smoke condensate (20). On the other hand, numerous *in vitro* and *in vivo* experiments in cell cultures and in animal models have shown that acetaldehyde has direct mutagenic and carcinogenic effects (9). It also induces nasal tumors in rats and an enhanced number of laryngeal carcinomas have documented in hamsters when acetaldehyde is administered by inhalation (21, 22).

We have previously shown that smokers, heavy drinkers, and patients with upper gastrointestinal tract cancer have increased salivary acetaldehyde production *in vitro* (23). We have also shown that tobacco smoking increases dramatically the salivary acetaldehyde concentration *in vivo*, because the acetaldehyde from the smoke is efficiently dissolving into the saliva (16). Thus, acetaldehyde dissolved into the saliva might exert its carcinogenic effect not only to the oral cavity but also to the larynx, the esophagus, and further on to the stomach when passed on by swallowing. This could explain the increased risks of the oral cavity, pharyngeal, and laryngeal cancers, as well as esophageal and stomach cancers among smokers (24). Critical carcinogenic factor is the local acetaldehyde in the upper digestive tract, because the systemic acetaldehyde levels are negligible during smoking and alcohol drinking (25).

In this study, we were able to eliminate all of the acetaldehyde from the oral cavity during smoking with a minimum of 5 mg of L-cysteine containing tablet. In addition, smaller concentrations of L-cysteine (2.5 and 1.25 mg) bound significantly although not totally the salivary acetaldehyde. L-cysteine as a thiol compound is known to be able to protect against acetaldehyde toxicity by reacting with acetaldehyde forming a nontoxic carboxylic acid–compound, 2-methylthiazolidine-4-carboxylic acid. On the other hand, even by the use of cysteine tablet during smoking, there still remain some important carcinogens in the tobacco smoke. Thus, we do not want to state that smoking will be safe with the use of L-cysteine tablet.

The idea of the chemopreventive mechanism of the L-cysteine tablet is that it is sucked during every tobacco smoking. Because of the resolving time of about 6 minutes, the tablet designed for tobacco is not ideal for longer smoking periods as it would be in the case of cigar smoking. The use of L-cysteine tablet is safe, because L-cysteine as a nonessential amino acid has no known adverse effects; furthermore, the daily dose of L-cysteine would be very small even if one is a chain smoker because one efficient tablet contains only 5 mg of L-cysteine.

In conclusion, numerous studies have shown the essential role of acetaldehyde in the carcinogenesis of the upper gastrointestinal tract in humans. We have shown that the major volatile substance of the tobacco smoke (acetaldehyde) is easily dissolved into saliva during smoking. This acetaldehyde could be totally removed by L-cysteine containing tablet, which is sucked during smoking. Hence, L-cysteine tablets could potentially be used in the prevention of upper gastrointestinal tract cancers among smokers. Although the numerous numbers of carcinogens in tobacco smoke limit these kind of experimental studies, our results warrant for further clinical trials on cancer prevention of smokers with L-cysteine tablet.

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