Elimination of Cigarette Smoke-derived Acetaldehyde in Saliva by Slow-release L-Cysteine Lozenge Is a Potential New Method to Assist Smoking Cessation. A Randomised, Double-blind, Placebo-controlled Intervention

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Abstract. *Background/Aim: Harmans are condensation products of acetaldehyde and biogenic amines in saliva. Like other monoamine oxidase inhibitors, harmans help maintain behavioral sensitization to nicotine and mediate the addictive potential of cigarette smoke-derived acetaldehyde. The aim of this study was to test the hypothesis that effective elimination of acetaldehyde in saliva by slow-release L-cysteine (Acetium™ lozenge; Biohit Oyj, Helsinki, Finland) blocks the formation of harmans and eliminates acetaldehyde-enhanced nicotine addiction in smokers. Study design: A double-blind, randomized, placebo-controlled trial comparing Acetium lozenges and placebo in smoking intervention was undertaken. Materials and Methods: A cohort of 423 cigarette smokers were randomly allocated to intervention (n=212) and placebo arms (n=211). Smoking-related data were recorded by questionnaires, together with nicotine dependence testing by Fagerström scale. The participants used a smoking diary to record the daily number of cigarettes, test lozenges and sensations of smoking. The data were analyzed separately for point prevalence of abstinence and prolonged abstinence endpoints. Results: Altogether, 110 study participants completed the trial per protocol, 234 had minor violations, and the rest (n=79) were lost to follow-up. During the 6-month trial, 65 participants quit smoking; 38 (17.9%) in the intervention arm and 27 (12.8%) in the placebo arm [odds ratio (OR)=1.48; 95% confidence intervals (CI)=0.87-2.54; p=0.143]. Success in the per protocol group was better (42.9% vs. 31.1%, respectively; OR=1.65, 95% CI=0.75-3.62; p=0.205) than in the modified intention-to-treat group: 13.5% vs. 7.4% (p=0.128). Conclusion: If the efficacy of Acetium lozenge can be confirmed in an adequately powered study, this new approach would represent a major breakthrough in smoking quit intervention because slow-release L-cysteine is non-toxic with no side-effects or limitations of use.*

The relative risk of lung cancer among cigarette smokers (1, 2) increases in a dose-dependent manner up to 30-40-fold among heavy smokers (2, 3), being amplified by exposure to other carcinogens, *e.g.* asbestos (4, 5) or oncogenic human papillomaviruses (HPV) (6). The risk of lung cancer remains increased for several years after smoking cessation (2-5), but gradually decreases to the level of non-smokers, making cessation meaningful even after long-term smoking (7, 8). Although smoking rates fell in many Western countries during the 1970s-1980s, this trend seems to be leveling off and instead is increasing in countries like China (9-11). It is estimated that 1.1 billion adults are smokers, making effective smoking cessation interventions essential to reduce the major public health impact (cancer, chronic obstructive pulmonary disease) of cigarette smoking (2, 5, 7, 11).

Smoking cessation can be achieved by two principally different approaches: i) with, and ii) without assistance from healthcare professionals (12, 13). Which of the available intervention methods is the most effective remains under debate (14, 15). Nicotine is the main psychoactive component of tobacco, and adolescents in particular seem to

be more sensitive to the rewarding effects of nicotine and develop nicotine addiction (16). This addiction develops when nicotine acts on nicotinic acetylcholine receptors in the central nervous system (CNS) to release neurotransmitters *e.g.* dopamine, glutamate and gamma-aminobutyric acid (16).

Dependence on smoking is much more complex than simply nicotine addiction (16). Recent experimental evidence implicates that acetaldehyde, a major carcinogenic (class I) compound of tobacco smoke (17, 18), enhances behavioral, endocrine and neuronal responses to nicotine in adolescent and adult rats (19-21). In these experiments, however, the acetaldehyde concentrations used far exceeded those reached in the saliva by humans while smoking (22, 23). Given that smoke-derived acetaldehyde is not absorbed into the circulation, its direct CNS interaction with nicotine described in these animal experiments can be excluded (19-21), suggesting that other indirect mechanisms must exist to explain the reinforcing effects of acetaldehyde in nicotine addiction.

In 2007, Talhout *et al*. (24) suggested that harman and salsolinol (two condensation products of acetaldehyde with biogenic amines), might be the mediators of smoking dependence-maintaining effects of acetaldehyde found in animal experiments (19-21). Both harman and salsolinol inhibit monoamine oxidase (MAO), and some other MAO inhibitors are known to increase nicotine self-administration and maintain behavioral sensitization to nicotine (24). Blood harman levels among smokers appear to be 2-10-times higher than in non-smokers, and since readily passing the blood–brain barrier, harmans are the prime culprits for the lower MAO activity observed in the brain of smokers (24). This led to the reasoning that acetaldehyde in cigarette smoke may increase the addictive potential of tobacco products *via* formation of acetaldehyde-biogenic amine adducts (harmans) *in vivo*.

This hypothesis has never been validated in human smokers until now. It is tempting to speculate, however, that elimination of acetaldehyde in the saliva during cigarette smoking using L-cysteine (25) might effectively i) block (or reduce) the formation of harmans, ii) reduce their high blood levels seen in smokers, and iii) by reducing MAO inhibition, minimize the reinforcing effects of acetaldehyde on smoking dependence. Indeed, a patented formulation based on slow-release L-cysteine lozenge is currently available (Acetium lozenge® 3 mg; Biohit Oyj, Helsinki, Finland), shown to be highly effective in eliminating cigarette smoke-derived acetaldehyde in the saliva by converting it to inactive 2-methylthiazolidine-4-carboxylic acid compound (23, 24).

The present study was a randomized, double-blind, placebo-controlled intervention trial designed to validate the concept that systematic use of Acetium lozenges concomitantly with cigarette smoking supports the decision to quit smoking.

Materials and Methods

Study design. This randomized, double-blind, placebo-controlled trial was designed to evaluate the efficacy of Acetium lozenge intervention in cessation of cigarette smoking. Current cigarette smokers (personally motivated to quit) were invited by public invitations (using different media) to participate in the trial, and randomly allocated to two study arms (Acetium and placebo). At baseline, all participants were requested to fill in a structured questionnaire recording their detailed smoking history and other clinical data pertinent to this study. An essential research tool was a smoking diary recorded on a daily basis by each participant and submitted to the study monitor at the end of each month, for recording study compliance and smoking-related covariates. The study was approved by the Helsinki University Hospital Coordinating Ethical Committee (DNo: 288/13/03/00/13; November 5, 2013).

Study participants. Between December 2013 and April 2015, a cohort of 423 current cigarette smokers were enrolled. Participants eligible for the study were current smokers (adult women and men) who were motivated to quit smoking, with no limitations in smoking duration and daily cigarettes (pack-years, PY). However, the following individuals were considered non-eligible: i) those who smoked a type of tobacco other than cigarettes, ii) those who refused to sign written consent, iii) those who were not motivated to quit smoking, and iv) those who did not commit themselves to not using other interventions during the 1-year follow-up.

The enrolled participants were randomly allocated into two study arms receiving either Acetium lozenges (n=212) or placebo (n=211), in a double-blind setting, where both the examiners and the participants were blinded to the test substance. For randomization, a random number generator was used, with a block size of 4 and creating unique randomization codes for each subject (https://www.sealedenvelope.com/simple-randomiser/v1/lists). A printed list (CSV Excel) of codes was sealed in an envelope and stored in a safety box until opened at completion of the study in October 2015. Before enrolment, all participants signed a written consent and agreed to use of the lozenges (Acetium or placebo) concomitantly with every single smoked cigarette throughout the whole intervention period, without adopting any other intervention methods.

Baseline data. Having consented to participate, each study participant was requested to fill in (with assistance of the study monitor) a structured questionnaire recoding their medical and smoking history, including details of previous intervention measures used to attempt quitting smoking. This questionnaire also included a more objective estimation of the nicotine dependence, evaluated by using the modified Fagerström Test for Nicotine Dependence (FTND) at baseline (26).

Follow-up records by smoking diary. For accurate monitoring of the smoking practices during the trial, all study participants were given a smoking diary. The participants were asked to submit the diaries to the study monitor on monthly basis, so as to confirm the compliance of each individual with the study protocol, to record the date of eventual smoking quit events, violations in the protocol or censoring due to other reasons. Apart from the detailed records on the number of cigarettes smoked per day and the number of

lozenges consumed concomitantly, the participants were asked to subjectively assess how they felt about each cigarette and estimate the degree of smoking-related pleasure, using the scale 1-10. This monthly diary provided an overall estimation of each month of smoking, recording all these variables at the conclusion of the month, total number of cigarettes and lozenges used, as well as the summary of pleasure scale. Two additional questions of each month were: i) Did your smoking habits change? ii) Has the sensation of smoking changed or not? iii) What is the smoking-associated pleasure using the scale 1-10?

Breath carbon monoxide (CO) monitoring. CO being a significant component of cigarette smoke, breath CO monitoring can be used to detect recent cigarette use (27). In the present study, CO concentration (ppm) and percentage of carboxy-hemoglobin (CO-Hb) in the blood were monitored at each follow-up visit using MicroCO monitor (Care Fusion, San Diego, CA, USA).

Application of the FTND. Originally introduced in 1978, the latest modification of this test is based on six simple questions recording the key variables of the smoker's daily practices (26). This test has been validated in several studies and shown to be valuable in monitoring the psychological dependence on nicotine. The FTND was recorded on each occasion when the participants returned their monthly smoking diaries. For statistical treatment, the FTND scores (from 1-10) were used as categorical variables: 0-2: very low; 3-4: low; 5: moderate; 6-7: high; and 8-10: very high (26).

Study compliance. Inherent to all longitudinal study designs, loss to follow-up is an inevitable outcome for a proportion of participants. Consisting of participants who terminated the trial due to a wide variety of reasons (extreme cases only completed interview but never initiated the trial), this group with no recordable outcome measure is not compatible with full or even partial data analysis, and was treated as a separate compliance category named LFU (lost to follow-up). As usual for randomized controlled trials (RCTs), the two categories of compliance as the target of the analysis were: i) per protocol (PP), and i) modified intention to treat (mITT). The former include all participants (in both arms) who were compliant with the study protocol, with only minor violations in taking the test substances (lozenges), and in recording their data. The mITT category includes all those who were not fully compliant with the protocol, but who completed the follow-up of at least 6 months and for whom the study endpoints were available. Following the usual practice for RCTs, the results were analyzed separately for the PP and mITT groups.

Primary study endpoints. The two most common outcome measures in smoking intervention trials are: i) prolonged abstinence (PA) and ii) point prevalence of abstinence (PPA) (28). PA, a sustained or continuous abstinence, is typically defined as not smoking for a period of several months after a quit attempt. PPA is typically defined as not smoking on the day of concluding the follow-up. In the present study, the results were analyzed separately for these two primary study endpoints, using the 2-month cut-off for a positive record of PA (28).

Statistical analysis. All statistical analyses were performed using SPSS 22.0.0.2 for Windows (IBM, NY, USA) and STATA/SE 14.1 software (STATA Corp., TX, USA). The descriptive statistics were procured according to routine procedures. Frequency tables were analyzed using the Chi-square test, with the likelihood ratio (LR) or Fisher's exact test for categorical variables. Differences in the means of continuous variables were analyzed using non-parametric (Mann–Whitney or Kruskal–Wallis) test for two and multiple independent samples, respectively. The risk estimates of PA and PPA in the two study arms were calculated using conventional univariate regression models, expressed as odds ratios (ORs), and their 95% confidence intervals (95% CI). The time to quit (TTQ) and duration of quit (quit time, equivalent to length of PA) in the two study arms (and compliance groups) were estimated using univariate survival (Kaplan–Meier) analysis, comparing the stratum-specific estimates using the log-rank (Mantel–Cox) statistics.

The data were arranged in the panel format, subjected to analyses using generalized linear models, *e.g.* generalized estimation equation or Poisson regression. In this study, the covariates of smoking quit were estimated using population-averaged Poisson regression model, where study subjects were clustered by their participant ID, follow-up visit (=monthly diary) as the time variable, and incident quit events (events/person days at risk) as the dependent count variable (29,30). The exchangeable within-group correlation structure for the Poisson model, with robust variance estimator (of 95% CI) to account for the within-participant correlation was the best-fit covariance pattern (29,30). The results for all covariates were expressed as the incidence rate ratio (IRR) statistics (with 95% CI). All covariates recorded at the baseline questionnaire (fixed variables) and all smoking-related variables from the smoking diaries (random variables) were first tested in univariate Poisson model. The final multivariate model was adjusted for the covariates that were significant in univariate analysis. All statistical tests were two-sided and declared significant at *p*-value of less than 0.05.

Results

The participants in the two arms were practically identical in most of their smoking-related variables, indicating an effective randomization (Table I). Importantly, this applies to their mean age, compliance with the intervention (PP, mITT, LFU groups), alcohol drinking habits, age of smoking onset, daily smoking habits and regularity, previous attempts to quit, type of interventions used for assisting quit, as well as compliance with previous interventions and their efficacy (*e.g.* the longest time of smoking abstinence). Most importantly, the participants in the intervention and the placebo arm were practically identical as to their PY of smoking (10.9 and 10.1 PYs, respectively (*p*=0.464), as well as to their nicotine dependence measured by the FTND score (*p*=0.895). In addition, the length of the follow-up of the two study arms was similar: 161 and 166 days, respectively, and an equal number of study participants in both arms reported adverse effects experienced during the intervention (*p*=0.396). The two arms were different in two respects only: i) gender distribution (more males in the placebo arm, *p*=0.020), and ii) weekly consumed alcohol equivalents (higher in the intervention arm, *p*=0.046).

The outcomes of intervention are summarized in Table II, separately for the two study arms, compliance groups (PP,

Table I. *Key characteristics of the study participants in the intervention and placebo arms.*

Variable	Intervention arm (n=212)	Placebo arm (n=211)	*p*-Value
Gender			
Women	109 (51.4%)	132 (62.6%)	0.020
Men	103 (48.6%)	79 (37.4%)	
Mean age (SD), years	43.8 (11.3)	44.6 (11.9)	0.504
Compliance			
PP	49 (23.1%)	61 (28.9%)	0.220
ITT	126 (59.4%)	108 (51.2%	
LFU	37 (17.5%)	42 (19.9%)	
Education			
Basic schooling only	28 (13.2%)	43 (20.5%)	0.153
Professional training	75 (35.4%)	64 (30.5%)	
Student examination (no further)	18 (8.5%)	26 (12.4%)	
High school/technical university	72 (34.0%)	62 (29.5%)	
Academic degree	19 (9.0%)	15 (7.1%)	
General health			
Healthy (no systemic disease)	161 (75.9%)	160 (75.8%)	0.978
Any systemic disease (regular med)	51 (24.1%)	51 (24.2%)	
Mental health			
No diagnosed ailment	190 (89.6%)	192 (91.0%)	0.633
Yes, on regular medication	22 (10.4%)	19 (9.0%)	
Alcohol intake			
Never	20 (9.4%)	22 (10.4%)	0.814
Social drinker	183 (86.3%)	178 (84.4%)	
Daily intake (moderate)	5 (2.4%)	8 (3.8%)	
Risk user (regular excessive)	4 (1.9%)	3 (1.4%)	
Mean weekly alcohol intake (SD)[1]	5.9 (7.3)	4.6 (5.3)	0.046
Mean age initiated smoking (SD), years	16.9 (4.6)	16.2 (3.4)	0.079
Regular smoker since start			
No	182 (85.8%)	168 (79.6%)	0.122
Yes	30 (14.2%)	43 (20.4%)	
If not regular			
Quit once (followed by relapse)	42 (23.1%)	38 (22.6%)	0.766
Quit twice (followed by relapse)	32 (17.6%)	35 (20.8%)	
Several attempts to quit (all failed)	108 (59.3%)	95 (56.5%)	
Smoking habits since initiation			
Daily no. of cigarettes remained stable	65 (30.7%)	53 (25.1%)	0.246
Daily no. of cigarettes increased	126 (59.4%	128 (60.7%	
Daily no. of cigarettes decreased	21 (9.9%)	30 (14.2%)	
Daily urgency to smoke			
No	23 (10.8%)	16 (7.6%)	0.244
Yes	189 (89.2%)	195 (92.4%)	
Wake up at night to smoke			
No	200 (94.3%)	198 (93.8%)	0.827
Yes	12 (5.7%)	13 (6.2%)	
Smoking at home (inside)			
No	193 (91.0%)	193 (91.5%)	0.875
Yes	19 (9.0%)	18 (8.5%)	
Smoking by household members			
Yes	131 (61.8%)	132 (62.5%	0.541
No	81 (38.2%)	79 (37.5%)	
Previous attempts to quit			
No	9 (4.2%)	17 (8.1%)	0.110
Yes	203 (95.8%)	194 (91.9%)	
Mean no. of previous quit attempts (SD)	7.6 (9.6)	7.8 (15.9)	0.849
Any intervention ever used for quit attempt			
No	7 (3.3%)	13 (6.3%)	0.175
Yes	203 (96.7%	193 (93.7%)	

Table I. *continued*

Variable	Intervention arm (n=212)	Placebo arm (n=211)	*p*-Value
Intervention type offered			
Personal techniques	107 (93.0%)	114 (95.0%)	0.526
Group-based techniques	8 (7.0%)	6 (5.0%)	
Compliance with the intervention methods			
Poor	11 (11.6%)	11 (12.1%)	0.910
Moderate	24 (25.3%)	20 (22.0%)	
Good	60 (63.2%)	60 (65.9%)	
Longest ever period without smoking (months)	16.8 (27.8)	14.1 (30.8)	0.364
Mean PY of smoking (SD)	10.9 (10.6)	10.1 (9.7)	0.464
FTND at baseline			
0-2	33 (15.6%)	35 (16.6%)	0.895
3-4	50 (23.6%)	48 (22.7%)	
5	33 (15.6%)	40 (19.0%)	
6-7	76 (35.8%)	70 (33.2%)	
8-10	20 (9.4%)	18 (8.5%)	
Mean blood CO (ppm) at baseline (SD)	24.9 (11.8)	24.8 (11.3)	0.965
Mean blood COHb (%) at baseline (SD)	4.0 (1.9)	3.9 (1.8)	0.968
Mean follow-up (days) (SD)[2]	160.9 (138.9)	165.9 (126.9)	0.729
Adverse effects during intervention[3]			
No	176 (88.4%)	166 (85.6%)	0.396
Yes	23 (11.6)	28 (14.4)	

PP, Per protocol; mITT, modified intention to treat; LFU, lost to follow-up; PY: pack-years; FTND, Fagerström test for nicotine dependence; CO: carbon monoxide; COHb, carboxyhemoglobin. [1]Glass of wine equivalent; [2]calculated for PP and ITT groups only; [3]recorded in detail in the smoking diary.

mITT) and stratified by the study arm and compliance. The distribution of the six possible outcomes was similar in the two arms. Altogether, 5.1% more participants quit smoking in the intervention arm (17.9% *vs*. 12.8%) than in the placebo arm, whereas a higher proportion of participants reduced smoking or quit but relapsed in the placebo arm (*p*=0.120). When stratified by study compliance, significantly more individuals stopped smoking in the PP group than in the mITT group, 36.4% and 10.7%, respectively.

When analyzed separately, in the PP group, 11.8% more participants (42.9% *vs*. 31.1%) quit smoking in the intervention arm than in the placebo arm, but the overall outcome pattern was not significantly different (*p*=0.107). In the mITT group, this difference in the effect size was only 6.1% in favor of the intervention arm, 13.5% and 7.4%, respectively.

The primary study endpoints (PPA and PA) in the two study arms and stratified by study compliance are shown in Table III. PPA and PA were found to be closely correlated (R=0.816; *p*=0.0001; Spearman's rho). PPA in the intervention arm as compared to the placebo arm had an OR of 1.48 (95% CI=0.87-2.54), PA was less frequent in the intervention than placebo group (OR=0.82; 95% CI=0.42-1.41). The probability of quitting smoking was significantly (*p*=0.0001) higher in the PP than in the mITT group

(OR=4.77), and the same is true for experiencing PA (OR=6.26). Of the two endpoints, PPA was a more consistent outcome measure than PA in both the PP and mITT groups, with OR=1.65 and OR=1.95, in favor of Acetium intervention over placebo.

To disclose the significant covariates of smoking quit, all smoking history-related variables recorded at baseline, as well as the study-level covariates (recorded by the smoking diaries), were analyzed using univariate and multivariate Poisson regression for panel data (Table IV). In this analysis, quit event was used as a count variable reported in the smoking diaries during the intervention. In the univariate model, six covariates proved to be significant predictors of the smoking quit event: i) age (older less likely to quit), ii) gender (male more likely to quit), iii) education (higher education, less likely to quit), iv) number of previous quit attempts (more attempts, more likely to quit), v) daily number of cigarettes smoked during intervention (higher number, less likely to quit), and vi) subjective sensations of smoking (changed sensations favor quit). When all these significant univariates were entered in the multivariate Poisson model, i) daily number of cigarettes during intervention, and ii) changed sensations of smoking remained the only significant independent predictors of the quit event, with IRR=0.89 (95% CI=0.82-0.97) and IRR=2.44 (95%CI=1.15-5.20), respectively.

Table II. *The study outcomes in the intervention and placebo arms and related to study compliance.*

	Study outcome, n (%)					
	Quit smoking	Reduced smoking	Quit but relapsed	No objective effect	Moved to other method	Lost to follow-up
*Study arm						
Intervention	38 (17.9%)	30 (14.2%)	7 (3.3%)	89 (42.0%)	11 (5.2%)	37 (17.5%)
Placebo	27 (12.8%)	39 (18.5%)	15 (7.1%)	73 (34.6%)	15 (7.1%)	42 (19.9%)
Total	65 (15.4%)	69 (16.3%)	22 (5.2%)	162 (38.3%)	26 (6.1%)	79 (18.7%)
			p=0.129 (Likelihood ratio statistics)			
Compliance						
PP	40 (36.4%)	22 (20.0%)	12 (10.9%)	29 (26.4%)	7 (6.4%)	NA
mITT	25 (10.7%)	47 (20.1%)	10 (4.3%)	133 (56.8%)	19 (8.1%)	NA
Total	65 (18.9%)	69 (20.1%)	22 (6.4%)	162 (47.1%)	26 (7.6%)	344
			p=0.0001 (Likelihood ratio statistics)			
Study arm by compliance						
PP						
Intervention	21 (42.9%)	6 (12.2%)	4 (8.2%)	17 (34.7%)	1 (2.0%)	NA
Placebo	19 (31.1%)	16 (26.2%)	8 (13.1%)	12 (19.7%)	6 (9.8%)	NA
Total	40 (36.4%)	22 (20.0%)	12 (10.9%)	29 (26.4%)	7 (6.4%)	110
			p=0.056 (Fisher's exact test)			
mITT						
Intervention	17 (13.5%)	24 (19.0%)	3 (2.4%)	72 (57.1%)	10 (7.9%)	NA
Placebo	8 (7.4%)	23 (21.3%)	7 (6.5%)	61 (56.5%)	9 (8.3%)	NA
Total	25 (10.7%)	47 (20.1%)	10 (4.3%)	133 (56.8%)	19 (8.1%)	234
			p=0.358 (Fisher's exact test)			

PP, Per protocol; mITT, modified intention to treat; NA, not applicable. Those lost to follow-up were omitted from analysis. *All enrolled participants (n=423) included.

Discussion

In a recent meta-analysis evaluating unassisted smoking intervention techniques, the quit rate from unaided methods was quite modest, only 7.3% after an average of 10 months of follow-up (31). These figures are substantially inferior to the efficacy of assisted intervention methods, with 25%-33% quit rates for over 6 months (12). In a recent comprehensive review and meta-analysis by Lemmens *et al*., evidence of effectiveness was found for the following assisted strategies: group behavioral therapy (OR=2.17, 95% CI=1.37-3.45), bupropion (OR=2.06, 95% CI=1.77-2.40), intensive physician advice (OR=2.04, 95% CI=1.71-2.43), nicotine replacement therapy (OR=1.77, 95% CI=1.66-1.88), individual counselling (OR=1.56, 95% CI=1.32-1.84), telephone counselling (OR=1.56, 95% CI=1.38-1.77), nursing interventions (OR=1.47, 95% CI=1.29-1.67) and tailored self-help interventions (OR=1.42, 95%CI=1.26-1.61) (14). Interestingly, comprehensive clean (smoke-free) indoor laws increased the quit rates by 12-38% (14).

This meta-analysis did not include the studies on varenicline tartrate (Pfizer), which is one of the most widespread intervention medications in the US (Chantix) and outside (Champix) (14). Two meta-analyses found varenicline to be more effective than nicotine replacement therapy or bupropion, varenicline (2 mg/day) giving the highest abstinence rate (33.2%) of any stand-alone therapy (32, 33). Superiority of varenicline over bupropion was also confirmed in a Cochrane review of 15 studies in 2011 (34). However, both these principal stand-alone medications have serious adverse effects or limitations for use. In double-blind studies, varenicline increased the risk of serious adverse cardiovascular events as compared with placebo (35). It may also cause neuropsychiatric side-effects, *e.g.* possible suicidal behavior, which should seriously limit its long-term use (35). Similarly, bupropion (Zyban, GSK), another FDA-approved medication in this indication is contraindicated *e.g.* in epilepsy and other seizures, anorexia/bulimia, in those taking antidepressant drugs (MAO inhibitors), and those with abrupt discontinuation of ethanol or sedatives (including benzodiazepines) (36). Needless to say, any new method with equal efficacy but devoid of these serious adverse effects would represent a major impact on the global public health burden due to smoking (2, 5, 7, 11).

The Acetium lozenge tested in the present RCT is based on a completely novel hypothesis, supported by animal experiments and convincing clinical results (18-25). In brief: i) acetaldehyde (class I carcinogen) (17) is the principal

Table III. *The primary study endpoints of point prevalence of abstinence (PPA) and prolonged abstinence (PA) in the intervention and placebo arms and related to study compliance.*

	Primary study endpoints					
	PPA			PA*		
	Yes, n (%)	No, n (%)	OR (95% CI), *p*-Value	Yes, n (%)	No, n (%)	OR (95% CI), *p*-Value
**Study arm						
Intervention	38 (17.9%)	174 (82.1%)	1.48 (0.87-2.54), *p*=0.143	29 (13.7%)	183 (86.3%)	0.82 (0.48-1.41), *p*=0.482
Placebo	27 (12.8%)	184 (87.2%)		34 (16.1%)	177 (83.9%)	
Compliance						
PP	40 (36.4%)	70 (63.6%)	4.77 (2.70-8.43), *p*=0.0001	42 (38.2%)	68 (61.8%)	6.26 (3.47-11.30), *p*=0.0001
mITT	25 (10.7%)	209 (89.3%)		21 (9.0%)	213 (91.0%)	
Study arm by compliance						
PP						
Intervention	21 (42.9%)	28 (57.1%)	1.65 (0.75-3.62), *p*=0.205	20 (40.8%)	29 (59.2%)	1.22 (0.56-2.64), *p*=0.610
Placebo	19 (31.1%)	42 (68.9%)		22 (36.1%)	39 (63.9%)	
mITT						
Intervention	17 (13.5%)	109 (86.5%)	1.95 (0.80-4.71), *p*=0.128	9 (7.1%)	117 (92.9%)	0.61 (0.24-1.52), *p*=0.290
Placebo	8 (7.4%)	100 (92.6%)		12 (11.1%)	96 (88.9%)	

*2-Month cut-off. **All enrolled participants (n=423) included.

carcinogenic substance in cigarette smoke (18), being present at a concentration half that of nicotine (37, 38); ii) a synergistic interaction seems to exists between nicotine and acetaldehyde in self-administration in rats (20); iii) acetaldehyde enhances behavioral, endocrine, and neuronal responses to nicotine in rats (19); iv) in concentrations reached in the saliva during cigarette smoking, acetaldehyde is not absorbed into circulation (22, 23), excluding the possibility of direct central interactions with nicotine; v) an indirect link mediating the central nicotine-reinforcing effects of salivary acetaldehyde might be provided by harmans - beta-carboline alkaloids exhibiting a wide range of biological, psychopharmacological and toxicological actions; vi) these beta-carbolines are known to be synthesized as condensation products of alcohol- or cigarette smoke-derived acetaldehyde and biogenic amines (*e.g.* tryptamine) (39, 40).

Among smokers, blood harman levels appear to be 2-10-times higher as compared to non-smokers. Both harman and salsolinol inhibit MAO. MAO inhibitors are known to increase nicotine self-administration and maintain behavioral sensitization to nicotine, and given that harmans readily pass the blood–brain barrier, these may be the agents contributing to the lower MAO activity observed in the brain of smokers (24). This led Talhout *et al.* to propose that acetaldehyde might increase the addictive potential of tobacco products *via* formation of these acetaldehyde-biogenic amine adducts (harmans) (24). The logical next step to validate this concept is to assess whether elimination of acetaldehyde in the saliva

could be an effective blocker of these central effects of acetaldehyde in cigarette smokers.

Some 40 years ago, it was demonstrated that cysteine (a non-essential amino acid) is highly effective in eliminating acetaldehyde by reacting covalently with it to form a stable 2-methylthiazolidine-4-carboxylic acid (25). Subsequently, this simple principle was patented by Biohit Oyj (Helsinki, Finland) in their Acetium™ products (capsule and lozenge) designed for reduction of harm due to alcohol intake and smoking, by eliminating acetaldehyde in the stomach and saliva, respectively (22, 23, 41).

The present RCT was designed to validate the concept that elimination of acetaldehyde in the saliva during cigarette smoking by sucking L-cysteine-containing lozenges might reduce the acetaldehyde-associated nicotine addiction among smokers (24). In this double blind, placebo controlled intervention trial, a cohort of 423 volunteer current smokers were randomly allocated to intervention (Acetium) and placebo arms, n=212 and n=211, respectively. This randomization was highly effective, building up two study arms with individuals who were practically identical in all key smoking-related variables, including the total PY and the level of nicotine dependence assessed by the FTND at baseline (Table I).

Following the adopted practice in many smoking intervention studies, two primary study endpoints were used: PPA and PA (28). Both PPA and PA are typically tied to the follow-up time (that continues a variable length after a recorded quit date), but both can also be tied to the end of

Table IV. *Predictors of smoking quit* in panel Poisson regression[1] run in univariate model and as adjusted for all significant univariates.*

	Quit smoking					
Covariate	Crude IRR	95% CI	*p*-Value	Adjusted IRR**	95% CI	*p*-Value
Age at study entry (cont.)	0.97	0.95-0.99	0.008	0.99	0.95-1.2	0.683
Intervention (placebo=ref)	1.46	0.90-2.35	0.120			
Compliance (PP=ref)	0.70	0.43-1.14	0.160			
Gender (women=ref)	1.79	1.11-2.86	0.016	1.03	0.43-2.47	0.937
Education (basic=ref)	0.75	0.63-0.91	0.003	0.88	0.65-1.17	0.373
General health (not=ref)	1.01	0.57-1.81	0.947			
Mental health (not=ref)	1.13	0.45-2.82	0.791			
Alcohol intake (social=ref)	0.85	0.50-1.43	0.547			
Alcohol weekly dose (cont.)	1.01	0.98-1.04	0.322			
Age initiated smoking (cont.)	0.97	0.91-1.03	0.371			
Regular smoker since start (no=ref)	0.56	0.23-1.33	0.195			
Attempts to quit (one=ref)	0.95	0.85-1.11	0.543			
Smoking habits since initiation (stable=ref)	0.93	0.60-1.44	0.761			
Daily urgency of smoking (no=ref)	0.89	0.45-1.79	0.765			
Nightly wake-up for smoking (no=ref)	0.53	0.13-2.14	0.378			
Smoking at home (inside) (no=ref)	0.31	0.07-1.30	0.111			
Smoking by household members (spouse=ref)	0.85	0.65-1.12	0.267			
Previous attempts to quit (no=ref)	1.36	0.43-4.32	0.595			
Number of previous quit attempts (cont.)	1.03	1.02-1.05	0.0001	1.00	0.96-1.04	0.812
Intervention ever used for quit attempt (no=ref)	1.49	0.37-6.00	0.573			
Type of intervention (personal=ref)	0.45	0.07-2.95	0.408			
Compliance with the intervention methods (poor=ref)	1.13	0.58-2.19	0.702			
Longest ever period without smoking (cont.)	1.00	0.99-1.01	0.212			
Pack years of smoking (cont.)	0.99	0.97-1.01	0.552			
FTND at FU visits (graded, 0-2 ref)	0.82	0.65-1.07	0.143			
CO blood level (ppm) at FU visits (cont.)	0.98	0.95-1.02	0.440			
COHb level (%) in blood at FU visits (cont.)	0.91	0.73-1.14	0.441			
Adverse effects during intervention (no=ref)	0.56	0.22-1.43	0.231			
Cigarettes per day during intervention (cont.)	0.88	0.81-0.95	0.002	0.89	0.82-0.97	0.009
Smoking habits changed during intervention (no=ref)	0.48	0.22-1.04	0.064			
Pleasure obtained from smoking (scale 1-10) (cont.)	0.92	0.75-1.12	0.427			
Sensations of smoking changed during intervention (no change=ref)	3.22	1.45-7.16	0.004	2.44	1.15-5.20	0.020
Level of pleasure (scale 1-10) obtained from smoking (cont.)	0.92	0.75-1.12	0.427			

*Count outcome (quit event), as defined by the quit event reported in the smoking diaries during intervention; [1]Population average model, clustered by subject ID number, monthly diaries, follow-up visits (FU) as the time variable, exchangeable within-group correlation structure, 95% CI=calculated by robust estimation. IRR= incidence rate ratio; cont., continuous variable.**Adjusted for age and all other significant covariates of smoking quit in univariate model.

intervention, or time prior to assessment of results. PA is typically defined as not smoking for a period of several months after a quit attempt. Both PPA and PA have their supporters in the literature (28). Albeit closely correlated, these two outcomes give somewhat different estimates for quit rates, and a recent meta-analysis recommends using both endpoints in smoking intervention studies (28). This was confirmed in the present study, where PPA and PA were closely correlated (R=0.816; *p*=0.0001), but despite this, slightly different results were obtained, PPA being more powerful (Table III). This is because PA (2-month cut-off) was calculated both for those who showed permanent quit, and those who quitted but relapsed. Duration of PA (7.1 and

1.9 months), respectively, among these two groups was significantly different (*p*=0.0001), and the likelihood of having a PA of more than 2 months was higher among those who quit rather than those who relapsed: 83.1% and 36.4%, respectively (OR=8.6, 95% CI=2.9-25.4; *p*=0.0001). Otherwise, TTQ was shorter (3.7 months) in the intervention arm than that (4.5 months) in the placebo arm (*p*=0.214), but the duration of quit (PA) did not differ significantly between the two arms: 5.7 and 5.9 months, respectively (*p*=0.860).

As evident from Table II, the six outcomes had a similar distribution in the two study arms (*p*=0.129). Altogether, 38 individuals (17.9%) in the Acetium arm and 27 (12.8%) in the placebo arm reported smoking quit, *i.e.*, 5.1% difference

in favor of intervention. Study compliance was significantly related to quitting smoking (*p*=0.0001), being higher in the PP than the mITT group. Importantly, much of this difference in compliance seems to be attributable to the intervention itself, as shown in analysis of the PP group, where 42.9% reported smoking quit in the intervention arm as compared with 31.1% in the placebo arm (*i.e.* 11.8% difference). The difference in quit prevalence between the two study arms is less marked (6.1%) in the mITT group, but still in favor of the Acetium intervention. When applied to primary endpoints (PPA, PA), the likelihood of PPA for intervention had an OR=1.65 and OR=1.95, in the PP and mITT groups, respectively (not significant).

Finally, the significant covariates of smoking quit were estimated using Poisson regression in univariate and multivariate mode. Altogether, 65 quit events were reported during a total of 55.730 pdr (quit rate: 1.16/1000 pdr), being higher in the intervention arm (1.37/1000 pdr) than in the placebo arm (0.96/1000 pdr) (IRR=1.42; 95% CI=0.84-2.42; *p*=0.079). Six variables proved to be significant covariates of smoking quit in univariate analysis (Table IV). When all significant univariates were entered in the multivariate Poisson model, only two covariates remained significant: daily number of cigarettes smoked during intervention, and the changed sensation of smoking (Table IV).

The present RCT testing the efficacy of Acetium lozenge intervention in smoking cessation provided evidence substantiating the study hypothesis (24). Accordingly, elimination of cigarette smoke-derived acetaldehyde in the saliva with slow-release L-cysteine might block the central acetaldehyde–nicotine interactions mediated by potent MAO inhibitors, harmans, borne as condensation products of acetaldehyde and biogenic amines and maintaining smoking dependency (24, 42). Once smoke-derived acetaldehyde is eliminated in the saliva, harmans are not formed, their blood level is decreased and the central interactions with nicotine are inhibited. The direct evidence to demonstrate this can be obtained by measuring the blood (and urine) levels of harmans (39), with and without concomitant use of slow-release L-cysteine during the smoking session.

The efficacy of Acetium lozenge, with OR ranging from 1.48-1.95 compared to placebo (Table III) favorably competes with most of the commonly used smoking intervention methods, including medications (14, 32-36). One weakness of the present RCT is the insufficient statistical power to confirm the significance of these results. The power calculations were based on more conservative estimates on i) the effect size and ii) the effect size difference between the two arms. The original cohort size of 500 (n=250 per study arm) was calculated to be adequately powered to detect a true difference (in PPA or PA) of 10% between the two arms, within the PPA/PA prevalence range of 10%-20%. Within this range, the power was shown to be sensitive to any decrease in

the effect size difference, but would allow less difference (7.5%) if the quit rate in the two arms was between 5% and 15%. Although this was almost the case in the crude comparison between the two arms (17.9% *vs.* 12.8%), and in the mITT group (13.5% *vs.* 7.4%), the success rates (42.9% *vs.* 31.1%) in the PP group far exceeded our assumptions. With the current effect size difference of 5.1% between the two study arms, an adequate statistical power would necessitate a minimum of 782 participants in both arms. In the PP group, however, current PPA rates would ensure adequate power with 262 participants in both study arms.

Another (potential) weakness of the present RCT is the imbalance in the gender distribution in the intervention arm (Table I). Albeit due to pure chance only, it can be argued that this might have an impact on the success rate in the intervention arm because male gender proved to be a significant (*p*=0.003) covariate of quit events (IRR>1.7; Table IV). Although this effect was confounded by the other covariates in the multivariate model, this potential bias is better handled by also randomizing the study arms by gender. Needless to say, the results of the present proof-of-concept RCT are encouraging enough to prompt a design of such an adequately powered study in the near future.

Conflicts of Interest

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