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Slow-release L-cysteine capsule prevents gastric mucosa exposure to carcinogenic acetaldehyde: results of a randomised single-blinded, cross-over study of *Helicobacter*-associated atrophic gastritis

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ABSTRACT

Introduction: *Helicobacter*-induced atrophic gastritis with a hypochlorhydric milieu is a risk factor for gastric cancer. Microbes colonising acid-free stomach oxidise ethanol to acetaldehyde, a recognised group 1 carcinogen.

Objective: To assess gastric production of acetaldehyde and its inert condensation product, non-toxic 2-methyl-1,3-thiazolidine-4-carboxylic acid (MTCA), after alcohol intake under treatment with slow-release L-cysteine or placebo.

Methods: Seven patients with biopsy-confirmed atrophic gastritis, low serum pepsinogen and high gastrin-17 were studied in a cross-over single-blinded design. On separate days, patients randomly received 200 mg slow-release L-cysteine or placebo with intragastric instillation of 15% (0.3 g/kg) ethanol. After intake, gastric concentrations of ethanol, acetaldehyde, L-cysteine and MTCA were analysed.

Results: Administration of L-cysteine increased MTCA ($p < .0004$) and decreased gastric acetaldehyde concentrations by 68% ($p < .0001$). The peak L-cysteine level was 7552 ± 2687 μmol/L at 40 min and peak MTCA level 196 ± 98 μmol/L at 80 min after intake. Gastric L-cysteine and MTCA concentrations were maintained for 3 h. The AUC for MTCA was 11-fold higher than acetaldehyde, indicating gastric first-pass metabolism of ethanol. With placebo, acetaldehyde remained elevated also at low ethanol concentrations representing 'non-alcoholic' beverages and food items.

Conclusions: After gastric ethanol instillation, slow-release L-cysteine eliminates acetaldehyde to form inactive MTCA, which remains in gastric juice for up to 3 h. High acetaldehyde levels indicate a marked gastric first-pass metabolism of ethanol resulting in gastric accumulation of carcinogenic acetaldehyde. Local exposure of the gastric mucosa to acetaldehyde can be mitigated by slow-release L-cysteine capsules.

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Alcohol; carcinogenesis; ethanol; prophylaxis; stomach

Introduction

There is strong evidence supporting the crucial role of acetaldehyde in gastric carcinogenesis. In a meta-analysis including total of 34 557 gastric cancer cases derived from 44 case control and 15 cohort studies, heavy drinking (more or equal to four drinks per day) was significantly associated with increased risk for stomach cancer.[1] In other studies, the highest risk has been demonstrated in ALDH2-deficient alcohol consumers and especially among those with chronic atrophic gastritis.[2,3] Moreover, ALDH2 deficiency combined with atrophic gastritis has been shown to increase also the risk for oesophageal squamous cell carcinoma.[4] Recently, it was demonstrated that ALDH2 deficiency results in a more than 5-fold increased exposure of the gastric mucosa to acetaldehyde after intragastric administration of a moderate dose of ethanol.[5] A recent study of interactions between alcohol, ALDH2 and risk for gastric cancer has also confirmed an increased risk with low ALDH2 and high alcohol consumption.[6] When combined with results from epidemiological studies, these findings provide concrete evidence for a causal relationship of acetaldehyde not only with the pathogenesis of oesophageal but also with gastric cancer. ALDH2 deficiency has been calculated to affect at least 540 million individuals of Eastern Asian descent.[7] Thus, local carcinogenicity of acetaldehyde in the human upper digestive tract is a world-wide observable fact.

Many microbes colonising the acid-free or hypochlorhydric stomach possess alcohol dehydrogenase activity and are able to locally produce acetaldehyde not only from ethanol but also from glucose.[8] In addition to atrophic gastritis, *Helicobacter pylori* infection is also a major risk factor for gastric cancer.[9] Many *H. pylori* strains possess alcohol dehydrogenase and are able to enzymatically oxidise ethanol into carcinogenic

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acetaldehyde.[10] Intragastric acetaldehyde production from ethanol mediated by microbes is supplemented by gastric mucosal alcohol dehydrogenase.[11,12] The limited capacity of mucosal cells and microbes to eliminate acetaldehyde results in accumulation of acetaldehyde in gastric contents of achlorhydric patients.[5,8]

L-cysteine neutralises acetaldehyde carcinogenicity by forming direct covalent bond. The product of this binding is the non-toxic 2-methyl-1,3-thiazolidine-4-carboxylic acid (MTCA).[13] Preparations that slowly release L-cysteine locally in the oral cavity have been shown to successfully eliminate acetaldehyde from the saliva during alcohol consumption and smoking.[14,15] Our recent study shows that slow-release L-cysteine capsules effectively eliminate ethanol-derived acetaldehyde also from the gastric juice of patients with atrophic gastritis and the effect persisted at least for 40 min.[16] In that study, patients orally ingested 15% alcohol solution in a total dose of 0.3 g/kg. The effect of L-cysteine capsules (4×50 mg) was documented to last for at least for 40 min. To date, formation of MTCA from L-cysteine and acetaldehyde has not been documented *in vivo*. As primary end-point, after gastric instillation of alcohol in patients with achlorhydric atrophic gastritis, we examined the *intragastric* levels of acetaldehyde with or without concomitant administration of slow-release L-cysteine. As secondary end-point, *intragastric* levels of the resulting end-products L-cysteine and MTCA were analysed.

Materials and methods

The investigation was carried out at Uppsala University Hospital between June 2013 and May 2014 as an exploratory single-blinded cross-over study in seven *H. pylori*-positive patients, derived from a cohort of 27 patients with biopsy-confirmed atrophic gastritis who showed low fasting serum levels of GastroPanel tests (Biohit Oyj, Helsinki, Finland): pepsinogen (PG) $1 < 30$ μg/L, PG II < 3 μg/L, PGI/PGII < 3 and high fasting serum gastrin-17 > 10 pmol/L participated in the study (Figure 1). Supplementation treatment with cobalamin was received by all subjects. None had on-going treatment with either acid inhibitory pharmaceuticals (proton pump inhibitors, PPIs; H_2-receptor blockers), antidepressants or non-steroid anti-inflammatory drugs. Patients with previous or on-going alcohol or drug abuse were excluded, as were patients on gastroprokinetics or with symptoms of gastroparesis. None of the patients had symptoms of any gastrointestinal perturbation, constipation or diarrhoea.

All subjects completed this randomised cross-over study serving as their own controls. On two separate occasions with an interval of one week, each patient received 15% ethanol (0.3 g/kg) by gastric instillation, randomly assigned to treatment with 200-mg slow-release L-cysteine (Acetium, Biohit Oyj, Helsinki, Finland) or placebo in a patient-blinded fashion. Randomisation was done by envelope draw by research coordinating nurse.

The study (project 620070-SWE-2012) was conducted in accordance with the Helsinki Declaration, and approved by the Ethics Committee for Human Research at the Regional Investigational Review Board, Uppsala, Sweden (diary no. 2012/411). All subjects provided written informed consent prior to enrolment in the study. The study was registered at www.ClinicalTrials.gov no. NCT02524262.

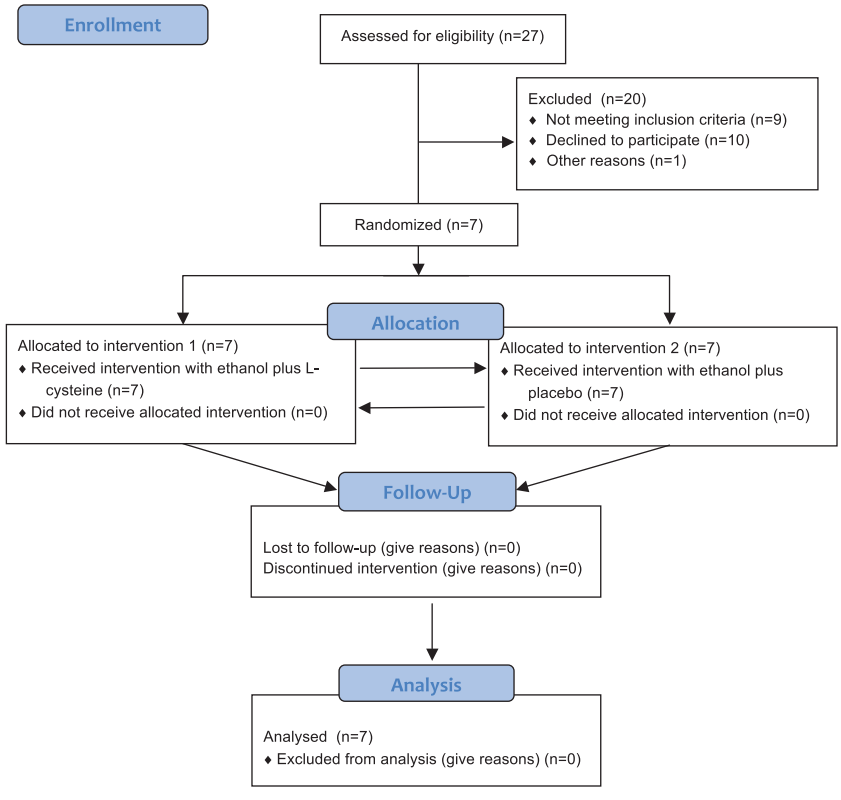

Figure 1. Study flow chart for subjects receiving either ethanol with L-cysteine (left) or ethanol without L-cysteine (right).

Nasogastric intubation and gastric aspiration

All subjects were instructed to refrain from consuming alcohol for 24 h and food for 12 h prior to each study session, conducted 8–11 am at the Clinical Trial Consultants research unit. After local anaesthesia of the nasal cavity with lidocaine gel (Xylocain 20 mg/mL, AstraZeneca, Södertälje, Sweden; without ethanol), a nasogastric tube was inserted through a nostril to a depth of 55 cm with 100 mL of water to facilitate positioning. The position of the tube was confirmed by aspiration of gastric contents. Immediately before administration of 15% ethanol through the nasogastric tube, the volunteers swallowed two slow-release L-cysteine capsules (Acetium; Biohit Oyj, Helsinki, Finland; in total 200 mg), or identical-appearing placebo capsules, with 50 mL of water. During the session, patients were in supine position on their left side to delay gastric emptying and facilitate the collection of adequate gastric juice samples. Gastric aspirates (5 mL) were collected at 20-min intervals for up to 240 min.

Acetaldehyde, ethanol, L-cysteine and MTCA analyses of gastric juice samples

For ethanol and acetaldehyde analysis, 5 mL of gastric juice was collected at each time point into cryo tubes containing 10% of 6 mol/L perchloric acid. This is known to be sufficient to stop microbial acetaldehyde production without hydrolysing L-cysteine-acetaldehyde binding.[16] Then, samples were immediately deep frozen and stored at −80 °C until transfer on dry ice to the Biohit laboratory for further analyses. Ethanol and acetaldehyde concentrations were analysed by headspace gas chromatography as duplicates, as previously described.[16,17]

For L-cysteine and MTCA analyses, 1 mL of gastric juice was immediately deep frozen and stored at −80 °C until transfer on dry ice to the Laboratory of Organic Chemistry, Åbo Akademi University (Turku, Finland) for analyses. The determination of L-cysteine and MTCA was performed by liquid chromatography – mass spectrometry (LC-MS/MS) with a Micromass Quattro Micro triple-quadrupole mass analyser (QqQ-MS) equipped with an electrospray ion source (Waters, Milford, MA). The mass analyser was operated in multiple reaction monitoring mode (MRM). Positive ions were recorded. Nitrogen was used as desolvation gas at 325 °C, and the flow rate was set to 640 L per hour. Argon was used as the collision gas with a collision cell pressure of 5.63×10^{-3} mbar. The source temperature was 120 °C, and the capillary voltage was set to 3.4 kV. The mass spectrometer was coupled to an Agilent 1100 series HPLC consisting of a binary pump, a vacuum degasser, an autosampler and a thermostatted column oven (30 °C).

An Atlantis T3 analytical column (3 μm, 2.1 × 100 mm; Waters, Ireland) equipped with a guard column of the same material (3 μm, 2.1 × 10 mm) was used. The mobile phase consisted of 0.5% aqueous acetic acid. The flow rate was 0.3 mL per minute. The run time was 4 min.

Prior to determination of L-cysteine and MTCA, samples were thawed and diluted 1:10 with a solution containing 4% formic acid, 0.25 mg/mL dithiothreitol and 1 μg/mL cysteine-D_3 as internal standard in water. All samples were analysed in triplicate. The injected volume was 10 μL.

Two transitions (122 > 86.9; 122 > 76.1 and 148.1 > 130.8; 148.1 > 102.2 mass units) were monitored for L-cysteine and MTCA, respectively. Quantification was performed using a 6-point linear calibration curve with $R^2 > .98$.

Calibration curves ranging between 0.1–40 μg/mL for MTCA and 0.5–200 μg/mL for L-cysteine were used. The repeatability was calculated as the relative SD from 10 injections and was 7.3% for L-cysteine and 5.6% for MTCA, for the concentrations of 50 μg/mL and 10 μg/mL, respectively. The matrix effect was determined by calculating the difference in peak area in Milli-Q® water compared to the peak area for the same concentration in matrix (five injections each). Due to the strong matrix effect for MTCA, a correction factor of 0.24 was used. The limit of quantification (LOQ) of L-cysteine and MTCA in the samples was defined as the concentration having signal-to-noise ratio (S/N) value of 10 and limit of detection (LOD) an S/N value of 3. The detection limit for the instrument was 1 ng/mL (8 nmol/L equivalent to 10 pg on column) for L-cysteine and 15 ng/mL (102 nmol/L equivalent to 151 pg on column) for MTCA.

L-cysteine slow-release capsules (acetium)

Each capsule (Acetium, Biohit Oyj, Helsinki, Finland) contains 100-mg L-cysteine in slow-release formulation as the active ingredient. L-cysteine is bound with matrix granules including Eudragit® RS-PO, hypromellose, calcium hydrogen phosphate and titanium dioxide. In dissolution tests, this formulation has been shown to release L-cysteine at a controlled rate, which is sufficiently fast permit time to react with acetaldehyde before the formulation leaves the stomach.[16] A placebo formulation where L-cysteine was replaced with the same amount of $CaHPO_4$ was prepared following exactly the same method.

Statistical analysis

Concentrations of ethanol, acetaldehyde, L-cysteine and MTCA were expressed as means ± SEM. Exposures of the gastric mucosa were expressed as the area under the curve (AUC) of acetaldehyde and MTCA, respectively. Statistical differences between the slow-release L-cysteine and placebo groups were analysed using the paired Student's *t*-test; $p < .05$ was considered statistically significant. All statistical analyses were exploratory and conducted using GraphPad Prism, version 6.03 (GraphPad Software Inc., La Jolla, CA).

Results

All the seven subjects completed the study serving as their own controls. No significant unintended adverse reactions were encountered, albeit some slight tipsiness occurred.

Ethanol in gastric juice

After intragastric instillation of 15 vol% alcohol (ethanol 0.3 g/kg), the peak ethanol concentration in gastric juice at 10 min reached 5.8 ± 0.8 vol% with placebo and 6.1 ± 0.9

vol% with L-cysteine (Figure 1). Thereafter, ethanol levels of gastric contents declined in parallel to 0.03 ± 0.003 vol% in placebo and to 0.06 ± 0.03 vol% in L-cysteine groups at 120 min, respectively (Figure 2). These levels correspond to 6.3–12.6 mmol/L ethanol, which is still more than enough for significant local acetaldehyde production.[17] No significant differences were found in gastric juice ethanol exposure in the placebo or L-cysteine setting. At 40 min, the mean gastric juice ethanol levels were 2.96 ± 0.66 vol% in placebo and 2.69 ± 0.52 vol% in L-cysteine groups. These values are close to the official ethanol limit (2.8 vol%) for alcoholic beverages in Finland.

Effect of slow-release L-cysteine on gastric juice acetaldehyde

In placebo experiments, peak acetaldehyde concentration in gastric juice (43.9 ± 8.8 μmol/L) was achieved at 40 min after intragastric alcohol infusion (Figure 2(A)). At 60 min, the acetaldehyde level was still markedly increased at 39.9 ± 10.5 μmol/L, thereafter levelling off to 9.6 ± 1.8 μmol/L at 120 min.

After treatment with slow-release L-cysteine capsules in addition to ethanol, the gastric acetaldehyde concentration was significantly reduced to 13.3 ± 2.7 μmol/L occurring already 20 min after intake, as compared to placebo with 39.9 ± 7.6 μmol/L ($p = 0.0063$). The peak acetaldehyde concentration of 43.9 ± 8.76 μmol/L at 40 min with placebo was markedly reduced with the addition of L-cysteine to 6.32 ± 1.80 μmol/L ($p = .0008$). The effect of L-cysteine was maintained over 120 min (Figure 3(A)). As estimated by the AUC, over the whole study period, slow-release L-cysteine reduced the gastric exposure of acetaldehyde by 68% ($p = .0005$) (Figure 3(B)).

Effect of slow-release L-cysteine on gastric juice L-cysteine and MTCA

Ten minutes after intake, the L-cysteine level in gastric contents was 83 ± 75 μmol/L. At 20 min after intake, it reached 3333 ± 1941 μmol/L (Figure 4). With L-cysteine, the MTCA levels were increased as compared to placebo ($p < .0004$) reaching 22 ± 14 μmol/L at 10 min and 60 ± 16 μmol/L at 20 min (Figure 4). The L-cysteine peak of 7552 ± 2687 μmol/L was reached 40 min after the intake of slow-release L-cysteine capsules, whereas the peak MTCA level 196 ± 98 μmol/L appeared first at 80 min. Both compounds remained elevated

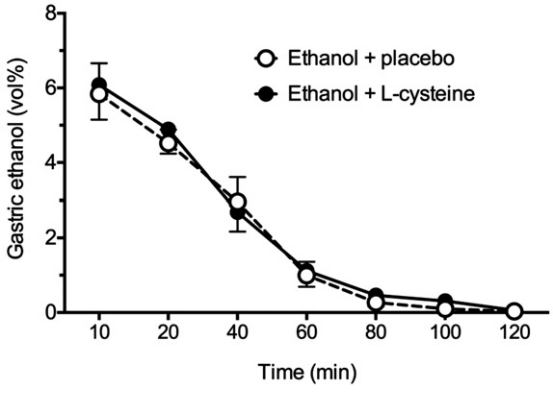

Figure 2. Ethanol kinetics (vol%) in the stomach after intragastric instillation of ethanol (15 vol%, 0.3 g/kg bodyweight) with placebo and slow-release L-cysteine capsule formulation (Acetium) (mean ± SEM; $n = 7$).

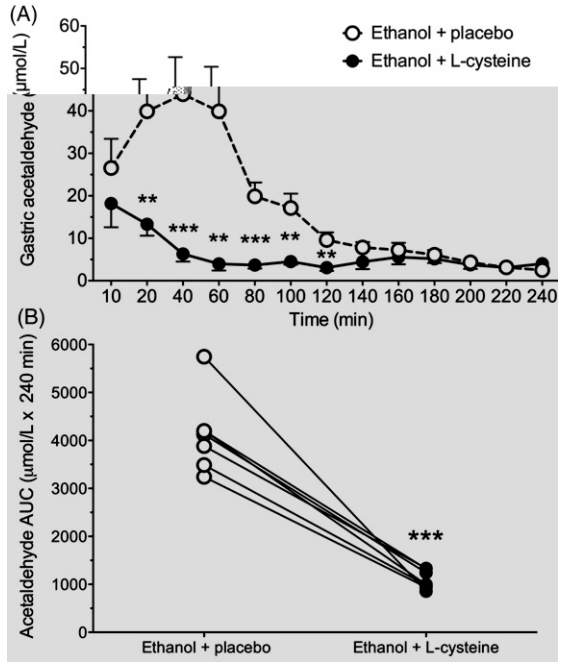

Figure 3. A. Effect of slow-release L-cysteine capsule formulation (Acetium) on acetaldehyde concentrations in gastric contents of achlorhydric patients with atrophic gastritis. $^{**}p < .01$, $^{***}p < .001$ (mean ± SEM; $n = 7$). B. Individual exposures of the gastric mucosa to acetaldehyde during 240 min after intragastric instillation of ethanol (15 vol%, 0.3 g/kg bodyweight) with or without slow-release L-cysteine capsule formulation (Acetium) (mean ± SEM; $n = 7$). $^{***}p < .001$. No error bars present in B.

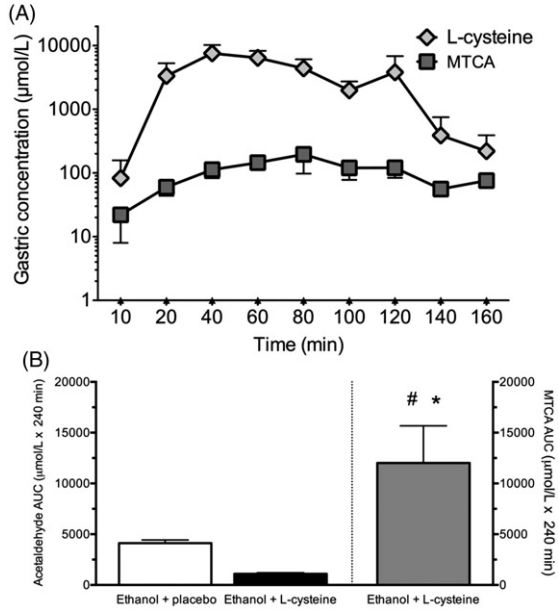

Figure 4. A. Intragastric concentrations of L-cysteine and the non-toxic reaction product 2-methyl-1,3-thiazolidine-4-carboxylic acid (MTCA) after covalent binding of L-cysteine to acetaldehyde in achlorhydric patients with atrophic gastritis after the intake of slow-release L-cysteine capsules (Acetium 2 × 100 mg) in conjunction with intragastric instillation of alcohol (15 vol%, 0.3 g/kg bodyweight) (mean ± SEM; $n = 7$). B. Gastric exposure to acetaldehyde after ethanol (15%vol) with and without L-cysteine in comparison with the presence of 2-methyl-1,3-thiazolidine-4-carboxylic acid (MTCA) in gastric contents. $\#p < .05$ in comparison with ethanol plus placebo; $^{*}p < .05$ in comparison with ethanol plus L-cysteine.

for at least 160 min (Figure 4(A)). In conjunction with this, the intragastric MTCA AUC was 3-fold higher than acetaldehyde AUC in the placebo-treated group ($p = .0469$) and 11-fold higher than the corresponding acetaldehyde AUC ($p = .0111$) when patients were treated with L-cysteine (Figure 4(B)). This indicated a marked local turnover of ethanol to acetaldehyde through gastric first-pass ethanol metabolism.

Discussion

According to Globocan 2012 statistics, stomach cancer is the fifth most common malignancy worldwide with the third highest mortality. The poor prognosis of gastric cancer underlines the importance of preventive measures.[18,19] A key to cancer prevention is the identification of the risk groups and early detection of precancerous conditions. Atrophic gastritis with hypochlorhydria is the most important independent risk factor for gastric cancer.[19,20] The normal acidic stomach contains few microbes, but in achlorhydric patients, bacteria and yeast colonise the gastric contents.[21] Some 35 years ago, it was proposed that gastric bacterial colonisation should lead to the intragastric conversion of nitrates to nitrites, resulting in elaboration of potentially carcinogenic N-nitroso compounds.[22] However, more recent cohort studies failed to provide conclusive evidence.[23] On the other hand, acetaldehyde is an underestimated risk factor for cancer development in humans.[24,25] It causes chromosomal damage, with sister-chromatid exchanges and chromosomal aberrations.[25,26] It also reacts with 2′-deoxyguanosine to form N_2-ethyl-2′-deoxyguanosine, leading to DNA adducts in animal models of ethanol exposure and in white blood cells of humans.[27] To this end, acetaldehyde inhibits DNA repair enzymes.[28]

Based on strong evidence derived from gene-epidemiological and gene-biochemical studies on alcohol drinking aldehyde dehydrogenase (ALDH)2-deficient individuals, acetaldehyde associated with the use of alcoholic beverages is classified as carcinogenic (Group 1) to humans, now being implicated as a risk factor for oral, pharyngeal and oesophageal cancers.[25] The new classification applies to both acetaldehyde present in alcoholic beverages and that formed endogenously from ethanol either by microbial or mucosal oxidation processes.

A point mutation in *ALDH2* gene resulting in deficient activity of the main acetaldehyde metabolising enzyme (ALDH2) is the most prevalent genetic health risk in the world, passing in frequency that of familiar hypercholesterolemia. The prevalence of familial hypercholesterolemia is 1:500, but that of ALDH2 deficiency 1:13. The *ALDH2* gene point mutation took place in South China over 2000 years ago. Its carrier frequency today is ~600 million people of East-Asian descent.[7,28] When drinking alcohol, those who are ALDH2-deficient are exposed via saliva to 2- to 3-fold, and via gastric juice to 5- to 6-fold higher acetaldehyde concentrations than those with active ALDH2 enzyme.[5,29,30] Parallel to increased local acetaldehyde exposure, the risk of ALDH2-deficient alcohol drinkers for oral, pharyngeal, oesophageal and gastric cancer is many fold compared to alcohol consumption in those with active ALDH2.[3,31,32] The difference in cancer risk between ALDH2 deficiency and active ALDH2 increases with increasing alcohol consumption. Thus, ALDH2 deficiency provides a unique human cancer model for acetaldehyde exposure, which proves the causal relationship between acetaldehyde and upper digestive tract cancers.

In this study, earlier findings on the powerful ability of slow-release L-cysteine to eliminate acetaldehyde formed through the gastric first-pass metabolism of ethanol in the achlorhydric stomach secondary either to atrophic gastritis or the use of PPIs, or H_2-receptor blockers were confirmed.[5,16] To this end, we found that orally administered slow-release L-cysteine intragastrically forms the non-toxic MTCA compound by binding to the reactive CHO-group of acetaldehyde. Peak MTCA concentrations of gastric juice were over 20-fold lower than MTCA levels found to be non-toxic to Caco-2 cell lines *in vitro*.[33] MTCA is an endogenous product that is detected in the blood after a moderate ethanol dose (0.5 g/kg).[34] Furthermore, it has been shown that MTCA can serve as a prodrug for L-cysteine and thus protect mice against acetaminophen toxicity.[35]

Under the present study conditions, sufficiently high L-cysteine levels for acetaldehyde neutralisation were achieved in the gastric juice at 10–20 min after L-cysteine (Acetium) capsule intake. If taken earlier, gastric emptying may sweep away some of the L-cysteine present in the stomach. L-cysteine and MTCA were shown to remain in gastric contents for up to 3 h after intake of slow-release L-cysteine formulation along with a moderate amount of ethanol corresponding to about two glasses of wine.

Ethanol itself is not carcinogenic, but acetaldehyde is. Due to its reactive aldehyde group, acetaldehyde is a cytotoxic, genotoxic and mutagenic compound.[24,25,36] Acetaldehyde is carcinogenic to experimental animals and, in conjunction with alcoholic beverages, strongly so to humans.[25] The classification of acetaldehyde as a group 1 carcinogen concerns both acetaldehyde present in alcoholic beverages and as well that formed endogenously from ethanol.[25] After alcohol intake, the highest concentrations of acetaldehyde in man are found in saliva and achlorhydric stomach.[5,29,37] This is due to the fact that parotid glands, oral microbes and upper digestive tract mucosal cells are able to oxidise ethanol to acetaldehyde, but are not as capable of its detoxification as liver.

The intragastric acetaldehyde concentrations found in our study (peak level 85.6 μmol/L) are considered to be mutagenic both *in vitro* and *in vivo*.[37,38] Increasing concentrations of acetaldehyde ranging from 25 to 500 μmol/L in the presence of 2′-deoxyguanosine or DNA and polyamines produce an exponential increase of mutagenic 1,N_2-propanodeoxyguanosine adducts.[37] Polyamine synthesis is tightly related to cellular proliferation, with the highest levels being found in rapidly dividing cells. This is characteristic for the regenerating upper digestive tract mucosa.[39] Increased formation of carcinogenic DNA adducts has also been demonstrated in the gastric mucosa of *ALDH2* gene knockout mice treated with ethanol.[38] In human volunteers, low doses of alcohol have been shown to produce a dose-dependent increase in mutagenic acetaldehyde-DNA damage in the oral cavity.[37] Corresponding doses of alcohol have been shown

to result in 18.7–143.4 μmol/L acetaldehyde levels in saliva,[17] which is similar to concentrations found in the stomach of patients with atrophic gastritis in this and earlier studies.[8,16]

A recent meta-analysis demonstrated that, compared with non-drinkers, heavy drinking, but not moderate, is significantly associated with gastric cancer.[1] Several other studies have reported an increased incidence of gastric cancer among ALDH2-deficient non-drinkers and among high consumers of foodstuffs produced through fermentation where ethanol, albeit at low concentrations, is elaborated.[40–43] The present results showing acetaldehyde production in gastric juice at low ethanol concentrations could explain the latter, and so far, unrecognised association between ALDH2 deficiency and gastric cancer. Many foodstuffs and 'non-alcoholic' beverages considered to be harmless may contain some ethanol and produce elevated levels of endogenous acetaldehyde.[44,45] In this study, the mean gastric ethanol concentrations from 40 min to 120 min throughout the study ranged from 3.0 to 0.1% which was associated with mutagenic acetaldehyde concentrations ranging from 28.5 to 85.6 μmol/L at 40 min. Thus, in achlorhydric subjects, even low ethanol levels in different foodstuffs and beverages are associated with formation of mutagenic concentrations of carcinogenic acetaldehyde via the gastric first-pass metabolism of ethanol. This conclusion is in accordance with our earlier findings in PPI-treated ALDH2-deficient subjects.[5] The fact that ethanol and acetaldehyde intake via widely used foodstuffs and beverages is not systematically recorded causes an obvious, but yet unrecognised, confounder and bias in cancer epidemiology of the upper digestive tract.

In the present study, with slow-release L-cysteine, the peak acetaldehyde concentrations in gastric contents at 20 min ranged from 2.8 to 20.2 μmol/L. Thus, the *in vitro* mutagenic concentration of 25 μmol/L acetaldehyde was not exceeded in any of the patients receiving slow-release L-cysteine capsules.[37] The Acetium capsule used in these studies is a CE-marked product that is registered in many countries as a medical device (class IIa). This classification is based on the nature of the active ingredient, L-cysteine, as a natural amino acid and the mode of action of the formulation. L-cysteine is a semi-essential sulphur-containing amino acid that is widely used as a food additive. It is classified as 'generally regarded as safe' by both the European Food Safety Administration (EFSA) and the US Food and Drug Administration (FDA). Characteristically, slow-release L-cysteine exerts its effect in the stomach, where acetaldehyde is also formed. Acetium capsules contain L-cysteine, which is bound to matrix granules with a matrix former. This causes L-cysteine to be released at a sustained rate locally in the stomach.[16] The use of a multi-particle system ensures that the formulation spreads to as large a part of the stomach as possible, even when the stomach contains solid or semi-solid content. Granules can also be assumed to remain longer in gastric mucosal folds, even in an upright position as gastric contents are usually retained in the fundus portion of the stomach. Aside from this, another interesting utility of the L-cysteine family is with N-acetyl cysteine, which when added to culture-based *H. pylori* eradication therapy has shown to achieve higher eradication rates than standard culture-based therapy in patients with a multi-resistant *H. pylori* infection.[46]

The markedly increased AUC for MTCA as compared to that for acetaldehyde in the presence of L-cysteine and ethanol confirmed substantial local oxidation of ethanol to acetaldehyde, i.e., gastric first-pass metabolism of ethanol mediated by gastric mucosal cells and oral microbiota colonising the acid-free stomach.[8,11,12] Atrophic gastritis results in decreased mucosal alcohol dehydrogenase activity and gastric first-pass metabolism of ethanol.[47] However, the decreased mucosal ethanol metabolism in patients with atrophic gastritis appears to be replaced by an enhanced microbial acetaldehyde production. In this study, patients were lying on their left side in order to enable sampling of gastric juice. This procedure may have delayed gastric emptying thereby significantly enhancing gastric first-pass metabolism of ethanol and consequently also acetaldehyde, L-cysteine and MTCA levels in gastric contents.[48]

An important limitation of our study is the fact that relatively few subjects were used. This should reduce the power of the study. However, using the patients as their own controls increases the validity of the pharmacological principle of L-cysteine capturing free acetaldehyde in the gastric lumen. Since we obtained stable values with limited variability in our acetaldehyde and MTCA assays reaching statistical significance with clear differences already with seven individuals, we decided to stop the recruitment at this point. Hence, the pharmacological interaction between L-cysteine and acetaldehyde was shown.

In conclusion, acetaldehyde is the most common cumulative human carcinogen.[49–51] It is present in most alcoholic beverages and foodstuffs produced or preserved by fermentation. Ethanol of alcoholic beverages is metabolised locally in the upper digestive tract to acetaldehyde. Acetaldehyde is widely used as an aroma agent and food additive. It is the most abundant carcinogen of tobacco smoke, which, in part, becomes dissolved in saliva and is in that manner distributed to the mucosal surfaces of the upper digestive tract. Generally accepted ALARA (As Low As Reasonably Achievable) principles should be implemented to all Group 1 human carcinogens including acetaldehyde. Most important is to avoid or decrease tobacco and alcohol consumption. In well-defined risk groups, slow-release L-cysteine formulations provide a novel and effective approach for the elimination of carcinogenic acetaldehyde locally in the stomach where it is formed. These risk groups include patients with achlorhydric atrophic gastritis and regular users of gastric acid secretion inhibitors (PPIs, H_2-receptor blockers) as well as ALDH2-deficient subjects, especially those with *H. pylori* infection or chronic atrophic gastritis as diagnosed either by gastric biopsy or by the GastroPanel blood test.[19] However, the actual effectiveness of slow-release L-cysteine (Acetium) formulation in cancer prevention remains to be evaluated in prospective intervention studies.

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Ethical standards

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions; ethics approval Dnr 2012/411. Informed consent or substitute for it was obtained from all patients for being included in the study.

Disclosure statement

M.S. is the board member, medical advisor and stock owner of Biohit Oyj. P.H., P.K., L.P., K.S. are employed by Biohit Oyj. However, the authors confirm that this does not alter their adherence to the policies of the journal on sharing data and materials. P.M.H., L.K., A.M., D.-L.W., H.S. are independent researchers and have no conflicts of interest.

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