

# Reducing Carcinogenic Acetaldehyde Exposure in the Achlorhydric Stomach With Cysteine

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**Background:** Acetaldehyde, associated with alcohol consumption, has recently been classified as a group 1 carcinogen in humans. Achlorhydric atrophic gastritis is a well-known risk factor for gastric cancer. Achlorhydria leads to microbial colonization of the stomach. Several of these microbes are able to produce significant amounts of acetaldehyde by oxidation from alcohol. Acetaldehyde can be eliminated from saliva after alcohol intake and during smoking with a semi-essential amino acid, L-cysteine. The aim of this study was to determine whether cysteine can be used to bind acetaldehyde in the achlorhydric stomach after ethanol ingestion.

**Methods:** Seven volunteers with achlorhydric atrophic gastritis were given either slow-release L-cysteine or placebo capsules in a double-blinded randomized trial. Volunteers served as their own controls. A naso-gastric tube was inserted to each volunteer. The volunteers ingested placebo or 200 mg of L-cysteine capsules, and ethanol 0.3 g/kg body weight (15 vol%) was infused intragastrically through a naso-gastric tube. Five-milliliter samples of gastric contents were aspirated at 5-minute intervals.

**Results:** During the follow-up period, the mean acetaldehyde level of gastric juice was 2.6 times higher with placebo than with L-cysteine (13 vs. 4.7  $\mu\text{M}$ ,  $p < 0.05$ ,  $n = 7$ ).

**Conclusions:** L-cysteine can be used to decrease acetaldehyde concentration in the achlorhydric stomach during alcohol exposure. Intervention studies with L-cysteine are needed on reducing acetaldehyde exposure in this important risk group for gastric cancer.

**Key Words:** Acetaldehyde, Ethanol, Cysteine, Achlorhydric Stomach, Gastric Cancer.

**G**ASTRIC CANCER IS currently the fourth most common cancer worldwide and has the second highest mortality rate because patients are most often diagnosed with advanced disease. The prognosis of gastric cancer patients is poor, 5-year survival being only 10 to 25%. However, the incidence of gastric cancer has been declining over the last decades, probably because of improved food preservation methods and general hygiene conditions and thereby lower *Helicobacter pylori* incidence rates. In 2002, 934,000 new cases were found worldwide, and the annual mortality was 700,000. Early detection has increased survival to 52% (obtained from overall mortality/incidence) in Japan, where mass screening by fluoroscopy has been used since the 1960s (Boyle and Levin, 2008; Parkin et al., 2005). With such a high mortality

rate and poor prognosis, it is essential to explore all possible means of prevention by identifying potential specific etiological factors and mechanisms of carcinogenesis and by intervening where possible.

*Helicobacter pylori* infection, atrophic gastritis, and smoking are the most important risk factors for gastric cancer (Chao et al., 2002; Gonzalez et al., 2003; International Agency for Research on Cancer, 1994; Sipponen et al., 1985). Furthermore, atrophic gastritis is also an independent risk factor for esophageal cancer and squamous dysplasia (Kamangar et al., 2009; de Vries et al., 2009; Ye and Nyren, 2003). The development of gastric cancer is a long-term process, with progression from normal tissue to intermediate stages of chronic gastritis, gastric atrophy, intestinal metaplasia, and dysplasia. The progression takes several decades, and the underlying mechanisms are largely unknown (Correa, 2004).

Gastric atrophy leads to achlorhydria and bacterial overgrowth in the stomach. Production of carcinogens by these bacteria is a plausible mechanism for the carcinogenesis associated with both autoimmune and *H. pylori*-induced atrophic gastritis. Acetaldehyde, associated with alcohol beverage drinking, has recently been classified as a group 1 carcinogen in humans (Secretan et al., 2009). Acetaldehyde is produced in the alimentary tract from ethanol by microbes representing normal flora (Jokelainen et al., 1996; Väkeväinen et al., 2001). The capacity of microbes to produce acetaldehyde largely exceeds their capacity to detoxify it, which results in the

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accumulation of acetaldehyde in saliva, the achlorhydric stomach, and the large intestine (Salaspuro, 2003). Some alcoholic beverages also contain acetaldehyde, which may even increase the total acetaldehyde level in the digestive tract (Lachenmeier et al., 2009).

Acetaldehyde is mostly eliminated by the low-K<sub>m</sub> mitochondrial aldehyde dehydrogenase-2 (ALDH2) enzyme. The liver is the main organ for acetaldehyde formation and elimination, but some acetaldehyde is also produced in the oral mucosa and salivary glands. A hereditary deficiency in ALDH2 enzyme activity leads to impaired acetaldehyde elimination and accumulation in the saliva and presumably also in other parts of the digestive tract (Salaspuro, 2009; Väkeväinen et al., 2000a, 2001). This low-activity *ALDH2* \*1/\*2 genotype is linked to markedly increased risk for ethanol-associated digestive tract cancers (Seitz and Stickel, 2010; Yokoyama et al., 1998). In Japanese alcoholics, chronic atrophic gastritis and *ALDH2* \*1/\*2 genotype have been found to be both independent and synergistic risk factors for gastric cancer, with a combined odds ratio of 39.2 (Yokoyama et al., 2007).

Acetaldehyde is produced from both ethanol and glucose in the achlorhydric stomach (Väkeväinen et al., 2002). *Helicobacter pylori* has also been shown to produce significant amounts of acetaldehyde when incubated with ethanol (Salmela et al., 1993, 1994). In addition, acetaldehyde is found in tobacco smoke, and it is easily dissolved into saliva during smoking (Salaspuro and Salaspuro, 2004). Furthermore, by modifying the oral flora, chronic smoking and heavy drinking are known to increase salivary acetaldehyde production from ethanol both in vitro and in vivo by 50 to 100% (Homann et al., 2000; Salaspuro and Salaspuro, 2004). Via swallowing, acetaldehyde can be expected to reach the stomach. Thus, acetaldehyde dissolved in saliva during smoking and produced microbially in the saliva and the achlorhydric stomach might be the common denominator in the pathogenesis of gastric cancer (Salaspuro, 2009).

Cysteine, a semi-essential amino acid, is able to eliminate the toxicity of acetaldehyde by binding to it covalently. The product is stable 2-methylthiazolidine-4-carboxylic acid (Sprince et al., 1975). Orally administered L-cysteine has been successfully used to bind acetaldehyde in saliva during smoking and ethanol consumption (Salaspuro et al., 2002, 2006). Cysteine might also bind acetaldehyde in the achlorhydric stomach. Therefore, the aim of this study was to determine whether cysteine can be used to eliminate carcinogenic acetaldehyde in the stomach of subjects with achlorhydric atrophic gastritis after ethanol ingestion.

## MATERIALS AND METHODS

### *Preparation of Study Capsules*

The capsules, prepared at the University Pharmacy in Helsinki, contained 50 mg of L-cysteine as an active ingredient. Five hundred grams of L-cysteine (Gonmisol S.A., Barcelona, Spain), 500 g of Eudragit RS-PO forming the matrix structure (Evonik Röhm GmbH, Damstadt, Germany), and 1 kg of calcium hydrogenphosphate as an inactive additive (CaHPO<sub>4</sub>, Emcompress® Anhydrous;

Mendell a Penwest Company, Lakeville, MN) were mixed in a Turbula Powder Blender (Glen Mills Inc., Clifton, NJ) for 10 minutes, and the mixture was wet-granulated using ethanol. The wet granules were sieved using a 2-mm sieve and thereafter allowed to dry at room temperature in a fume hood for 24 hours. The dried granules were sieved using 1.68 mm and 1.18 mm sieves, and the fraction between 1.68 mm and 1.18 mm was collected for capsulation. Simultaneously, a placebo formulation where L-cysteine was replaced with the same amount of CaHPO<sub>4</sub> was prepared following exactly the same method.

The matrix granules formed were weighed into hard gelatin capsules to ease the administration such that each capsule contained 200 mg of granules, equaling 50 mg of L-cysteine. The L-cysteine concentration of the granules was determined using a capillary method (400 mg of granules contained 98 mg of L-cysteine).

### *Dissolution Test for the Capsules*

Dissolution tests for the capsules were performed according to the USP I method (USP 24) (The United States Pharmacopeia 2001). A standard curve was prepared between 0.01 and 0.6 mg/ml ( $y = 2.196 + 0.0016x$ ,  $r^2 = 0.9999$ ). The medium used was 500 ml of pH 1.2 HCl buffer. The rotation rate of the baskets was 100 rpm, and the temperature of the medium was +37°C (±0.5). Samples were taken at 5-minute intervals for the first half hour and thereafter at 10-minute intervals for the remaining 2 hours. L-cysteine was detected in flow-through cells (10 mm) at a wavelength of 213 nm. The results were calculated by using dissolution software. The system was equipped with a bath and pump (Sotax AT7 UV Dissolution System; Sotax, Allschwil, Switzerland) and a spectrophotometer (Perkin-Elmer, Lambda 25; PerkinElmer, Inc., Waltham, MA); the software used for the test and for calculating the results was WinSotax (Sotax).

## SUBJECTS

Seven volunteers (2 men, 5 women) with achlorhydric atrophic gastritis participated in the study. Their mean age ± SD was 57 ± 7 years and mean body weight 75 ± 22 kg. The mean serum gastrin level of the subjects was 417 pM (range 192 to 968 pM, upper normal limit 50 pM). Pepsinogen-1 level was below the detection threshold of 25 µg/l in all volunteers. A routine follow-up gastroscopy with biopsies had been performed on each participant within 1 year prior to the study, and chronic atrophic corpus gastritis without *H. pylori* had been confirmed histologically in all subjects. All volunteers were nonsmokers and normal social drinkers, with an average consumption of 50 g or less of ethanol per week. Five of the subjects were receiving vitamin B12 substitution, 1 had medication for hypertension and hypercholesterolemia, and 2 had medication for hypothyreosis; otherwise, the volunteers were clinically healthy. None of the volunteers had received any antibiotics or medication that influences the acidity of the stomach for 1 month preceding the study.

### *Study Design*

The study protocol was approved by the Ethics Committee of the Department of Medicine, Helsinki University Hospital, and the Finnish National Agency for Medicines. An informed consent to participate in the study was obtained from each subject. A randomized double-blinded placebo-controlled study design was used, and each participant served as his/her own control. The 2 study days were separated by at least a 3-day interval. The volunteers were admitted to the Department of Gastroenterology of Helsinki University Hospital, and all studies started between 8 and 10 AM. The volunteers were told to refrain from alcohol intake for 24 hours and food intake for 12 hours prior to the study. The subjects were asked to report all possible side effects of the study formulations used both during and after the experiments.

A nasogastric tube (Duodenal tube Levin, CH 10; Unomedical, Birkeroed, Denmark) was inserted into subjects to a depth of 55 cm at the beginning of both study days. The correct positioning of the gastric tube was confirmed by aspiration of gastric contents. The tube was lubricated with Xylocain gel (AstraZeneca, Södertälje, Sweden) containing no ethanol. During the tube placement, the volunteers were given 100 ml of water to facilitate swallowing of the tube. For the rest of the study, the volunteers laid on their left side to delay gastric emptying. Four capsules containing either cysteine 50 mg each or placebo were given to the subjects double blindly with 200 ml of water orally. Immediately thereafter, ethanol (0.3 g/kg body weight) diluted in water to 15 vol% solution was infused via the nasogastric tube into the stomach of volunteers. Samples of gastric juice (5 ml) were aspirated through the tube at 5-minute intervals up to 60 minutes after the ethanol infusion or until the stomach had emptied, as indicated by unsuccessful aspiration. The samples were analyzed for pH and acetaldehyde, ethanol, and cysteine concentrations. The pH of the gastric juice was measured using a glass electrode and a digital pH meter (WTW pH-521, Weilheim, Germany).

#### Acetaldehyde and Ethanol Analysis of Gastric Juice Samples

To measure acetaldehyde concentration, 450  $\mu$ l of gastric juice was immediately transferred into a headspace vial containing 50  $\mu$ l of 6 mol/l perchloric acid. In our unpublished tests, perchloric acid has been determined not to hydrolyze cysteine-acetaldehyde binding. For ethanol analysis, the gastric juice was diluted 10-fold in purified water, and 500  $\mu$ l of diluted gastric juice was transferred into a headspace vial. Two parallel samples were used for measurements, and the mean value was calculated. Acetaldehyde and ethanol levels were analyzed by headspace gas chromatography, as previously described (Väkeväinen et al., 2002).

#### L-Cysteine Analysis of Gastric Juice Samples

L-cysteine concentration of the gastric juice samples was determined by using a HPLC method. A standard curve was prepared between concentrations of 0.0625 and 2.0 mg/ml ( $y = 851.06x + 8.52$ ,  $r^2 = 0.9704$ ). Two parallel samples of each gastric juice sample were prepared. Sixty microliters of gastric juice was measured into a test tube, and 30  $\mu$ l of pH 7.4 phosphate-buffered saline solution (Ph.Eur.) and 30  $\mu$ l of 20 vol% Tri-n-butylphosphine in dimethylformamide were added. The samples were incubated for 30 minutes at +4°C, after which 90  $\mu$ l of cold 10% trichloroacetic acid containing 1 mM Na<sub>2</sub>EDTA was added, and the samples were vortexed for 2 minutes and then centrifuged (10 minutes, 2,490 $\times$ g). Fifty microliters of supernatant was pipetted into a test tube containing 125  $\mu$ l of pH 9.5 borate buffer with 4 mM Na<sub>2</sub>EDTA, 10  $\mu$ l of 1.55 M sodium hydroxide, and 50  $\mu$ l of 2 mg/ml 4-Fluoro-7-Sulfobenzofurazan, Ammonium salt (SBD-F) solution in borate buffer. The samples were incubated for 60 minutes at +60°C so that a yellow derivat was formed. Thereafter, 150  $\mu$ l of the solution was pipetted into HPLC inserts. Injection volume was 10  $\mu$ l. The system was equipped with a Waters Model 501 piston pump (Waters, Milford, MA), a Waters 717 Auto-sampler, a Waters 484 tunable absorbance detector, and a Millennium 32 Chromatography Manager workstation. The isocratic mobile phase was pH 7.0 phosphate buffer and methanol (95:5). The flow rate was 1 ml/min and retention time was 6 minutes. L-cysteine concentration was determined using a fluorescence detector (excitation 385 nm, emission 515 nm).

#### Statistical Analysis

Statistical significance between placebo and cysteine administrations was analyzed by Wilcoxon's nonparametric test for paired samples. Correlations were tested by using Spearman's rho. A  $p$  value of less than 0.05 was considered significant. Statistical analysis was

carried out using SPSS statistical software (SPSS 15.0.1.; SPSS Inc., Chicago, IL).

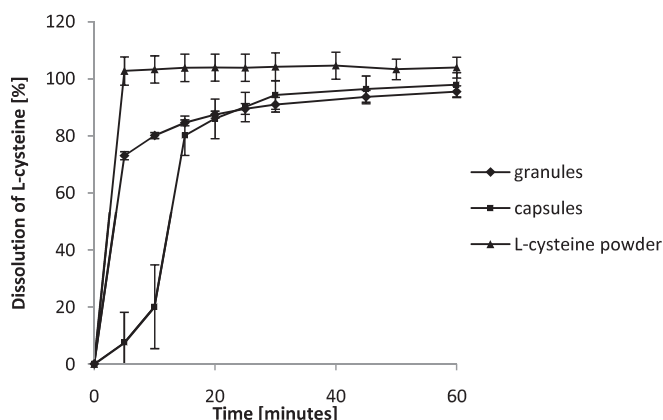
## RESULTS

The dissolution tests showed that the formulation released L-cysteine at a controlled rate, yet still fast enough to have time to react with acetaldehyde before the formulation is assumed to leave the stomach (Fig. 1). When not granulated, L-cysteine was dissolved rapidly (100% in 5 minutes).

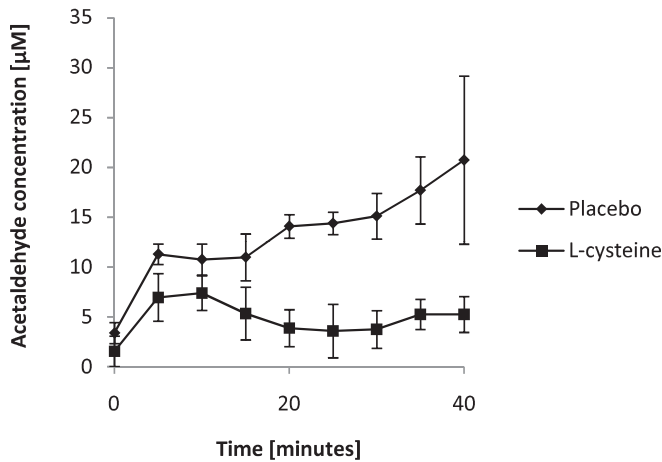
No difference was found in gastric juice pH between placebo and cysteine administrations, and no significant change in pH was observed during the follow-up. The mean pH  $\pm$  SD of the gastric juice samples was  $6.9 \pm 0.7$ .

In all measurements, the average acetaldehyde concentration of the gastric juice was 2.6 times higher with placebo than with cysteine (13 vs. 4.7  $\mu$ M,  $p < 0.05$ ,  $n = 7$ ). We calculated the area under the curve (AUC) up to 40 minutes for 5 subjects with data up to 40 minutes. The average AUC for acetaldehyde was significantly higher with placebo than with cysteine (531 vs. 197  $\mu$ M  $\times$  min,  $p < 0.05$ ,  $n = 5$ , Fig. 2). No significant differences existed in ethanol concentrations between cysteine and placebo treatments. The average ethanol concentration in the gastric juice was 5.0 vol% (range 1.1 to 7.6) in the first sample, declining to 0.9 vol% (range 0.1 to 2.7) in the 40-minute sample. A positive correlation emerged between acetaldehyde concentration and ethanol concentration ( $r = 0.40$ ,  $p < 0.01$ ). None of the volunteers reported having any side effects during the study, but 1 did describe having slight joint pain after the experiment with placebo.

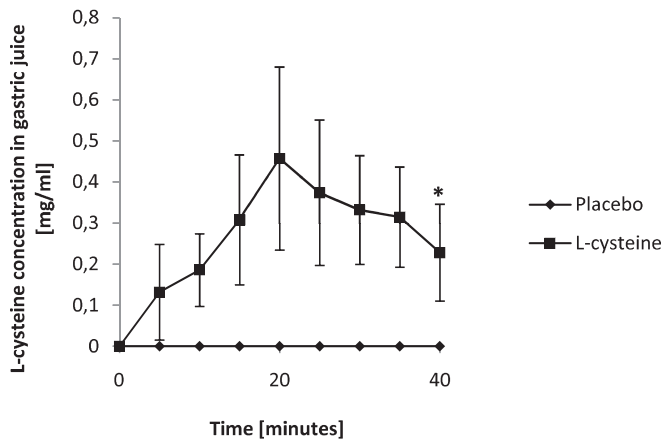
L-cysteine was detected in the gastric juice of all volunteers after the administration of study formulations containing L-cysteine. The mean cysteine concentrations are represented in Fig. 3. After administration of placebo formulations, no L-cysteine was detected. No significant correlation was found between cysteine concentration and acetaldehyde concentration ( $r = -0.12$ ,  $p = 0.34$ ).



**Fig. 1.** Results from the dissolution test for L-cysteine capsules, L-cysteine granules without the capsule shell, and L-cysteine powder (dissolution %  $\pm$  SD).



**Fig. 2.** Acetaldehyde concentration ( $\mu\text{M} \pm \text{SEM}$ ) after intragastric ethanol (0.3 g/kg body weight) and placebo or cysteine (200 mg) administration in 5 subjects with atrophic gastritis.



**Fig. 3.** Mean cysteine concentration  $\pm$  SEM in gastric juice after intragastric ethanol (0.3 g/kg body weight) and placebo or cysteine (200 mg) administrations in 5 subjects with atrophic gastritis (\* $N = 4$  at 40 minutes because of insufficient sample volume for cysteine analysis in 1 subject).

## DISCUSSION

Although alcohol is a major risk factor for esophageal cancer (Secretan et al., 2009; Zeka et al., 2003), the epidemiological evidence for an association between alcohol consumption and stomach cancer is controversial. However, in a meta-analysis of alcohol-related cancers, the relative risk for cancer of the stomach was 1.32/100 g alcohol daily (Bagnardi et al., 2001). The markedly increased risk for stomach cancer among ALDH2-deficient heavy drinking Asian subjects also strongly supports the direct role of acetaldehyde in gastric carcinogenesis (Yokoyama et al., 1998, 2007). It is noteworthy that epidemiological studies on gastric cancer published to date are biased by the alcohol and acetaldehyde content of beverages classified as nonalcoholic beverages and various fermented food stuffs not being included in the calculations (Lachenmeier et al., 2010). However, they may contain up to 500 mM of ethanol, constituting a powerful and long-term mechanism

for exposure to the acetaldehyde formed by microbes surviving in an achlorhydric stomach.

Atrophic gastritis is a well-known premalignant condition of gastric cancer (Morson et al., 1980; Sipponen et al., 1985). In epidemiological follow-up studies, the incidence of gastric cancer among atrophic gastritis patients has been 4.6 to 10% and approximately 20 to 40% of gastric cancer cases are associated with atrophic gastritis (Siurala et al., 1974; Testoni et al., 1987). The risk for gastric cancer is correlated with the severity of gastric atrophy (Sipponen et al., 1985). Although the increased gastric cancer risk in atrophic gastritis has long been recognized, the exact pathogenetic mechanism behind this is still unknown.

Acetaldehyde has recently been classified as a group 1 carcinogen in humans (Secretan et al., 2009). The carcinogenicity of acetaldehyde is based on many epidemiological, genetic, biochemical, and microbial studies (Salaspuro, 2009; Seitz and Stickel, 2010). By and large, all known risk factors for gastric cancer are associated with increased exposure of the gastric mucosa to acetaldehyde. Acetaldehyde is an important component of tobacco smoke, which is transported to the stomach via saliva. Microbially mediated acetaldehyde formation from ethanol and glucose occurs in atrophic gastritis (Väkeväinen et al., 2002). In addition, *H. pylori* can produce acetaldehyde from ethanol (Salmela et al., 1993, 1994). Therefore, carcinogenic acetaldehyde could be a common denominator and a plausible explaining factor for gastric cancer.

In this study, we showed that cysteine can be used to reduce acetaldehyde concentration in the achlorhydric stomach of subjects with atrophic gastritis. The differences in gastric acetaldehyde concentrations between placebo and cysteine administrations were statistically significant at time-points from 20 to 40 minutes. Because of individual stomach emptying times of the study subjects, the follow-up time was ranged from 15 to 60 minutes. At 45 minutes, we were able to obtain samples from only 3 subjects, and thus, there is insufficient data to statistically show the effect of cysteine on gastric acetaldehyde level beyond 40 minutes. However, the data obtained from these 3 volunteers suggest that the binding effect of cysteine on intragastric acetaldehyde remains at least 60 minutes. The fasted volunteers laid on their left side throughout the experiment to slow down gastric emptying. At the moment, we do not have data on the effect of a meal on acetaldehyde concentrations and the role of L-cysteine in this situation.

The overall acetaldehyde concentration was lower than expected based on our previous study with atrophic gastritis patients. In our earlier study with the same ethanol dose as in this study, the mean gastric juice acetaldehyde concentration at 30 minutes varied from 34 to 45  $\mu\text{M}$  (Väkeväinen et al., 2002). The lower figures in the current study are probably because of more water being given to subjects to facilitate swallowing of the capsules, resulting in dilution of ethanol, acetaldehyde, and the microbes producing acetaldehyde in the stomach. Furthermore, in this study, we opted to take more frequent samples, and thus, there was an increased removal of ethanol, acetaldehyde, and microbes from the stomach.

The cysteine granules used were designed to release L-cysteine at a controlled rate. As L-cysteine is rapidly dissolved when not bound to a formulation (solubility 280 g/l at +25°C of water), it was important to prove that the matrix structure decreased the release rate. Nevertheless, the L-cysteine should be released rapidly enough that the formulation would not leave the stomach before the whole amount, or at least the majority, of L-cysteine is released and dissolved into the stomach content. The gelatin capsules caused a short lag-time in the release, but as this was only 5 to 10 minutes, it was not considered relevant for the *in vivo* functionality of the matrix formulation.

L-cysteine was detected in the gastric juice of all volunteers already in the first sample taken at 5 minutes. This indicated that the lag-time seen in the *in vitro* study was not significant *in vivo*. L-cysteine was also present in the gastric juice of all volunteers at the last time-point of the experiment, which proved that the formulations stayed in the stomach for the duration of the study and released L-cysteine at a controlled rate. In addition, this showed that the chosen dose of L-cysteine (200 mg) was adequate to bind the amount of acetaldehyde produced in the stomach from the dose of ethanol (0.3 g/kg body weight) administered for at least 40 to 60 minutes. The effect of cysteine may even be longer if the cysteine formulation is taken during meals, as solid content slows stomach emptying and the granules mixed with the stomach content are likely to leave the stomach only with the digested meal. Presumably, the gastric acetaldehyde concentration will start to rise again as the administered cysteine is transported to the small bowel by gastric emptying. Therefore, if alcohol consumption is continued, a new dose of cysteine would have to be taken every 1 to 2 hours to keep gastric acetaldehyde at a low level.

The cysteine concentrations of the gastric juice seen in this study were as expected. Taking into account that the dose of cysteine was 200 mg and the capsules were administered with 200 ml of water and the volume of ethanol dose used was 120 to 200 ml, the estimated concentration of cysteine would be approximately 0.8 to 1 mg/ml if L-cysteine was evenly distributed to the whole stomach content. Thereby, our results indicate that a nonsignificant amount of L-cysteine was absorbed from the stomach or transferred to the small intestine. Absorption was not expected because it has been shown that LAT2 transporters, which are mainly responsible for the active absorption of L-cysteine in the digestive tract, are not present in the stomach (del Amo et al., 2008). Because we could not define the exact location of the gastric tube or the part of the stomach in which the capsule disintegrated, calculating the true concentration of L-cysteine in the stomach was impossible.

In our previous study, we were able to detect small amounts of endogenous acetaldehyde and ethanol in the gastric juice of some of our study subjects with atrophic gastritis after intragastric glucose infusion (Väkeväinen et al., 2002). Their endogenous acetaldehyde levels had varied from 2.3 to 15.7  $\mu\text{M}$ . The amounts of endogenous acetaldehyde and

ethanol produced in the achlorhydric stomach from food intake are unknown. Considering our previous study, it can, however, be assumed that some endogenous ethanol and acetaldehyde are formed from carbohydrates of ingested food in the achlorhydric stomach. We have also shown that hypochlorhydria induced by proton pump inhibitors leads to microbial colonization of the stomach and microbial production of acetaldehyde from ethanol (Väkeväinen et al., 2000b). Moreover, small amounts of endogenous ethanol have been found in patients receiving cimetidine (Bode et al., 1984). The amount of cysteine needed to bind acetaldehyde in these situations remains to be elucidated. Taking into account previous results and the results of this present study, the dose of cysteine needed is likely the same or lower than that used here.

An estimated 4 mg/kg body weight of cysteine is required per day in the human diet. This translates to 320 mg per day in a person weighing 80 kg (Joint FAO/WHO/UNU Expert Consultation, 2002). Cysteine has long been used as a food supplement. Based on data from the 1988 to 1994 Third National Health and Nutrition Examination Survey, the mean cysteine intake from food and supplements in the United States was 1.0 g/d (Food and Nutrition Board/Institute of Medicine 2002, 2005). If cysteine and methionine are taken together, an adult in a Western country consumes, on the average, proteins equivalent to 2 to 4 g cysteine per day (Breitkreutz et al., 2000).

Most clinical studies on long-term cysteine supplementation have been performed either with the relatively stable synthetic cysteine derivative N-acetylcysteine or with a naturally derived cysteine-rich undenatured whey protein isolate. However, in our unpublished experiments, the acetaldehyde-binding ability of N-acetylcysteine has been minimal. Cysteine supplementation has been shown to improve skeletal muscle functions, decrease the body fat/lean body mass ratio, improve immune functions, and increase plasma albumin levels. Dietary consumption of cysteine has been suggested in general to be suboptimal and further that everybody is likely to have a cysteine deficiency sooner or later (Droge, 2005).

N-acetylcysteine has been considered to be safe and well tolerated when administered orally (Demedts et al., 2005; Dodd et al., 2008). However, single large doses (5 to 10 g) of cysteine have been shown to induce nausea, light-headedness, and dissociation in man (Carlson et al., 1989). Reports of chronic administration of cysteine to humans are not available, and it has been concluded that insufficient data exist on the long-term effects of increased intake of cysteine to establish a safe upper limit for intake (Food and Nutrition Board/Institute of Medicine 2002, 2005).

An excess of L-cysteine has proved neurotoxic *in vivo* only in developing animals (Janáky et al., 2000). However, because mammalian liver tightly regulates its free cysteine pool, L-cysteine capsules in recommended doses for adults can be considered to be safe (Stipanuk et al., 2006). Furthermore, no major adverse effects have been reported even with continuous use of cysteine as a food supplement. Cysteine itself tastes unpleasant, and it therefore is formulated and covered so that

this does not limit its use. None of the study subjects described any adverse effects of the cysteine formulation and dose (200 mg) used in this study, indicating that a similar formulation and dose could be used in general in humans.

## CONCLUSIONS

Our findings indicate that L-cysteine can be used to bind acetaldehyde in the achlorhydric stomach of fasted patients with atrophic gastritis during alcohol exposure. Acetaldehyde associated with alcohol consumption is a group I carcinogen in humans, and atrophic gastritis has long been recognized as a major risk factor for gastric cancer. Medical devices releasing cysteine in the stomach in a controlled manner can be used to minimize the exposure to carcinogenic acetaldehyde in this important risk group for gastric cancer. It remains to be investigated whether reduced acetaldehyde exposure is reflected in the reduction in malignant or premalignant transformation of gastric epithelia. Because of the slow course of cancer development, this will take years.

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## CONFLICT OF INTEREST

Mikko Salaspuro is a Board member of Biohit Oyj. Tuuli Marvola is an employee at Biohit Oyj. Patents and pending patents for L-cysteine capsules (Acetium, CE marked Medical Device) are owned by Biohit Oyj.

## REFERENCES

- del Amo EM, Urtti A, Yliperttula M (2008) Pharmacokinetic role of L-type amino acid transporters LAT1 and LAT2. *Eur J Pharm Sci* 35:161–174.
- Bagnardi V, Blangiardo M, La Vecchia C, Corrao G (2001) Alcohol consumption and the risk of cancer: a meta-analysis. *Alcohol Res Health* 25:263–270.
- Bode JC, Rust S, Bode C (1984) The effect of cimetidine treatment on ethanol formation in the human stomach. *Scand J Gastroenterol* 19:853–856.
- Boyle P, Levin B (2008) World Cancer Report 2008. International Agency for Research on Cancer, Lyon, France.
- Breitkreutz R, Holm S, Pittack N, Beichert M, Babylon A, Yodoi J, Droge W (2000) Massive loss of sulfur in HIV infection. *AIDS Res Hum Retroviruses* 16:203–209.
- Carlson HE, Miglietta JT, Roginsky MS, Stegink LD (1989) Stimulation of pituitary hormone secretion by neurotransmitter amino acids in humans. *Metabolism* 38:1179–1182.
- Chao A, Thun MJ, Henley SJ, Jacobs EJ, McCullough ML, Calle EE (2002) Cigarette smoking, use of other tobacco products and stomach cancer mortality in US adults: The Cancer Prevention Study II. *Int J Cancer* 101:380–389.
- Correa P (2004) Is gastric cancer preventable? *Gut* 53:1217–1219.
- Demedts M, Behr J, Buhl R, Costabel U, Dekhuijzen R, Jansen HM, MacNee W, Thomeer M, Wallaert B, Laurent F, Nicholson AG, Verbeken EK, Verschakelen J, Flower CD, Capron F, Petruzzelli S, De Vuyst P, van den Bosch JM, Rodriguez-Becerra E, Corvasce G, Lankhorst I, Sardina M, Montanari M, IFIGENIA Study Group (2005) High-dose acetylcysteine in idiopathic pulmonary fibrosis. *N Engl J Med* 353:2229–2242.
- Dodd S, Dean O, Copolov DL, Malhi GS, Berk M (2008) N-acetylcysteine for antioxidant therapy: pharmacology and clinical utility. *Expert Opin Biol Ther* 8:1955–1962.
- Droge W (2005) Oxidative stress and ageing: is ageing a cysteine deficiency syndrome? *Philos Trans R Soc Lond B Biol Sci* 360:2355–2372.
- Food and Nutrition Board/Institute of Medicine 2002 (2005) Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). The National Academies Press, Washington DC, USA.
- Gonzalez CA, Pera G, Agudo A, Palli D, Krogh V, Vineis P, Tumino R, Panico S, Berglund G, Siman H, Nyren O, Agren A, Martinez C, Dorronsoro M, Barricarte A, Tormo MJ, Quiros JR, Allen N, Bingham S, Day N, Miller A, Nagel G, Boeing H, Overvad K, Tjonneland A, Bueno-De-Mesquita HB, Boshuizen HC, Peeters P, Numans M, Clavel-Chapelon F, Helen I, Agapitos E, Lund E, Fahey M, Saracci R, Kaaks R, Riboli E (2003) Smoking and the risk of gastric cancer in the European Prospective Investigation Into Cancer and Nutrition (EPIC). *Int J Cancer* 107:629–634.
- Homann N, Tillonen J, Meurman JH, Rintamäki H, Lindqvist C, Rautio M, Jousimies-Somer H, Salaspuro M (2000) Increased salivary acetaldehyde levels in heavy drinkers and smokers: a microbiological approach to oral cavity cancer. *Carcinogenesis* 21:663–668.
- International Agency for Research on Cancer Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7–14 June 1994 (1994) Schistosomes, liver flukes and *Helicobacter pylori*. IARC Monogr Eval Carcinog Risks Hum 61:1–241.
- Janáky R, Varga V, Herrmann A, Saransaari P, Oja SS (2000) Mechanisms of L-cysteine neurotoxicity. *Neurochem Res* 25:1397–1405.
- Joint FAO/WHO/UNU Expert Consultation (2002) Protein and amino acid requirements in human nutrition. WHO Technical Report Series 935.
- Jokelainen K, Siitonen A, Jousimies-Somer H, Nosova T, Heine R, Salaspuro M (1996) In vitro alcohol dehydrogenase-mediated acetaldehyde production by aerobic bacteria representing the normal colonic flora in man. *Alcohol Clin Exp Res* 20:967–972.
- Kamangar F, Diaw L, Wei WQ, Abnet CC, Wang GQ, Roth MJ, Liu B, Lu N, Giffen C, Qiao YL, Dawsey SM (2009) Serum pepsinogens and risk of esophageal squamous dysplasia. *Int J Cancer* 124:456–460.
- Lachenmeier DW, Kanteres F, Rehm J (2009) Carcinogenicity of acetaldehyde in alcoholic beverages: risk assessment outside ethanol metabolism. *Addiction* 104:533–550.
- Lachenmeier DW, Uebelacker M, Hensel K, Rehm J (2010) Acetaldehyde in the human diet: an underestimated risk factor for cancer. *Deut Lebensm Rundsch* 106:30–35.
- Morson BC, Sobin LH, Grundmann E, Johansen A, Nagayo T, Serck-Hanssen A (1980) Precancerous conditions and epithelial dysplasia in the stomach. *J Clin Pathol* 33:711–721.
- Parkin DM, Bray F, Ferlay J, Pisani P (2005) Global cancer statistics, 2002. *CA Cancer J Clin* 55:74–108.
- Salaspuro MP (2003) Acetaldehyde, microbes, and cancer of the digestive tract. *Crit Rev Clin Lab Sci* 40:183–208.
- Salaspuro M (2009) Acetaldehyde as a common denominator and cumulative carcinogen in digestive tract cancers. *Scand J Gastroenterol* 44:912–925.
- Salaspuro V, Hietala J, Kaihovaara P, Pihlajarinne L, Marvola M, Salaspuro M (2002) Removal of acetaldehyde from saliva by a slow-release buccal tablet of L-cysteine. *Int J Cancer* 97:361–364.
- Salaspuro VJ, Hietala JM, Marvola ML, Salaspuro MP (2006) Eliminating carcinogenic acetaldehyde by cysteine from saliva during smoking. *Cancer Epidemiol Biomarkers Prev* 15:146–149.
- Salaspuro V, Salaspuro M (2004) Synergistic effect of alcohol drinking and smoking on in vivo acetaldehyde concentration in saliva. *Int J Cancer* 111:480–483.

- Salmela KS, Roine RP, Höök-Nikanne J, Kosunen TU, Salaspuro M (1994) Acetaldehyde and ethanol production by *Helicobacter pylori*. *Scand J Gastroenterol* 29:309–312.
- Salmela KS, Roine RP, Koivisto T, Höök-Nikanne J, Kosunen TU, Salaspuro M (1993) Characteristics of *Helicobacter pylori* alcohol dehydrogenase. *Gastroenterology* 105:325–330.
- Secretan B, Straif K, Baan R, Grosse Y, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L, Coglianò V, WHO International Agency for Research on Cancer Monograph Working Group (2009) A review of human carcinogens – part E: tobacco, areca nut, alcohol, coal smoke, and salted fish. *Lancet Oncol* 10:1033–1034.
- Seitz HK, Stickel F (2010) Acetaldehyde as an underestimated risk factor for cancer development: role of genetics in ethanol metabolism. *Genes Nutr* 5:121–128.
- Sipponen P, Kekki M, Haapakoski J, Ihamäki T, Siurala M (1985) Gastric cancer risk in chronic atrophic gastritis: statistical calculations of cross-sectional data. *Int J Cancer* 35:173–177.
- Siurala M, Lehtola J, Ihamäki T (1974) Atrophic gastritis and its sequelae. Results of 19–23 years' follow-up examinations. *Scand J Gastroenterol* 9:441–446.
- Sprince H, Parker CM, Smith GG, Gonzales LJ (1975) Protective action of ascorbic acid and sulfur compounds against acetaldehyde toxicity: implications in alcoholism and smoking. *Agents Actions* 5:164–173.
- Stipanuk MH, Dominy JE, Jeong-Inn L, Coloso RM (2006) Mammalian cysteine metabolism: new insights into regulation of cysteine metabolism. *J Nutr* 136:1652S–1659S.
- Testoni PA, Masci E, Marchi R, Guslandi M, Ronchi G, Tittobello A (1987) Gastric cancer in chronic atrophic gastritis. Associated gastric ulcer adds no further risk. *J Clin Gastroenterol* 9:298–302.
- The United States Pharmacopeia (2001) USP 24 in the United States Pharmacopeia, pp. 2049–2050. The United States Pharmacopeial Convention Inc., Rockville, MD, USA.
- Väkeväinen S, Mentula S, Nuutinen H, Salmela KS, Jousimies-Somer H, Färkkilä M, Salaspuro M (2002) Ethanol-derived microbial production of carcinogenic acetaldehyde in achlorhydric atrophic gastritis. *Scand J Gastroenterol* 37:648–655.
- Väkeväinen S, Tillonen J, Agarwal DP, Srivastava N, Salaspuro M (2000a) High salivary acetaldehyde after a moderate dose of alcohol in ALDH2-deficient subjects: strong evidence for the local carcinogenic action of acetaldehyde. *Alcohol Clin Exp Res* 24:873–877.
- Väkeväinen S, Tillonen J, Blom M, Jousimies-Somer H, Salaspuro M (2001) Acetaldehyde production and other ADH-related characteristics of aerobic bacteria isolated from hypochlorhydric human stomach. *Alcohol Clin Exp Res* 25:421–426.
- Väkeväinen S, Tillonen J, Salaspuro M, Jousimies-Somer H, Nuutinen H, Färkkilä M (2000b) Hypochlorhydria induced by a proton pump inhibitor leads to intragastric microbial production of acetaldehyde from ethanol. *Aliment Pharmacol Ther* 14:1511–1518.
- de Vries AC, Capelle LG, Looman CW, van Blankenstein M, van Grieken NC, Casparie MK, Meijer GA, Kuipers EJ (2009) Increased risk of esophageal squamous cell carcinoma in patients with gastric atrophy: independent of the severity of atrophic changes. *Int J Cancer* 124:2135–2138.
- Ye W, Nyren O (2003) Risk of cancers of the oesophagus and stomach by histology or subsite in patients hospitalised for pernicious anaemia. *Gut* 52:938–941.
- Yokoyama A, Muramatsu T, Ohmori T, Yokoyama T, Okuyama K, Takahashi H, Hasegawa Y, Higuchi S, Maruyama K, Shirakura K, Ishii H (1998) Alcohol-related cancers and aldehyde dehydrogenase-2 in Japanese alcoholics. *Carcinogenesis* 19:1383–1387.
- Yokoyama A, Yokoyama T, Omori T, Matsushita S, Mizukami T, Takahashi H, Higuchi S, Maruyama K, Ishii H, Hibi T (2007) *Helicobacter pylori*, chronic atrophic gastritis, inactive aldehyde dehydrogenase-2, macrocytosis and multiple upper aerodigestive tract cancers and the risk for gastric cancer in alcoholic Japanese men. *J Gastroenterol Hepatol* 22:210–217.
- Zeka A, Gore R, Kriebel D (2003) Effects of alcohol and tobacco on aerodigestive cancer risks: a meta-regression analysis. *Cancer Causes Control* 14:897–906.