CURRENT
THERAPEUTIC
RESEARCH®
VOL. 58, NO. 10, OCTOBER 1997

EFFECTS OF *N*-ACETYL-L-CYSTEINE IN PATIENTS WITH CHRONIC ATROPHIC GASTRITIS AND NONULCER DYSPEPSIA: A PHASE III PILOT STUDY

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ABSTRACT

Several studies have suggested that oxidative damage may contribute to gastritis. This damage is counteracted by various scavengers including glutathione (GSH), which may help defend the gastric mucosa. *N*-acetyl-L-cysteine (NAC), a GSH precursor, could thus be of therapeutic interest. This multiple-dose, double-masked, randomized, parallel-group, phase III pilot study was designed to assess the effects of NAC in 18 patients undergoing upper gastrointestinal endoscopy for dyspepsia who had endoscopically and histologically confirmed chronic atrophic gastritis but no peptic ulcer. Patients were randomly allocated to one of three treatment groups (NAC 1 g/d at bedtime, 1 g two times a day [2 g/d], or 2 g two times a day [4 g/d]) for 4 weeks. After treatment, patients underwent a second endoscopy. During both endoscopies, multiple biopsies were taken for histologic examination (based on a semiquantitative score according to the Sydney system). Reduced/oxidized glutathione (GSH/GSSG) and malondialdehyde (MDA) were also measured (using high-performance liquid chromatography and fluorometric assay). At recruitment, 6 patients tested negative and 12 tested positive for *Helicobacter pylori*. Serologic findings and symptoms (semiquantitatively scored) were collected at the beginning and end of the trial. After treatment, 13 (72%) of 18 patients showed improvement on endoscopy, irrespective of NAC dose, and 5 (28%) showed no change. Histologically, polymorph infiltration was significantly reduced in patients who received 2 g of NAC. The 5-point total symptom score was lower, but not significantly so, in patients who received the other doses. Because of a high variability, no significant change in GSH and MDA was found. No difference was observed between *H pylori*–positive and –negative patients. No relevant changes were detected in laboratory findings, and the most common adverse events were constipation, abdominal pain, and flatulence. Our findings suggest that in patients with gastritis and nonulcer

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Received for publication on June 4, 1997. Printed in the U.S.A.

0011-393X/97/$3.50

dyspepsia, NAC is fairly well tolerated and apparently leads to endoscopic, symptomatic, and to some extent histologic improvement, unrelated to changes in mucosal GSH levels. ***Key words:*** **gastritis, glutathione, *N*-acetyl-L-cysteine, nonulcer dyspepsia, malondialdehyde.**

INTRODUCTION

Several studies have suggested that oxidative damage may contribute to chronic gastritis and that antioxidants could act as defense mechanisms.[1–5] In particular, glutathione (GSH) may be one of the most important means of defense for the gastric mucosa.[6–9] A number of sulfhydryl compounds have been shown to protect the gastric mucosa from injury by various noxious substances.[10,11] One such agent is *N*-acetyl-L-cysteine (NAC), a source of cysteine that is a key substrate for GSH synthesis.[12] Several studies have clearly shown that NAC, when administered orally, can effectively prevent or markedly attenuate the extent of gastric injury induced by various noxious substances applied to the gastric mucosa.[12–19] This study was carried out to investigate the effects of a new oral formulation of NAC* (in l-g sachets) in 18 patients with gastritis and nonulcer dyspepsia.

PATIENTS AND METHODS

Clinical Trial Design

This phase III pilot study was performed at a single center (Cattedra Malattie Apparato Digerente, Istituto di Medicina Interna, Università di Padova, Padova, Italy) according to an uncontrolled, multiple-dose, double-masked, randomized, parallel-group design. Patients participated in the study for approximately 5 weeks; the first patient was screened on December 3, 1993, and the last completed the trial June 30, 1994. Patients received one of the three doses of NAC: 1 g/d at bedtime (mean dose, 13.8 ± 3.3 mg/kg body weight), l g two times per day (2 g/d) (25.8 ± 4.7 mg/kg body weight), or 2 g two times per day (4 g/d) (53.3 ± 21 mg/kg body weight) for 4 weeks. All patients consumed two sachets a day of an orange-flavored preparation, irrespective of their assigned dose. The typical NAC smell was masked by the orange flavoring.

Trial Population

Eighteen patients undergoing gastroduodenal endoscopy for dyspepsia and presenting a gastric morphology compatible with chronic gastritis ac-

* Trademark: Fluimucil (Zambon Group S.p.a., Bresso-Milano, Italy).

Table I. Patient characteristics.

Characteristics	1 g/d (n = 6)	2 g/d (n = 6)	4 g/d (n = 6)
Sex (n)			
Male	3	5	4
Female	3	1	2
Age (y)			
Mean	48.8	56.3	48
Range	24–65	38–65	25–63

companied by varying degrees of atrophy were required by the protocol to complete the study. Patients were enrolled after undergoing endoscopy and laboratory tests if they were between 18 and 65 years of age; had normal physical examination, laboratory test, and urinalysis results before the study; provided written informed consent before the first endoscopy; and could comply with the study protocol. Patients with peptic ulcer, those allergic to NAC or to other substances, those with significant disease (eg, cardiovascular, respiratory), drug abusers, and those taking disqualifying medication (nonsteroidal anti-inflammatory drugs, corticosteroids, H_2-receptor antagonists, or omeprazole) were excluded from the trial. No patient was taking anti–H_2-receptor antagonists or proton pump inhibitors before entering the study, and only one was taking antacids.

Three patients subsequently dropped out: 1 withdrew consent, 1 because of adverse events, and 1 because of adverse events and refusal to cooperate. They were replaced by 3 other patients who received a second series of NAC sachets provided for patient replacements. Demographic features are shown in Table I.

Variables Examined and Methods

At the screening visit the following variables were recorded: demographic data, smoking habits, drug allergies, contraceptive practices, alcohol intake, and medical history. Gastric symptoms were assessed at the screening visit and at the end of the study by using a semiquantitative score. A total symptoms score (TSS) was obtained by combining the scores for five gastrointestinal symptoms (nausea, bloating/distention, abdominal pain, anorexia, early satiety) measured by a horizontal, 100-mm visual analogue scale with end points of "absent" and "severe." This approach was used to simplify analysis of the data, given the small number of subjects included in this pilot study.

Patients underwent hematologic and chemical laboratory tests and urinalysis before starting treatment and again at the end of the study.

Gastroduodenal endoscopy was always performed by the same gastroenterologist to standardize the macroscopic description. During endoscopy,

multiple biopsies were taken for histologic examination (at least two from the antrum and two from the body), and four biopsies were taken for GSH and malondialdehyde (MDA) determination. Biopsy samples were fixed in 5% buffered formaldehyde, stained with hematoxylin & eosin (H&E) and a modified Giemsa stain, and observed by the same pathologist. The extent of gastric inflammation was semiquantitatively scored according to the Sydney system,[20] based on the following five variables: polymorph infiltration of the lamina propria, polymorph infiltration of the epithelium, mononuclear cell infiltration of the mucosa, mucin depletion, and epithelial degeneration. Each of these five features was graded from 0 to 3 (0 = absent, 1 = mild, 2 = moderate, 3 = severe) to reflect disease "activity." The presence of *Helicobacter pylori* infection was also semiquantitatively scored (0, +, ++, +++) on biopsies stained with H&E, modified Giemsa, or the Warthin Starry stain. The control endoscopy was always performed within 48 hours after patients consumed their last sachet. The endoscopist (who also collected the TSS), the pathologist, and the physician who carried out the biochemical determinations were masked as to each patient's dose. Compliance was assessed by controlling the number of sachets in the unit dose boxes returned at the end of treatment. Three patients returning more than 20% of the sachets were withdrawn from the study, considered not assessable, and replaced.

For reduced/oxidized glutathione (GSH/GSSG) determination, described in detail elsewhere,[21] samples were processed by thawing in 0.15 M KCl and homogenizing with an ice-cooled Teflon pestle, and then precipitating in 5% perchloric acid at 3000 rpm. The sample then underwent chromophore derivatization of amino groups with Sanger's reagent (1-fluoro-2,4-dinitrobenzene) according to Reed et al.[22] GSH and GSSG levels were then determined by means of Shimadzu high-performance liquid chromatography and expressed in nanomoles per milligram of tissue. Interassay and intraassay coefficients of variation for the method were lower than 15%. MDA was measured using the thiobarbituric acid method described by Masugi and Nakamura[23] and was expressed in nanomoles per gram of tissue. Coefficients of variation for the method were lower than 10% for MDA.

Statistical Analysis

In general, all findings were summarized by using means and standard deviations or frequency tables, as appropriate. Analysis of variance was used for the following variables with a normal distribution (age, body weight, TSS, GSH, GSSG, percent GSSG, and MDA); the Kruskal-Wallis test was applied where the distributional assumption did not hold (ie, gastric histology results). All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant.

Table II. Gastric histology results: Degree of inflammation expressed as a semiquantitative score (mean ± SD).

	1 g/d		2 g/d		4 g/d	
	A	B	A	B	A	B
Polymorph infiltration of lamina propria	1.2 ± 0.9	1.2 ± 0.7	2.2 ± 0.4	1.7 ± 0.5	1.5 ± 0.8	1.2 ± 0.4
Polymorph infiltration of epithelium*	0.8 ± 0.9	1.2 ± 0.7	2.0 ± 0.6	1.5 ± 0.5	0.8 ± 0.7	0.8 ± 0.7
Mononuclear cell infiltration of mucosa	2.0 ± 0.6	1.8 ± 0.7	2.2 ± 0.4	2.2 ± 0.4	1.8 ± 0.7	1.3 ± 1.0
Mucin depletion	1.0 ± 0.8	0.8 ± 0.7	1.5 ± 0.5	1.2 ± 0.4	0.8 ± 0.7	0.8 ± 1.1
Epithelial degeneration	0.8 ± 0.9	0.5 ± 0.5	1.2 ± 0.4	1.2 ± 0.4	0.8 ± 0.7	0.7 ± 1.0

A = baseline; B = week 4.
* Kruskal-Wallis test for baseline score, $P = 0.04$ in the 2-g/d group; Fisher's exact test for "improval" between baseline and week 4, $P = 0.048$.

RESULTS

There were no statistically significant differences between treatment groups in sex, age, body weight, height, smoking habit, alcohol intake, drug allergy, or number of biopsies performed at endoscopy (data not shown).

Endoscopic Features

At the end of the study, 13 (72%) of 18 patients showed improvement on endoscopy (eg, disappearance of erosions, hyperemic areas on the gastric mucosa) and 5 (28%) showed no change.

Gastric Histology

The subgroup of patients taking 2 g/d of NAC had a higher score of polymorph infiltration at baseline with respect to the other two groups ($P = 0.048$). In the same subgroup of patients, comparing baseline versus end point, a statistically significant reduction ($P = 0.04$) was found in polymorph infiltration of the epithelium (Table II).

Total Symptoms Score

When all patients were considered together, there was a statistically significant difference between findings at the screening visit and at the second visit, with a general decrease in TSS in all three treatment groups ($P = 0.04$). TSS decreased in 15 of 18 patients, remained stable in 1, and increased in 2. When the three groups were considered separately, the

Table III. Evaluated patients: Total symptoms score (mean ± SD).

	1 g/d	2 g/d	4 g/d
Baseline	155.5 ± 68.8	79.2 ± 31.7*	113.8 ± 92.3
Range	68–247	52–125	6–245
Week 4	107.7 ± 104.4	39.8 ± 20.9*	68.8 ± 78.5
Range	0–230	17–76	2–171

General analysis of variance between visits; $P = 0.04$.
* In particular, 2-g/d group: baseline versus week 4, $P = 0.01$.

decrease proved statistically significant only in the 2-g/d group ($P = 0.01$), in which the mean TSS at the fourth week was halved compared with baseline (79.2 ± 31.7 vs 39.8 ± 20.9). Of the 18 patients, only 2 (in the 1-g/d group) showed no decrease in TSS after treatment with NAC. In three patients in each group, symptom score improved more than 50% at the fourth week; two patients in the 1-g/d group showed a complete remission of symptoms (Table III).

Helicobacter pylori *Infection*

Twelve (67%) of 18 patients (3 of 6 in the 1-g/d group, 5 of 6 in the 2-g/d group, and 4 of 6 in the 4-g/d group) tested positive for *H pylori*. No difference was observed between positive and negative patients in either endoscopic or histologic findings or any changes in symptoms. Two of 3 patients in whom TSS was not reduced after treatment were positive for *H pylori* and 1 was negative (not statistically significant).

Glutathione and Malondialdehyde Determination

No difference was observed between GSH (Figure 1), GSSG (Figure 2), and MDA (Figure 3) levels at baseline and at week 4, either in general or when the three treatment groups were considered separately.

Adverse Events

Of 21 patients who participated in the study, 14 reported at least one adverse event—6 in the 1-g/d group, 5 in the 2-g/d group, and 3 in the 4-g/d group. Adverse events mainly involved the gastrointestinal tract and totaled 33 episodes. These events all had a positive outcome, because they either "regressed" (84%) or "improved" (16%). They were mild in 68% of cases and moderate in the rest. They lasted from 1 to 26 days, with constipation generally lasting the longest (20 to 26 days). All three groups had a similar number of adverse events (Table IV).

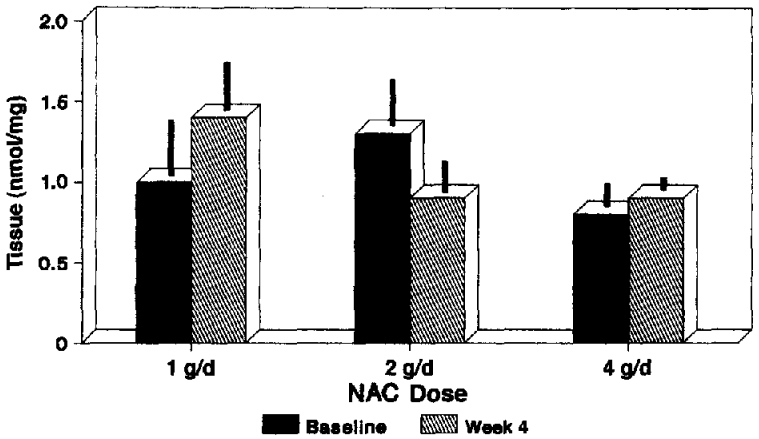

Figure 1. No difference (mean ± SD) was observed between glutathione (GSH) levels at baseline and at week 4. NAC = *N*-acetyl-L-cysteine.

DISCUSSION AND CONCLUSIONS

This study investigated the effects of a new oral formulation of NAC (in 1-g sachets) on gastric morphology in patients with gastritis and dyspepsia but no peptic ulcer. Gastric GSH and MDA concentrations and tolerability were measured after 4 weeks of treatment at three different doses. The three treatment groups were comparable in all baseline characteristics except for polymorph infiltration of the epithelium on gastric mucosal biopsy. This difference could be explained by the relatively higher percentage of *H pylori*–positive patients in this subset or could be due to chance, and it does not appear to be of any relevance.

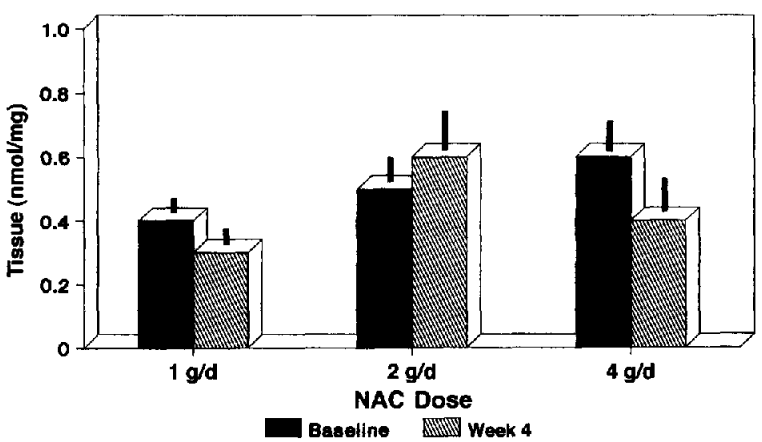

Figure 2. No difference (mean ± SD) was observed between oxidized glutathione (GSSG) levels at baseline and at week 4. NAC = *N*-acetyl-L-cysteine.

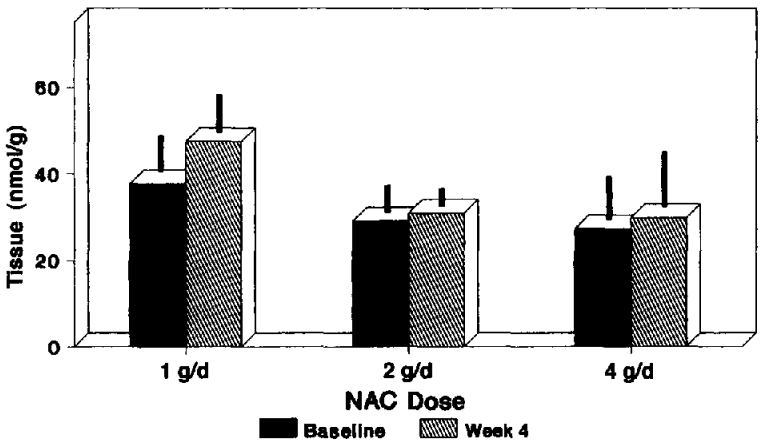

Figure 3. No difference was observed between malondialdehyde (MDA) levels at baseline and at week 4. NAC = *N*-acetyl-L-cysteine.

On endoscopy, 72% of patients showed improvement at the end of the trial, while histologic findings revealed a statistically significant difference in the extent of polymorph infiltration of the epithelium in the 2-g/d treatment group compared with the 1-g/d and 4-g/d groups.

The TSS at week 4 was decreased in all three groups and reached statistical significance in the 2-g/d group. No difference was found between *H pylori*–positive and –negative patients.

Table IV. Adverse events by body system.

Body System	No. of Patients		
	1 g/d	2 g/d	4 g/d
Cardiovascular	1	—	1
Hypertension	1	—	1
Respiratory	1	1	—
Bronchitis	—	1	—
Cough increased	1	1	—
Sinusitis	1	—	—
Gastrointestinal	4	3	3
Diarrhea	2	1	—
Dyspepsia	1	—	—
Flatulence	3	—	2
Constipation	1	2	1
Nausea	1	—	—
Vomiting	1	—	—
Body as a whole	4	3	1
Abdominal pain	2	1	1
Fever	1	2	—
Malaise	—	—	1
Headache	1	—	—
Patients experiencing at least one adverse event	6	5	3

All comparisons were found to be not statistically significant.

No statistically significant difference was found in GSH, GSSG, or MDA levels. The lack of any statistical significance for these findings is probably due to the considerable biologic (and not methodologic) variability of the measurements. No important changes emerged in the laboratory findings, and reported adverse events mainly involved the gastrointestinal system.

In conclusion, these data suggest that in patients with gastritis and nonulcer dyspepsia, NAC is fairly well tolerated and apparently leads to an endoscopic, symptomatic, and to some extent histologic improvement that is not clearly related to changes in mucosal GSH and MDA levels.

Acknowledgment

The study was sponsored by Zambon Group S.p.a., Bresso-Milano, Italy.

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