Chemoprevention of Gastric Dysplasia: Randomized Trial of Antioxidant Supplements and Anti-*Helicobacter pylori* Therapy

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Background: **Previous research has identified a high risk of gastric carcinoma as well as a high prevalence of cancer precursor lesions in rural populations living in the province of Nariño, Colombia, in the Andes Mountains.** ***Methods:*** **A randomized, controlled chemoprevention trial was conducted in subjects with confirmed histologic diagnoses of multifocal nonmetaplastic atrophy and/or intestinal metaplasia, two precancerous lesions. Individuals were assigned to receive anti-*Helicobacter pylori* triple therapy and/or dietary supplementation with ascorbic acid, β-carotene, or their corresponding placebos. Gastric biopsy specimens taken at baseline were compared with those taken at 72 months. Relative risks of progression, no change, and regression from multifocal nonmetaplastic atrophy and intestinal metaplasia were analyzed with multivariate polytomous logistic regression models to estimate treatment effects. All statistical tests were two-sided.** ***Results:*** **All three basic interventions resulted in statistically significant increases in the rates of regression: Relative risks were 4.8 (95% confidence interval [CI] = 1.6–14.2) for anti-*H. pylori* treatment, 5.1 (95% CI = 1.7–15.0) for β-carotene treatment, and 5.0 (95% CI = 1.7–14.4) for ascorbic acid treatment in subjects with atrophy. Corresponding relative risks of regression in subjects with intestinal metaplasia were 3.1 (95% CI = 1.0–9.3), 3.4 (95% CI = 1.1–9.8), and 3.3 (95% CI = 1.1–9.5). Combinations of treatments did not statistically significantly increase the regression rates. Curing the *H. pylori* infection (which occurred in 74% of the treated subjects) produced a marked and statistically significant increase in the rate of regression of the precursor lesions (relative risks = 8.7 [95% CI = 2.7–28.2] for subjects with atrophy and 5.4 [95% CI = 1.7–17.6] for subjects with intestinal metaplasia).** ***Conclusions:*** **In the very high-risk population studied, effective anti-*H. pylori* treatment and dietary supplementation with antioxidant micronutrients may interfere with the precancerous process, mostly by increasing the rate of regression of cancer precursor lesions, and may be an effective strategy to prevent gastric carcinoma. [J Natl Cancer Inst 2000;92: 1881–8]**

Helicobacter pylori infection is recognized as a cause of gastric carcinoma *(1),* with estimates of attributable risk ranging from 50% to 73% *(2,3).* Ample evidence supports the protective effect of consumption of fresh fruits and vegetables against gastric cancer. This association may be related to the effects of antioxidants, particularly ascorbic acid and β-carotene *(4,5).*

A robust test of the causal association of *H. pylori* infection with gastric cancer and of the protective effect of ascorbic acid and β-carotene would be to cure the infection, to provide antioxidant supplements, and to observe the risk of developing cancer relative to the risk in a control group. Intervention studies utilizing cancer diagnosis as the primary end point are not feasible because they require following tens of thousands of subjects for several decades *(6).* Using intermediate steps in the progression of gastric carcinogenesis as end points allows inferences based on observations of a smaller number of subjects followed over a shorter time period.

Previous studies *(7–10)* in the Nariño region of southeastern Colombia documented high gastric cancer rates and identified the sequence of histologic lesions that currently define the gastric precancerous process, the nosologic complex referred to as multifocal atrophic gastritis (the main components of which are multifocal nonmetaplastic atrophy [gland loss], intestinal metaplasia, and dysplasia). The goal of the current randomized trial was to determine if treatment of the *H. pylori* infection and/or supplementation with ascorbic acid and β-carotene prevents the progression of the gastric precancerous process.

Materials and Methods

Study Subjects

This study was approved by the Institutional Review Board of Louisiana State University Health Sciences Center and the Committees on Ethics of Universidad del Valle and Hospital Departamental de Nariño in Colombia. Study subjects were recruited in Pasto and Túquerres, two communities in the province of Nariño in the Andes Mountains of Colombia. After signing informed consent documents approved previously by the U.S. Department of Health and Human Services, potential study subjects between 29 and 69 years of age provided a medical history and a blood sample and underwent physical examination, upper gastrointestinal endoscopy, and gastric biopsies prior to determining eligibility. Subjects with a preliminary histologic diagnosis of multifocal atrophic gastritis with or without intestinal metaplasia and dysplasia, but otherwise in good health, were randomly assigned in a single step, using a permuted block design, to one of eight different treatment regimens. Computer-generated lists were produced in

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See "Notes" following "References."

New Orleans and applied in the fieldwork in Pasto. Before randomization, subjects were classified into one of three strata—atrophy (without metaplasia), intestinal metaplasia, or dysplasia—according to baseline histologic diagnosis. After a factorial design, a double-blind approach—i.e., study investigators and subjects were unaware of treatment assignments—was used to assign subjects to a dietary supplement of β-carotene (30 mg once per day) and/or ascorbic acid (1 g twice a day) or their corresponding placebos, provided in identical coded tablets by Hoffmann-La Roche Inc. (Nutley, NJ). The prevalence of *H. pylori* infection among all gastric biopsy specimens was 97%. Anti-*H. pylori* treatment consisting of amoxicillin (500 mg three times per day), metronidazole (375 mg three times per day), and bismuth subsalicylate (262 mg three times per day) was given for 14 days to half of the study subjects assigned randomly. This treatment was not blinded or placebo controlled because an appropriate placebo was not available for bismuth subsalicylate.

Compliance with treatment was constantly encouraged and monitored by social workers who interviewed the participants and recorded pill counts every 3 months. Blood levels of β-carotene and ascorbic acid were measured in the entire study cohort at baseline and after 36 and 72 months of follow-up. In addition, blood levels of β-carotene and ascorbic acid were measured every 3 months in a 20% random sample of participants. Blood samples were protected from light and centrifuged at 3000*g* for 5 minutes at 10 °C–12 °C immediately after collection. Aliquots of serum for ascorbic acid analysis were immediately mixed 1 : 1 with a freshly prepared 10% metaphosphoric acid solution. Serum aliquots for β-carotene and ascorbic acid analysis were transported frozen to Louisiana State University Health Sciences Center, New Orleans. β-Carotene and ascorbic acid were measured by high-performance liquid chromatography following methods published previously *(11–13).*

H. pylori infection was monitored by the ^{13}C-urea breath test *(14,15)* administered once a year in subjects assigned to anti-*H. pylori* treatment. Breath samples were collected before and 30 minutes after administering a 175-mg oral dose of ^{13}C-urea dissolved in 25% dextrose solution and transported to the Pennington Biomedical Research Center (Baton Rouge, LA) for analysis. Urea-derived $^{13}CO_2$ was measured by mass spectrometry, and a positive result was defined by a ^{13}C enrichment of eight parts per thousand or greater at 30 minutes. Gastric biopsy specimens were taken at 36 months, the mid-point of the trial, and at 72 months of follow-up and were evaluated for *H. pylori* infection. The modified Steiner Silver technique *(16)* was used to detect *H. pylori* in tissue sections. Subjects assigned to anti-*H. pylori* treatment who tested positive for *H. pylori* at 36 months were treated again for 14 days with amoxicillin (1 g twice a day), clarithromycin (500 mg twice a day), and either omeprazole (20 mg twice a day) or lansoprazole (30 mg twice a day). Except for this 14-day treatment limited to 143 patients in whom the original triple therapy failed to eradicate the infection, the subjects did not receive proton-pump inhibitors or H_2 blockers.

Histopathology

At the time of each endoscopy, four biopsy specimens were obtained for histologic evaluation from the following locations: antrum, adjacent to incisura angularis; antrum, greater curvature, 5 cm above the pylorus; antrum, anterior wall; and corpus, anterior wall. These biopsy samples were fixed in 10% buffered formalin, dehydrated, and paraffin embedded within 24 hours. At embedding, tissues were oriented on edge, positioning the mucosal plane perpendicular to the cutting surface. Embedded tissues were transported to New Orleans for processing. Multiple 4-μm-thick histologic sections were also obtained from each biopsy fragment. Sections were stained with hematoxylin–eosin for regular histologic examination and with Alcian blue–periodic acid Schiff (pH 2.5) *(16)* to detect intestinal metaplasia. When intestinal metaplasia was observed, sections were stained with the high-iron diamine–Alcian blue procedure to detect sulfated mucins *(17).* Three sections were stained with the modified Steiner technique *(18)* to detect *H. pylori.*

A preliminary histopathologic evaluation was conducted in Colombia soon after baseline biopsy specimen collection to assess each subject's eligibility for enrollment and randomization. This evaluation was reviewed in New Orleans at the beginning of the study. At the end of the study, a single experienced pathologist (J. C. Bravo), blinded to treatment assignment and all other study variables, examined all biopsy specimens collected at baseline and after 72 months of follow-up, following the guidelines published by Dixon et al. *(19)* for the classification and grading of chronic gastritis. Each biopsy specimen fragment was evaluated separately for the following parameters: type of mucosa (antral, oxyntic, or transitional); biopsy adequacy (full or partial thickness of the mucosa represented); severity and distribution of polymorphonuclear and lymphocytic infiltrates; multifocal nonmetaplastic atrophy, defined as loss of glands, graded as absent, mild, moderate, or severe; intestinal metaplasia, graded (0–3) according to the proportion of the gastric mucosa being replaced by the metaplastic tissue *(19);* presence and severity of dysplasia, defined by atypical cytologic and architectural derangement independent of the degree of inflammation; and presence and abundance of *H. pylori* organisms. Intestinal metaplasia was further subclassified as either complete (small intestinal type or type I), defined by the presence of columnar cells with brush border alternating with goblet cells, or incomplete (colonic type or type III), defined by the presence of areas of columnar cells with foamy cytoplasm, lacking brush border *(20).* A global histologic diagnosis was made for each participant corresponding to the most advanced lesion observed in all biopsy fragments collected on each occasion *(21).* Quality control of the global diagnosis was made by independent review by a second expert pathologist, reviewing a set of slides from every 20th study subject as well as all dysplasias and all case subjects considered to be doubtful at the initial review. The pathologist read the slides blind to all identifiers, including treatment assignment. He received from the statistician scrambled and relabeled batches that included equal numbers of slides from the baseline and biopsy specimens at 72 months to minimize temporal differences between observers.

The histologic classification of the gastric biopsy specimens has two main sources of variability: 1) intrareader (biopsy specimens were examined twice by the same reader) and 2) sampling (five biopsy specimens were taken at each endoscopy, at preselected sites in the stomach). The kappa statistic for overall diagnosis was 0.75, which is better than the values usually obtained in similar exercises *(22).* The two readings were completely independent (blind duplicates read in a random order at different occasions). The discordant pairs were examined by a panel of four expert pathologists who produced a final diagnosis for that pair, which was used in the analysis. Only 5.4% of the variability of overall diagnosis can be attributed to intrareader discrepancies.

Potential adverse effects of β-carotene supplementation in smokers *(23)* were reported during the last year of this trial, and the National Cancer Institute (Bethesda, MD) asked for a review of this trial by the Safety and Data Monitoring Board (SDMB). Active participants on β-carotene supplementation who were current smokers were asked to stop smoking for the remainder of the trial, following recommendations of the SDMB. At the time of this recommendation, 141 (21.8%) of 648 active participants were smokers. Most of those subjects were light smokers (median, three cigarettes/day). Of these, 65 of the smokers were receiving β-carotene; 34 stopped smoking and 31 elected to continue smoking and were reassigned to a β-carotene placebo. Twenty-eight of the 31 subjects who were switched to a β-carotene placebo completed the 72-month follow-up. On average, these participants took β-carotene for approximately 64 months and β-carotene placebo for approximately 8 months (median, 7.8 months; range, 4.5–14.0 months). For purposes of analysis, these subjects were retained in the β-carotene treatment group to which they were originally assigned. Blood levels of β-carotene at 72 months in subjects reassigned to receive placebo were statistically significantly higher than their baseline readings (39.7 versus 14.2 μg/dL) ($P<.001$).

Treatment groups were compared with respect to the following potential confounders: age, sex, town of residence (Pasto versus Túquerres), baseline diagnosis, baseline *H. pylori* status, current smoking status, baseline levels of β-carotene, and average duration of treatment in months.

The hypothesis tested in this trial was that treating *H. pylori* infection and/or supplementing the diet with antioxidant micronutrients prevents the progression—i.e., favors no change or regression—of the gastric precancerous process. For each study participant, progression, no change, or regression status was defined by comparing outcome and baseline global histologic diagnoses in relation to the sequence of lesions that define the gastric premalignant process *(9):* normal histology, nonatrophic gastritis, atrophic gastritis, intestinal metaplasia, and dysplasia.

In subjects with a baseline diagnosis of multifocal atrophic gastritis without intestinal metaplasia, progression was defined as an outcome diagnosis of metaplasia, dysplasia, or cancer, while regression was defined as an outcome diagnosis of nonatrophic gastritis. In subjects with intestinal metaplasia, progression was defined as an outcome diagnosis of dysplasia or cancer, while regression was defined as an outcome diagnosis of multifocal atrophic gastritis or nonatrophic gastritis. In subjects with a baseline diagnosis of dysplasia, only regression rates were calculated because too few events (i.e., cancer) occurred to evaluate progression.

Statistical Analysis

A global chi-square test was performed to ascertain the overall treatment effect for histologic regression. Each treatment was compared with placebo. The placebo group was used as the comparison group because it is expected to represent the natural history of the disease over a 6-year period. All statistical tests were two-sided and were assumed to be statistically significant at the .05 level.

Because of the 2^3 factorial design, the assumption of additivity of the combination therapies was tested. There were three two-treatment combinations and one three-treatment combination. The effects of all treatment combinations were less than additive, one (β-carotene plus anti-*H. pylori* treatment) statistically significantly so ($P = .05$). The cumulative effects of the lack of additivity of all treatment combinations in a 2^3 factorial design produces a marked bias toward no effect when any main-effects model, either univariate or multivariate, is tested.

A polytomous multivariate logistic regression model *(24)* was used to ascertain which treatment, and treatment combinations, with covariates, jointly distinguished regression, no change, or progression. The statistical model was designed with the no-change group considered to be the baseline for parameter estimation; comparison between the regression and progression groups was made by assessing the difference between the logistic slope parameters.

Next, binary logistic regression was used to obtain the odds of histologic regression given progression/no change. Stepwise inclusion and exclusion decisions regarding covariates were based on the two-sided .05 level of significance. The final model contained all relevant covariates and provided risk estimates and their 95% confidence intervals (CIs).

Both main effects and full factorial models were considered. The final model from which relative risks are produced was the full factorial model. The lack of additivity of the combination therapies not only produces a poor fit of the main-effects model but also widens the CIs of the combination therapies. Not surprisingly, the larger the lack of additivity of a given combination therapy, the larger the CI.

Serum β-carotene and ascorbic acid levels are expressed as means ± standard deviations (SDs) and are compared with discrete points in time, baseline, and 72 months. Regression, no change, and progression rates were calculated and comparisons expressed as relative risks. Analyses were conducted separately by sex, but statistical power was limited. The proportions of subjects whose lesions progressed or regressed were not statistically significantly different between males and females, although the proportion with regressing lesions tended to be higher in males. Sex was included in all models as a covariate.

RESULTS

A total of 1219 volunteers were screened by endoscopy as potential candidates for study. Of these, 243 were determined at the preliminary histopathologic evaluation to be ineligible. After randomization, an additional seven refused to begin treatment, two were determined to have cancer on the rereview of the baseline biopsy specimens in New Orleans, and 115 were found to be ineligible on review of the preliminary diagnosis as shown in Fig. 1. Table 1 and Fig. 1 show the distributions of the 852 eligible (treated) subjects by age and sex and by treatment assignment and follow-up status, respectively. Two hundred twenty-one participants withdrew from the study before their 72-month evaluation: 102 quit treatment, 59 were lost to follow-up, 34 dropped out of the study because of pregnancy and other medical conditions, 18 died of causes unrelated to gastric cancer, and eight developed cancer other than gastric cancer. In one participant, the 72-month biopsy specimen was inadequate for histologic evaluation and determination of outcome. The average rate of loss was 4.3% per year over the 6-year trial. The dropout rate was influenced by two main issues: 1) the fact that the participants had to take treatment for a long time (6 years) and 2) the invasive procedure required (endoscopy). More than half of the dropouts occurred during the first 2 years of treatment. A total of 684 participants came to the 36-month biopsy; of those, 92% (631) came for the 72-month biopsy, a dropout rate of 2.6% per year for the last 3 years of the trial. The demographic characteristics of the subjects who withdrew from the study were compared with those who completed follow-up. No statistically significant differences were found, although a somewhat higher proportion were from Tuquerres than from Pasto. For those who completed the trial, there were no statistically significant differences among treatment groups in age, sex, time of follow-up, baseline diagnosis, current smoking status, and baseline serum β-carotene levels. A statistically nonsignificant difference was found in those completing the trial with respect to

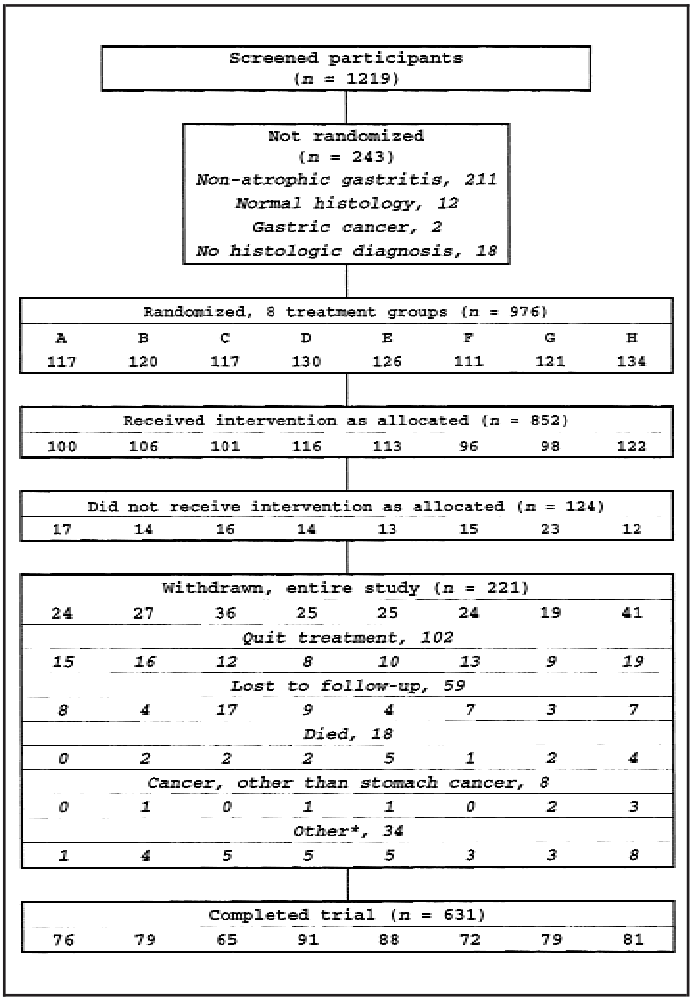

Fig. 1. Consort flow chart. Eight possible treatment combinations: **A**) placebo only, **B**) anti-*Helicobacter pylori* (HP), **C**) β-carotene (BC), **D**) ascorbic acid (AA), **E**) HP + BC, **F**) HP + AA, **G**) BC + AA, and **H**) HP + BC + AA. * = Pregnancy and other medical indications for withdrawal.

Table 1. Subjects screened, percent men, and mean age (years) by histology diagnosis*

	No. of subjects	% men	Mean age ± SD, y
Treated	852	46.1	51.1 ± 8.5
Multifocal nonmetaplastic atrophy	194	44.3	48.8 ± 8.3
Intestinal metaplasia	579	44.6	52.0 ± 8.6
Dysplasia	79	62.0	50.9 ± 8.1

*Subjects with baseline diagnosis of dysplasia were randomly assigned and treated but are not included in estimates of progression rates. SD = standard deviation.

town of residence, with a higher proportion of subjects from Tuquerres receiving placebo than other treatment groups (63.2% versus 44.0%; $P = .06$).

Estimation of treatment compliance based on pill count among participants who completed the study was high for all intervention modalities (mean compliance ± SD for ascorbic acid, 91.8% ± 7.3%; for β-carotene, 92.3% ± 8.8%; and for anti-*H. pylori* treatment, 99.1% ± 2.6%). Side effects were monitored closely, and none of clinical importance was found. Some patients on bismuth noted stool darkening, an expected but innocuous side effect. Table 2 compares serum levels of β-carotene and ascorbic acid and *H. pylori* infection status by treatment assignment at baseline, at 36 months, and at 72 months of follow-up. Baseline mean levels of serum β-carotene were not significantly different between subjects assigned to placebo or active ingredient ($P = .910$). The mean levels (log transformed and untransformed) increased significantly in subjects who received β-carotene supplement versus placebo at 36 months, and the difference was even more pronounced at 72 months ($P<.001$). Baseline levels of ascorbic acid are not available for comparison because methods used at baseline (colorimetric) were incompatible with those used later in the study (high-performance liquid chromatography). Nevertheless, the difference between mean levels in subjects who received ascorbic acid supplement versus placebo was statistically significantly higher at both the 36- and the 72-month evaluations ($P<.001$). The difference in *H. pylori* infection at baseline was not statistically significant ($P = .466$), but it was highly significant at 72 months ($P<.001$).

Table 3 compares the baseline diagnosis with the outcome (72 months) diagnosis. The diagonal (bold numbers) represents subjects with no change (identical baseline and outcome diagnosis). To the left of the diagonal are subjects whose gastric lesions regressed and to the right those whose lesions progressed during the 6 years of observation. Similar tables were prepared for each intervention category; for brevity they are not presented here.

Table 2. Serum β-carotene and ascorbic acid levels and *Helicobacter pylori* status by treatment assignment and follow-up time*

	Serum β-carotene, μg/dL				
	Active		Placebo		
	No.	Mean ± SD	No.	Mean ± SD	*P*†
Baseline	405	23.9 ± 18.1	368	24.0 ± 32.6	.910
36 mo	299	136.1 ± 122.7	287	31.5 ± 44.1	<.001
72 mo	271	359.2 ± 311.1	271	35.5 ± 36.2	<.001
	***Serum ascorbic acid, mg/dL*‡**				
	Active		Placebo		
	No.	Mean ± SD	No.	Mean ± SD	*P**
36 mo	287	1.50 ± 0.51	289	1.20 ± 0.34	<.001
72 mo	265	1.27 ± 0.33	272	1.04 ± 0.33	<.001
	H. pylori *status*				
	Treated		Not treated		
	No.	% negative	No.	% negative	*P*§
Baseline	387	3.6	386	4.7	.466
36 mo	300	52.3	310	4.8	<.001
72 mo	285	74.0	283	15.2	<.001

*SD = standard deviation.
†Independent samples: Student's *t* test.
‡Baseline ascorbic acid levels not available.
§Chi-square test.

Table 3. Number of subjects by baseline and outcome (72 months) diagnoses*

	Outcome					
Baseline	NAG	MA	IM	Dysplasia	Cancer	Total
MA	36	**61**	45	1	0	143
IM	31	47	**254**	88	4	424
Dysplasia	1	4	31	**26**	1	63
Total	68	112	330	115	5	630

*NAG = nonatrophic gastritis, MA = multifocal nonmetaplastic atrophy, and IM = intestinal metaplasia. **Bold values** = subjects with no change.

The initial analysis compared active treatment and placebo with histologic progression, no change, and regression. Fig. 2 shows the 6-year rates of progression, no change and regression for the eight treatment categories. A clear pattern emerges: Regression rates in the nonintervention (placebo) category are less than half of those in each intervention group, and there are no statistically significant differences ($P \geq .05$ for all comparisons) between single and combined treatments. The proportions of those with no change and progression are similar across treatment groups. This analysis showed that the primary effect of treatment was to increase the number of participants who show histologic regression of gastric lesions.

Multivariate analysis by use of polytomous and binary logistic regression shows that the participants on single intervention agents were, on average, three times more likely to have histologic regression than the subjects on placebo. Subjects on combination therapy were three to five times more likely to have histologic regression; however, some of the combinations were not statistically significant. The effect of the two-drug combination therapy is not additive but roughly the same as the one-drug effect. Regression was more marked in the antrum, but it is not clear if this finding is a biologic phenomenon or a reflection of the sampling because a larger number of biopsy specimens were obtained from the antrum.

The covariates sex, age, town of residence, and smoking were not statistically significantly related to outcome ($P>.05$) and were not included in the final model. Baseline diagnosis, atrophy, or intestinal metaplasia was the initial stratification variable and was, therefore, included in the final model. The magnitude of the difference in regression rates between treated and untreated subjects is greater in subjects with a baseline diagnosis of atrophy than those with a baseline diagnosis of metaplasia, but the direction of the differences is the same. The inclusion of the baseline diagnosis in the final model increased slightly (0.1–0.2) the relative risks of the single treatment effects.

Multifocal Nonmetaplastic Atrophy

The proportions of subjects with progression and regression after a baseline diagnosis of atrophy without intestinal metaplasia for each treatment category are shown in Fig. 2. The relative risks and CIs for histologic progression and regression in the multivariate model are shown in Table 4. When compared with subjects on placebo, subjects on single treatments are approxi-

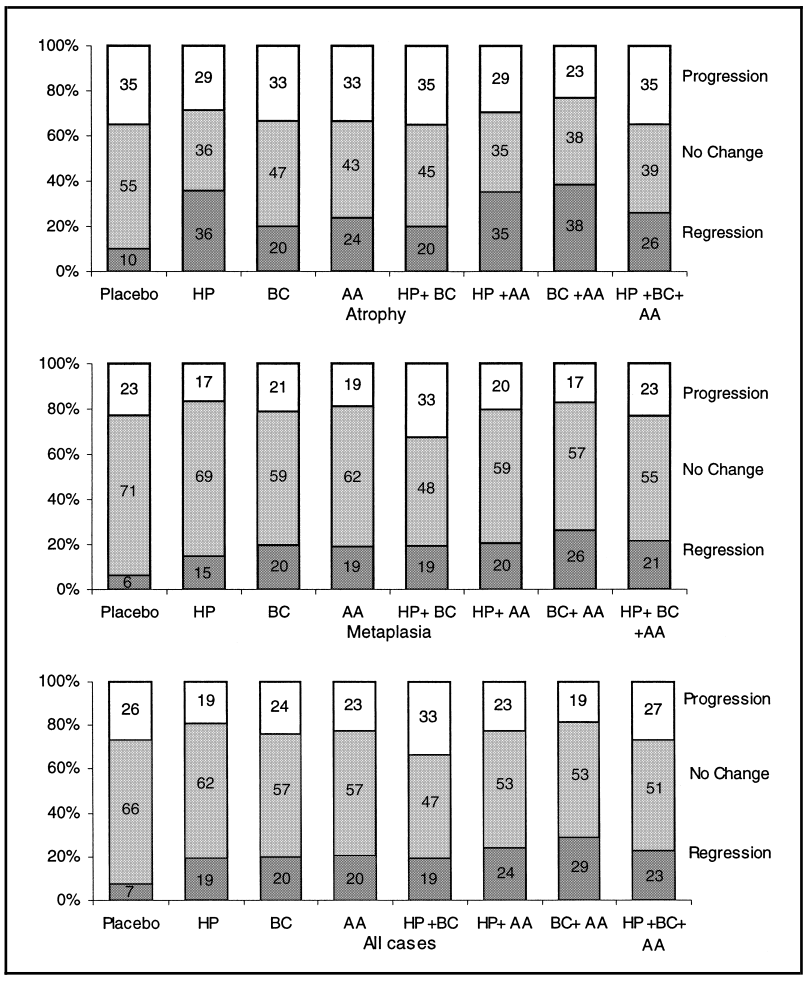

Fig. 2. Rates of progression, no change, and regression for this 6-year interval for each intervention. **Upper set**—case subjects with baseline diagnosis of multifocal nonmetaplastic atrophy; **middle set**—subjects with intestinal metaplasia; and **lower set**—all participants. HP = anti-*Helicobacter pylori* treatment; BC = β-carotene supplementation; and AA = ascorbic acid supplementation.

mately five times more likely to have histologic regression. All combinations statistically significantly increase regression. Removing from these calculations the smokers on β-carotene who were reassigned to placebo did not change the results.

Intestinal Metaplasia

The progression and regression rates in subjects with a baseline diagnosis of intestinal metaplasia can be seen in Fig. 2. The relative risks of histologic progression and regression as well as the CIs are shown in Table 5. All interventions resulted in a reduced risk of progression, statistically significant only for anti-*H. pylori* treatment (CI does not include 1.0). In a separate analysis taking into account changes in the extent of atrophy and in the type of metaplasia (complete versus incomplete), the three basic interventions resulted in statistically significantly decreased rates of progression ($P<.05$). Compared with those on placebo, participants on single treatments were approximately three times more likely to have histologic regression ($P<.05$). There were no additive effects of the combination treatments. The effects of the combination of antibacterial therapy with β-carotene supplementation, as well as triple-agent combination, were not statistically significant ($P>.05$).

Dysplasia

In the great majority of subjects with a diagnosis of dysplasia, the lesion was very mild. By present international standards, they would fall into the category of "indefinite for dysplasia." They should not, therefore, be considered directly comparable to patients diagnosed in clinical settings. This finding does, however, indicate a more advanced stage of the metaplastic process. Regression proportions for subjects with a baseline diagnosis of dysplasia did not show statistically significant differences among treatment categories, most probably because of small numbers. High proportions of patients with regression (33%–71%) were observed in most categories, which could reflect problems in histologic classification. Special attention was given to separate inflammatory and hyperplastic changes, which may be related to the resolution of the *H. pylori* infection, from architectural alterations associated with dysplasia. High proportions with regression were observed among those patients treated as well as those not treated for the infection and for those who cleared or did not clear the infection.

Cure of the Infection

The effect of *H. pylori* clearance on histologic regression was highly dependent on baseline diagnosis. Among those with nonmetaplastic atrophy at baseline, 40.7% (24 of 59) of those who cleared the bacteria showed histologic regression compared with

Table 4. Relative risks of regression and progresion from the final multivariate model for multifocal nonmetaplastic atrophy*

	Regression		Progression	
Treatment	RR	95% CI	RR	95% CI
Placebo	1.00	Referent	1.00	Referent
Anti-*Helicobacter pylori*	4.8	1.6–14.2	0.8	0.4–1.9
β-carotene	5.1	1.7–15.0	1.0	0.5–2.3
Ascorbic acid	5.0	1.7–14.4	1.0	0.5–2.3
Anti-*H. pylori* + β-carotene	4.7	1.2–18.5	1.8	0.7–6.8
Anti-*H. pylori* + ascorbic acid	6.3	1.6–24.3	1.1	0.4–4.2
Ascorbic acid + β-carotene	8.3	2.2–31.5	0.9	0.3–2.8
All treatments	5.8	1.0–33.7	1.3	0.1–3.3

*RR = relative risk; CI = confidence interval.

Table 5. Relative risks of regression and progresion from the final multivariate model for intestinal metaplasia*

	Regression		Progression	
Treatment	RR	95% CI	RR	95% CI
Placebo	1.00	Referent	1.00	Referent
Anti-*Helicobacter pylori*	3.1	1.0–9.3	0.4	0.2–0.9
β-carotene	3.4	1.1–9.8	0.5	0.2–1.1
Ascorbic acid	3.3	1.1–9.5	0.5	0.2–1.1
Anti-*H. pylori* + β-carotene	3.1	0.8–12.1	0.9	0.3–2.7
Anti-*H. pylori* + ascorbic acid	4.1	1.1–15.9	0.5	0.2–1.7
Ascorbic acid + β-carotene	5.4	1.4–20.6	0.4	0.1–1.4
All treatments	3.8	0.7–22.1	0.6	0.1–3.2

*RR = relative risk; CI = confidence interval.

14.3% (12 of 84) of those who still tested positive at the 72-month biopsy ($P = .001$). There was no statistically significant difference among those whose baseline diagnosis was metaplasia ($P>.05$). If the same analysis is done only for the patients who received anti-*H. pylori* treatment, then the proportions are 38% (19 of 50) for atrophy cleared and 8.3% (two of 24) for atrophy positive ($P = .008$) for the difference in proportions. In a multivariate model, patients who cleared *H. pylori* and had atrophy were 8.7 times more likely to show histologic regression (95% CI = 2.7–28.2). Patients with metaplasia were 5.4 times more likely to show histologic regression (95% CI = 1.7–17.6).

Discussion

The possible role of eradication of *H. pylori* infection in preventing gastric cancer is a major focus of attention at the present time. On the basis of extensive epidemiologic evidence, in particular, three independent nested case–control studies showing elevated cancer risk years after documented infection, the International Agency for Research on Cancer *(1)* in 1994 classified *H. pylori* infection as a cause of gastric cancer. Experimental evidence lacking at that time has been recently provided utilizing the Mongolian gerbil model. Gastric cancer is the second most common fatal cancer in the world *(25),* and the 5-year survival rates are under 20% in most countries *(26).* Prevention, therefore, has considerable public health potential.

In our study, comparison of proportions of subjects with progression, no change, and regression for the 6-year interval showed that, in subjects with intestinal metaplasia, anti-*H. pylori* treatment statistically significantly decreases the number with progression and increases the number with regression. In subjects with nonmetaplastic atrophy, all interventions studied resulted in statistically significant increases in the proportion with histologic regression. These findings suggest that the decades-long precancerous process provides the opportunity for prevention of the progression to its advanced stages, namely, dysplasia and carcinoma.

Curing the infection has a beneficial effect in preventing the advance of the gastric precancerous process. The 74% cure rate achieved demonstrates that, in developing populations of low socioeconomic status and with high prevalence of infection, bismuth-based triple therapy followed by treatment of those failing to respond to bismuth therapy with proton pump inhibitors and clarithromycin can be highly beneficial.

It is also noteworthy that, in our trial, approximately 15% of the subjects infected with *H. pylori* cleared their infection without having had documented anti-*H. pylori* therapy. This phenomenon is being reported in other populations as well as in some children of low socioeconomic strata *(27).* Although the causes of such "spontaneous" clearing of the infection are unknown, widespread use of antibiotics for other infections is suspected to play a role.

Unlike the majority of trials *(23,28)* that have found little or no beneficial effect of β-carotene for chemoprevention of cancers of the lung or esophagus, our study suggests a benefit in retarding progression of premalignant gastric lesions. This finding is consistent with that of the large general population trial conducted in Linxian, China *(29).* There, combined supplementation with β-carotene plus selenium plus α-tocopherol resulted in a statistically significantly reduced mortality from stomach cancer (relative risk = 0.79; 95% CI = 0.64–0.99). Although the end points in the two trials—the current trial and the Linxian trial—differ and although, in the Linxian trial, it was not possible to assess the effect of β-carotene alone, each trial was conducted in a population at high risk of gastric cancer, and their results suggest that supplementation with β-carotene in high-risk populations merits further examination.

Since the initiation of this trial, numerous reports *(12,30,31)* have documented an inverse relationship between *H. pylori* infection and ascorbic acid concentration in gastric juice; specifically, clearance of infection was accompanied by a statistically significant increase in gastric ascorbic acid. On the basis of this work, one might expect that a beneficial effect of ascorbic acid supplementation, if any, would be observed in individuals treated for *H. pylori.* A statistically significant increase in histologic regression of premalignant lesions was observed in our study for ascorbic acid supplementation, regardless of the *H. pylori* status.

To address the prevention issue, at least 10 prevention trials are under way in Europe, Japan, China, and Latin America *(6).* Five of these trials utilize cancer as the end point, and five are assessing the progression of the precancerous process by means of intermediate end points. To our knowledge, this study describes the first intervention completed and reported. Our results favor the notion that eradication of *H. pylori* infection is a promising option for the prevention of gastric cancer in high-risk populations.

Less industrialized, rural populations offer special opportunities for chemoprevention research. Access and follow-up are greatly facilitated by the low rate of migration and minimal temporal changes in lifestyle. Our study indicates that, in such populations, adequate planning and resources may make such studies feasible and economic. Our findings are relevant to populations of low socioeconomic strata with very high gastric cancer rates and very high prevalence of *H. pylori* infection. They may be less relevant to populations with low gastric cancer risk, lower infection prevalence, and different nutritional status. It is well known that infection of the gastric mucosa with *H. pylori* may lead to a wide variety of outcomes. Most infections are subclinical and do not lead to demonstrable gastroduodenal pathologic abnormalities *(32).* In others, the infection is associated with nonatrophic, predominantly antral, gastritis and duodenal ulcer, which are not associated with an increased risk of gastric cancer *(33).* In the Colombian (Nariño) population, the infection frequently leads to atrophy and intestinal metaplasia, with high risks of dysplasia and cancer *(7,9).*

The mechanisms underlying the differences in outcome of *H. pylori* infection are poorly understood. It appears that the host reaction to the infection is very complex, and in high-risk populations a decades-long process takes place. It has been hypothesized that many sequential events participate in the process, some of which are identifiable by means of histopathology and immunohistochemistry *(20).* Our study documents that both progression and regression events take place during the prolonged precancerous process. It also demonstrates that intervention strategies can decrease progression and enhance regression. Intervention should result in slowing the precancerous process and preventing the development of cancer in some individuals. The eventual outcome in a given individual probably depends on the interaction between the biologic forces controlling progression and regression over many years. Our study indicates that these competing biologic forces may differ in their influence on the precancerous process according to the stage of the process at

which intervention is initiated. In early stages of multifocal atrophic gastritis and intestinal metaplasia, curing the *H. pylori* infection and supplementing the diet with antioxidants have clear beneficial effects. In our study, such beneficial effects were not detected at the (late) stage of dysplasia.

Methodologic problems arising from the multifocality of the lesions were addressed in several ways. The treatment randomization and independent reading of the slides were designed to minimize such bias. In addition, biopsy specimens were taken from areas in the gastric mucosa identified in previous studies as having the highest probability of harboring the most advanced lesions. In contrast to the situation observed in northern European populations, in this study, most of the atrophic, metaplastic, and dysplastic lesions are in the gastric antrum, especially in the area of the incisura angularis. This topographic distribution of lesions is applicable to all high-risk populations not of northern European extraction, including China, Japan, and Latin America *(8,34)*.

In our population, dysplasia was diagnosed more frequently than in clinical series. The diagnosis was based on architectural and cellular abnormalities independent of the inflammatory infiltrate. The great majority represented mild dysplasias, which would not be reported as such in a clinical setting, in which such a diagnosis may have led to clinical intervention. In the new international classification, those cases would have been classified as "indefinite for dysplasia" *(35);* they may represent a step between intestinal metaplasia and dysplasia. The great majority (112 of 115) of the cases classified as dysplasia were accompanied by areas of incomplete metaplasia. Both groups may represent stages between metaplasia and severe dysplasia. In the present series, as in previous observations in the same population, "spontaneous" regression of dysplastic lesions is a frequent observation *(9)*.

The interpretation of our finding requires consideration of their generalizability. The possible international differences in the magnitude of the cancer risk and in the prevalence of infection have been mentioned. In the population under study, the age at first infection is very young: 50% prevalence at age 2 years and 90% at age 9 years *(36)*. Young age at *H. pylori* infection has been suggested as a risk factor for cancer *(37)*. Infections that do not increase cancer risk, such as those leading to duodenal ulcer, may have occurred at a later age. The genotype and dose of infective bacteria, the nutritional status of the host, and the presence of comorbid conditions may also determine the outcome of infection and may, therefore, have relevance to prevention strategies *(8,32)*.

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