

Randomized, Placebo-Controlled, Esophageal Squamous Cell Cancer Chemoprevention Trial of Selenomethionine and Celecoxib

PAUL J. LIMBURG,* WENQIANG WEI,[†] DENNIS J. AHNEN,[§] YOULIN QIAO,[†] ERNEST T. HAWK,[¶] GUOQING WANG,[†] CAROL A. GIFFEN,^{||} GUIQI WANG,[†] MARK J. ROTH,[¶] NING LU,[†] EDWARD L. KORN,[¶] YURONG MA,[#] KATHLEEN L. CALDWELL,** ZHEIWEI DONG,[†] PHILIP R. TAYLOR,[¶] and SANFORD M. DAWSEY[¶]

*Mayo Clinic College of Medicine, Rochester, Minnesota; [†]Cancer Institute, Chinese Academy of Medical Sciences, Beijing, People's Republic of China; [§]University of Colorado, Denver, Colorado; [¶]National Cancer Institute, Bethesda, Maryland; ^{||}Information Management Services, Inc, Silver Spring, Maryland; [#]Dalian Medical College, Dalian, People's Republic of China; and **Centers for Disease Control and Prevention, Atlanta, Georgia

Background & Aims: Esophageal squamous cell carcinoma remains a leading cause of cancer death worldwide. Squamous dysplasia, the accepted histological precursor for esophageal squamous cell carcinoma, represents a potentially modifiable intermediate end point for chemoprevention trials in high-risk populations.

Methods: We conducted a randomized, controlled trial of selenomethionine 200 µg daily and/or celecoxib 200 mg twice daily (2 × 2 factorial design) among residents of Linxian, People's Republic of China. Subjects had histologically confirmed mild or moderate esophageal squamous dysplasia at baseline. Esophagogastroduodenoscopy was performed before and after a 10-month intervention. Per-subject change (regression, stable, or progression) in the worst dysplasia grade was defined as the primary end point. Results were compared by agent group (selenomethionine vs placebo; celecoxib vs placebo). **Results:** Two hundred sixty-seven subjects fulfilled all eligibility criteria, and 238 (89%) completed the trial. Overall, selenomethionine resulted in a trend toward increased dysplasia regression (43% vs 32%) and decreased dysplasia progression (14% vs 19%) compared with no selenomethionine ($P = .08$). In unplanned stratified analyses, selenomethionine favorably affected a change in dysplasia grade among 115 subjects with mild esophageal squamous dysplasia at baseline ($P = .02$), but not among 123 subjects with moderate esophageal squamous dysplasia at baseline ($P = 1.00$). Celecoxib status did not influence changes in dysplasia grade overall ($P = .78$) or by baseline histology subgroup. **Conclusions:** After a 10-month intervention, neither selenomethionine nor celecoxib inhibited esophageal squamous carcinogenesis for all high-risk subjects. However, among subjects with mild esophageal squamous dysplasia at baseline, selenomethionine did have a protective effect. Although it is based on unplanned stratified analyses, this finding is the first report of a

possible beneficial effect for any candidate esophageal squamous cell carcinoma chemopreventive agent in a randomized controlled trial.

Esophageal cancer is the sixth most common cause of cancer death in the world, with more than 400,000 new cases diagnosed each year.¹ Because symptoms typically remain absent until late in the course of disease, most esophageal cancer patients present with advanced-stage tumors. According to data from the US Surveillance, Epidemiology, and End Results Program, only approximately 1 in 5 esophageal cancer patients survives ≥ 3 years beyond the date of the initial diagnosis.^{2,3} To reduce the public health burden associated with esophageal cancer, investigation of novel prevention strategies, such as chemoprevention, has been advocated among high-risk patient populations.^{4,5}

Although subtype-specific incidence patterns are shifting in some global regions, esophageal squamous cell carcinoma (ESCC) is substantially more common than esophageal adenocarcinoma worldwide.^{1,2,6} In Linxian, People's Republic of China (PRC), ESCC rates are among the highest in the world, exceeding 100 deaths per 100,000 population annually.⁷ Previous data from Linxian have shown that squamous dysplasia is a strong predictor of ESCC risk in this population.^{8,9} Because squamous dysplasia can be accurately visualized and targeted for biopsy by mucosal iodine staining during

Abbreviations used in this paper: CI, confidence interval; COX, cyclooxygenase; EGD, esophagogastroduodenoscopy; ESCC, esophageal squamous cell carcinoma; PRC, People's Republic of China; RR, relative risk.

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esophagogastroduodenoscopy (EGD),¹⁰ this lesion represents an excellent surrogate end point biomarker for the initial assessment of candidate chemoprevention agents.

Currently, the standard of care for treating patients with esophageal squamous dysplasia in Linxian is based on the histological grade of the disease. Histologically severe lesions are usually treated either by endoscopic resection (mucosectomy) or by thermal ablation (argon plasma coagulation), whereas mild or moderate lesions are generally monitored by observation only. In this context, we designed a chemoprevention trial to assess the effects of 2 promising agents—selenomethionine (a synthetic form of organic selenium) and celecoxib (a selective inhibitor of cyclooxygenase [COX]-2)—among asymptomatic adults with histologically confirmed mild or moderate grades of esophageal squamous dysplasia. Selenium compounds^{11–20} and COX-2 inhibitors^{21–25} have shown potential chemopreventive effects on esophageal carcinogenesis in previous observational studies and multinutrient intervention trials. However, these agents have not been directly evaluated in ESCC chemoprevention trials.

Materials and Methods

This randomized, double-blind, placebo-controlled chemoprevention trial was approved by the institutional review boards of the US National Cancer Institute and the Cancer Institute, Chinese Academy of Medical Sciences. Informed consent was obtained from all study subjects before enrollment. An impartial Medical Monitor received all reports of grade 3 or 4 adverse events and adjudicated their potential association to the intervention agents. Additionally, an independent Data and Safety Monitoring Board reviewed the trial data at 6-month intervals, beginning 3 months before the baseline evaluation and continuing until 11 months after the intervention period ended.

Subject Recruitment and Screening Evaluation

Potential subjects were recruited in Rencun Commune, in the northern part of Linxian County, beginning in November 1998. Local health officials and investigators from the Cancer Institute of the Chinese Academy of Medical Sciences went to each village, met with the residents, and described the details of the chemoprevention trial. At the end of each village meeting, residents who agreed to undergo an initial screening evaluation were registered as potential subjects. On the basis of a combination of registration rates and geographic locales, 8 villages were chosen for the screening evaluation. Two thousand two hundred thirteen residents, aged 26–73 years, from these 8 villages underwent a screening EGD with mucosal iodine staining in April 1999. Participants who had at least 1 grossly visible lesion with biopsy-proven

mild or moderate dysplasia, according to histological interpretation by a single pathologist, were potentially eligible for the chemoprevention trial. Trial participation was limited to a maximum of 600 subjects because of practical limitations regarding the volume of endoscopic procedures that could be performed in a timely fashion at the field site outpatient clinic. All patients with moderate dysplasia were invited to enroll in the study. Patients with mild dysplasia were then enrolled until the preset threshold of 600 subjects was achieved.

Baseline Evaluation

Baseline evaluations were conducted in October 1999, 6 months after the initial screening examinations. Willing participants completed a questionnaire, focused physical examination, venipuncture collection, and confirmatory EGD and agreed to abstain from chronic nonsteroidal anti-inflammatory drug (NSAID) use (defined as ≥ 3 times per week for > 2 weeks) while on study (aspirin ≤ 100 mg/day was allowed, and acetaminophen was made available for analgesic use as needed). Women of childbearing potential (defined as premenopausal or < 2 years postmenopausal and not surgically sterile) documented a negative pregnancy test and agreed to use adequate contraceptive methods during the trial. Inclusion criteria for the study required that all subjects must have a grossly visible lesion with histologically confirmed mild or moderate squamous dysplasia (worst histological diagnosis) at the baseline EGD. Exclusion criteria consisted of a history of cancer (except nonmelanoma skin cancer), symptoms suggestive of an upper gastrointestinal tract malignancy, recently treated peptic ulcer disease, or contraindications to the intervention agent(s) or study-related procedures. Subjects were also excluded if a grossly visible lesion could not be confirmed during the baseline EGD or if the worst histological diagnosis at baseline was less than mild or greater than moderate dysplasia. Because full tissue processing facilities were not available at the field site, documentation of mild or moderate dysplasia at baseline was not possible until biopsy samples were histologically reviewed after randomization.

Randomization and Intervention

Intervention groups were randomly assigned and stratified by sex by using a variable-block approach with an allocation ratio of 1:1:1:1—active selenomethionine and active celecoxib (group 1)/placebo selenomethionine and active celecoxib (group 2)/active selenomethionine and placebo celecoxib (group 3)/placebo selenomethionine and placebo celecoxib (group 4). The random allocation sequences by sex were generated by the US data-coordinating center before the baseline evaluation. After all of the baseline evaluations were complete, final intervention group assignments were performed on site by data-coordinating center staff. The masked code linking agent bottles to intervention group assignments was retained by data-coordinating center staff and concealed from all but the study statisticians until completion of the study analyses. All subjects began intervention on the same day in December 1999 and continued for 10 months. Doses for the active agents

were 200 μg of selenium as selenomethionine once per day and celecoxib 200 mg twice per day.

On-Study Evaluations, Compliance Monitoring, and Adverse Events

An interim evaluation was performed 5 months after the intervention began, at which time subjects completed a focused physical examination and an electrocardiogram. The end-of-trial evaluation was performed 10 months after the intervention began and consisted of a follow-up questionnaire, focused physical examination, venipuncture collection, electrocardiogram, and third EGD.

To ensure proper compliance and adverse event monitoring in this rural area, 30 local health care providers (village doctors) and 3 quality-control monitors were selected and trained to participate in this study. Each village doctor was responsible for approximately 12 subjects. The quality control monitors made unannounced visits to both the subjects and the village doctors to confirm the reported information.

Compliance was assessed by pill counts and by direct observation by the village doctors, who watched all subjects take their 2 morning pills each day throughout the intervention period. Compliance was further assessed biochemically by comparing baseline and end-of-trial serum selenium concentrations. Selenium analyses were performed at the inorganic toxicology laboratory of the Centers for Disease Control and Prevention by using inductively coupled plasma–mass spectrometry protocols established for the National Health and Nutrition Examination Survey.²⁶

Throughout the intervention period, adverse events and concomitant medications were recorded at least weekly. Whenever possible, adverse events were graded according to the National Cancer Institute's Common Toxicity Criteria, version 2.0 (modified slightly to reflect differences in Chinese reference ranges for hemoglobin concentration, leukocyte count, and platelet count). Otherwise, adverse events were graded according to their effect on the subject's ability to perform usual activities: no limitation, grade 1; some limitation, grade 2; inability to perform, grade 3; and life-threatening or fatal event, grade 4. All adverse events were followed up according to sound medical practice.

Endoscopic Examinations and Tissue Processing

Screening, baseline, and end-of-trial EGDs were performed at the field site outpatient clinic with Pentax videoendoscopy equipment (Pentax Precision Instrument Corporation, Orangeburg, NY). After initial inspection, the esophageal mucosa was sprayed with 1.2% Lugol's iodine solution, after which dysplastic lesions appear unstained relative to the surrounding normal mucosa.¹⁰ Unstained lesions ≥ 5 mm in diameter were recorded by site in centimeters from the incisors and biopsied for histological assessment according to the following protocol: 5–19 mm, 1 biopsy sample; 20–39 mm, 2 biopsy samples; and ≥ 40 mm, 3 biopsy samples. One additional biopsy sample was obtained from macroscopically nor-

mal mucosa located at least 10 mm away from any grossly visible lesion. Biopsy samples were immediately fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned in 5- μm thicknesses, and stained with H&E. The biopsy slides from the baseline and end-of-trial endoscopy examinations of each patient were paired and reviewed together, with the dates of the examinations masked. The slides were independently reviewed by 2 blinded gastrointestinal pathologists who were unaware of the intervention group assignments. Cases with discrepant results were adjudicated by a third blinded gastrointestinal pathologist.

Primary End Point

The primary end point was defined as the change in histological grade of squamous dysplasia (determined by comparing the most advanced histological diagnosis for each subject at the baseline and end-of-trial evaluations) and was categorized as regression, stable, or progression. For subjects whose most advanced histological diagnosis at baseline was mild dysplasia, regression was defined as no evidence of dysplasia, and progression was defined as moderate dysplasia, severe dysplasia, or invasive cancer at the end-of-trial evaluation. For subjects whose most advanced histological diagnosis at baseline was moderate dysplasia, regression was defined as no evidence of dysplasia or mild dysplasia, and progression was defined as severe dysplasia or invasive cancer at the end-of-trial evaluation. For all subjects, stable disease was defined as no change in the most advanced histological diagnosis between the baseline and the end-of-trial evaluations. Because a substantial number of the dysplastic lesions identified in this subject cohort were either irregularly shaped or linear in appearance at endoscopy, "dysplasia area" could not be reliably assessed and was therefore not included as a trial end point.

Power Calculation and Statistical Analysis

This complex, first-ever chemoprevention trial in Linxian, PRC, was limited to a maximum of 600 participants for logistic reasons. Power was calculated over a range of anticipated initial sample sizes (240–600 randomized subjects), as specified in the study protocol. We further estimated that approximately 20% of the randomized subjects would later be withdrawn because of false inclusion (ie, found not to satisfy all entry criteria after postrandomization review of prandomization materials, including baseline esophageal biopsy samples) or missing data (ie, failure to complete the end-of-trial evaluation), resulting in an a priori expectation that the final analytic cohort size would range from 192 to 480 subjects. Power calculations showed $\geq 90\%$ power to detect a very large true difference in the combined primary end point (regression from 20% to 35% coupled with progression from 16% to 4%) throughout this range of analytic cohort sizes. To detect a smaller true difference in the combined primary end point (regression from 20% to 30% combined with progression from 16% to 8%), the power was 57% for an analytic cohort of 192, 79% for a cohort of 336, and 91% for a cohort of 480 subjects.

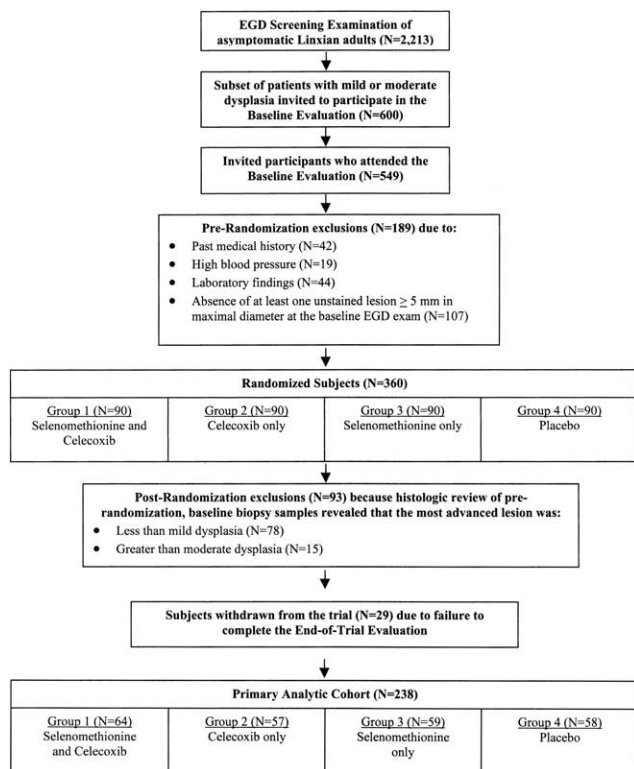


Figure 1. Trial flow diagram.

The primary data analyses were based on the intention-to-treat principle.²⁷ Because determination of trial eligibility was based on tissue samples obtained before randomization for each potential subject, the final analytic cohort included all subjects with histologically confirmed mild or moderate dysplasia at baseline who completed the end-of-trial evaluation. Randomized subjects who were later withdrawn because of false inclusion or missing end-of-trial data were excluded blind to the trial outcome and before the final data analyses. Of note, eligible randomized subjects who were excluded as a result of missing end-of-trial data were equally distributed across the originally assigned intervention groups, so it is unlikely that these exclusions biased the reported trial results. The primary end point was compared by selenium treatment status (active vs placebo, ie, intervention groups 1 and 3 vs intervention groups 2 and 4) and celecoxib treatment status (active vs placebo, ie, intervention groups 1 and 2 vs intervention groups 3 and 4) by using a 2-sample permutation *t* test with codes of -1 for regression, 0 for stable disease, and 1 for progression.²⁸ We also performed analyses stratified by baseline histology, although this was not prespecified in the study protocol. Proportions were compared by using the Fisher exact test. All reported *P* values are 2 sided and are unadjusted for multiple comparisons.

Results

A schematic overview of subject recruitment and retention at key study time points is shown in Figure 1.

Of the 600 patients originally invited to participate in the chemoprevention trial, 549 (92%) attended the baseline evaluation. Before randomization, 189 patients were excluded on the basis of their medical history ($n = 42$), high blood pressure ($n = 19$), laboratory findings ($n = 44$), and/or the absence of at least 1 grossly visible lesion ≥ 5 mm in maximal diameter at the baseline EGD ($n = 107$). Some patients were excluded for multiple reasons. The remaining 360 subjects were stratified by sex and randomly assigned to 1 of the 4 intervention groups. Baseline characteristics of the randomized cohort are provided in Table 1 by intervention group.

After randomization, 93 subjects were withdrawn from analysis because, after blinded review of their pre-randomization baseline biopsy samples, the most advanced histological diagnosis was either less than mild dysplasia (ie, no evidence of dysplasia; $n = 78$) or greater than moderate dysplasia (ie, severe dysplasia or carcinoma; $n = 15$). These randomized subjects who were withdrawn because of false inclusion included 21 in group 1, 23 in group 2, 22 in group 3, and 27 in group 4. After these postrandomization exclusions, 267 subjects fulfilled all entry criteria.

Twenty-nine study participants (11%) did not complete the end-of-trial evaluation, including 5, 10, 9, and 5 from intervention groups 1, 2, 3, and 4, respectively. The primary analytic cohort therefore consisted of 238 subjects. Within the analytic cohort, a mean of 4.8 biopsy samples (range, 2–14) were obtained from each subject at baseline, and histological review of these samples showed that the most advanced histological diagnosis was moderate dysplasia in 123 of 238 (52%) subjects and mild dysplasia in 115 of 238 (48%) subjects. Age, sex, body mass index, tobacco use, alcohol consumption, serum selenium concentration, the mean number of biopsy samples, and the distribution of dysplasia grades at baseline were comparable across the intervention groups (Table 2). A mean of 6.5 biopsy samples (range, 2–11) were obtained from each subject at the end-of-trial EGD, and these numbers were comparable across intervention groups as well.

Compliance

Compliance with the single daily dose of selenomethionine (or placebo) and 1 of the 2 daily doses of celecoxib (or placebo) was in excess of 99% by both direct observation and pill counts. No subjects reported any aspirin use while taking the study agent, although 13 of 267 (5%) subjects did report use of traditional Chinese remedies containing varying amounts of other NSAIDs during the intervention period (for ≤ 1 week on each occasion).

Table 1. Baseline Characteristics of the Randomized Cohort (n = 360) by Intervention Group

Variable	Group 1 (n = 90) (selenomethionine and celecoxib)	Group 2 (n = 90) (celecoxib only)	Group 3 (n = 90) (selenomethionine only)	Group 4 (n = 90) (placebo)
Age (y)				
Mean (SD)	47 (6.6)	48 (6.4)	47 (5.6)	48 (6.2)
Range	34–67	36–68	35–64	37–68
Sex				
Women, n (%)	53 (59)	52 (58)	52 (58)	52 (58)
Body mass index (kg/m ²)				
Mean (SD)	22.0 (3.2)	22.0 (2.3)	22.5 (2.6)	22.5 (2.7)
Range	15.6–38.5	16.9–28.4	16.8–29.2	17.3–31.1
Tobacco use				
Ever, n (%)	26 (29)	22 (24)	23 (26)	26 (29)
Alcohol consumption				
Yes, n (%)	45 (50)	42 (47)	42 (47)	36 (40)

SD, standard deviation.

Serum selenium concentrations were measured to confirm the selenomethionine compliance data obtained by direct observation. The median serum selenium concentration in the primary analytic cohort was 82 µg/L (interquartile range, 70–99 µg/L) at baseline, with comparable distributions for each intervention group (Table 2). After the 10-month intervention, the median serum selenium concentration increased from 83 to 201 µg/L (142%) among subjects assigned to receive active selenomethionine and from 80 to 83 µg/L (4%) among subjects assigned to receive placebo selenomethionine. In light of these data, additional expenditure of limited serum resources to confirm the celecoxib compliance data was not pursued. Celecoxib treatment status had no

appreciable effect on the change in serum selenium concentration (data not shown).

Adverse Events

Adverse events were reported by 193 of 267 (72%) subjects (Table 3) and ranged in number from 1 to 5 per subject. The proportions of patients who reported any adverse event were not associated with either selenomethionine or celecoxib status ($P > .05$ for each comparison). Most (68%) reported adverse events were asymptomatic grade 1 laboratory abnormalities, including 137 reports of grade 1 serum creatinine levels (81–128 µmol/L; 1.0–1.5 times the upper limit of normal). There were 2 grade 2 labora-

Table 2. Baseline Characteristics of the Analytic Cohort (n = 238) by Intervention Group

Variable	Group 1 (n = 64) (selenomethionine and celecoxib)	Group 2 (n = 57) (celecoxib only)	Group 3 (n = 59) (selenomethionine only)	Group 4 (n = 58) (placebo)
Age (y)				
Mean (SD)	48 (6.8)	48 (6.4)	48 (5.6)	48 (6.3)
Range	34–67	36–68	37–64	37–68
Sex				
Women, n (%)	38 (59%)	32 (56%)	31 (53%)	33 (57%)
Body mass index (kg/m ²)				
Mean (SD)	21.9 (2.9)	21.7 (2.3)	22.4 (2.6)	22.7 (2.8)
Range	15.6–29.1	16.9–27.8	17.7–28.9	17.3–31.1
Tobacco use				
Ever, n (%)	19 (30%)	13 (23%)	17 (29%)	15 (26%)
Alcohol consumption				
Yes, n (%)	32 (50%)	24 (42%)	31 (53%)	21 (36%)
Serum selenium (µg/L) ^a				
Median (IQR)	82 (67–100)	81 (70–101)	85 (75–98)	79 (68–97)
Biopsy samples				
Mean	6.0	5.8	6.1	5.9
Range	3–10	2–15	3–11	3–12
Histological diagnosis				
Moderate dysplasia, n (%)	35 (55%)	29 (51%)	29 (49%)	30 (52%)
Mild dysplasia, n (%)	29 (45%)	28 (49%)	30 (51%)	28 (48%)

IQR, interquartile range; SD, standard deviation.

^aBecause of missing selenium values, n = 61, 56, 57, and 58 for groups 1, 2, 3, and 4, respectively.

Table 3. Reported Adverse Events (AEs) Overall and by Intervention Group

Variable	Overall (n = 267)	By intervention group			
		Group 1 (n = 69) (selenomethionine and celecoxib)	Group 2 (n = 67) (celecoxib only)	Group 3 (n = 68) (selenomethionine only)	Group 4 (n = 63) (placebo)
Reported AEs (including laboratory AEs), n (%)	193 (72)	52 (75)	50 (75)	48 (71)	43 (68)
Reported AEs (not including laboratory AEs), n (%)	59 (22)	17 (25)	21 (31)	13 (19)	8 (13)
Highest grade 1–2	56 (21)	17 (25)	19 (28)	12 (18)	8 (13)
Highest grade 3–4	3 (1)	0 (0)	2 (3)	1 (1)	0 (0)
Withdrawn because of AEs, n (%)	6 (2)	0 (0)	3 (4)	1 (1)	2 (3)

tory adverse events (2 serum aspartate aminotransferase levels of 152 U/L; 3.8–4.1 times the upper limit of normal) and no laboratory adverse events of grade 3 or higher. Nonlaboratory adverse events were reported by 59 of 267 (22%) subjects. Of these, there were 3 serious adverse events, including 1 grade 3 event (lung cancer; group 2), and 2 grade 4 events (1 fatal myocardial infarction, group 3; 1 life-threatening stroke, group 2). None of these 3 serious adverse events was judged by the Medical Monitor as likely related to the intervention agents. Overall, 6 patients were withdrawn from the study agents before the end of the intervention period, including the 3 with grade 3 or 4 adverse events and 3 patients with grade 1 cardiac abnormalities (heart murmurs or electrocardiogram changes). There were 9 grade 2 nonlaboratory adverse events, 7 of which were instances of hypertension. Although the incidence of hypertension events by celecoxib status did not reach the level of statistical significance, there was a significant increase in mean systolic (but not diastolic) blood pressure among patients receiving active celecoxib vs placebo celecoxib

(17.9 vs 12.4 mm Hg; $P = .026$). This effect was not seen in the selenomethionine analysis.

Primary Analysis

Overall, neither intervention agent significantly changed the dysplasia grade on the basis of comparison of the most advanced histological diagnosis per subject at the baseline and end-of-trial evaluations (Table 4 and Figure 2). Active selenomethionine resulted in a trend toward increased regression (43% vs 32%; relative difference of 34%) and decreased progression (14% vs 19%; relative difference of 28%) compared with placebo selenomethionine ($P = .08$ for the combined primary end point incorporating regression, stable disease, and progression). Subjects treated with active vs placebo celecoxib experienced similar regression (36% vs 40%) and progression (16% vs 17%) rates overall ($P = .78$ for the combined primary end point). Trial results by intervention group, rather than intervention agent, are shown in Figure 3. No obvious interaction was observed between selenomethionine and celecoxib, al-

Table 4. Change in Histological Grade of Esophageal Squamous Dysplasia, by Intervention Agent

Baseline histology	Outcome ^a	Celecoxib			Selenomethionine		
		Active	Placebo	<i>P</i> value ^b	Active	Placebo	<i>P</i> value ^b
All subjects (n = 238)	Number in group	121	117		123	115	
	Regression, n (%)	43 (36)	47 (40)	.78	53 (43)	37 (32)	.08
	Stable, n (%)	59 (49)	50 (43)		53 (43)	56 (49)	
	Progression, n (%)	19 (16)	20 (17)		17 (14)	22 (19)	
Moderate dysplasia (n = 123)	Number in group	64	59		64	59	
	Regression, n (%)	28 (44)	27 (46)	1.00	30 (47)	25 (42)	1.00
	Stable, n (%)	32 (50)	28 (47)		28 (44)	32 (54)	
	Progression, n (%)	4 (6)	4 (7)		6 (9)	2 (3)	
Mild dysplasia (n = 115)	Number in group	57	58		59	56	
	Regression, n (%)	15 (26)	20 (34)	.71	23 (39)	12 (21)	.02
	Stable, n (%)	27 (47)	22 (38)		25 (42)	24 (43)	
	Progression, n (%)	15 (26)	16 (28)		11 (19)	20 (36)	

^aBased on comparison of the most advanced histological diagnosis per subject at the baseline and end-of-trial evaluations.

^bTwo-sided, 2-sample permutation *t* test for the combined primary end point (regression/stable/progression).

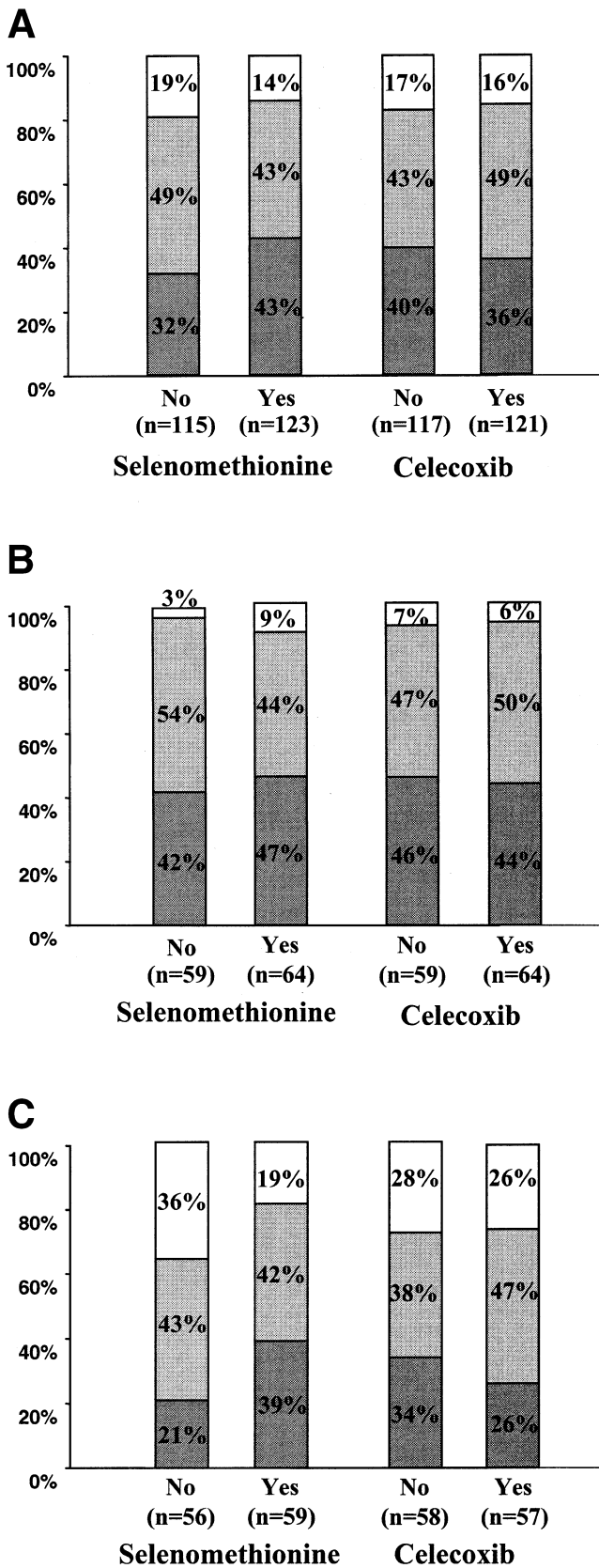


Figure 2. Change in severity of esophageal squamous dysplasia by intervention agent. □, Progression; ▨, stable; ■, regression. (A) All subjects; (B) moderate dysplasia at baseline; (C) mild dysplasia at baseline.

though the number of subjects limits our ability to test this meaningfully.

Stratified Analyses

Among the 115 patients with mild dysplasia at baseline, active selenomethionine led to a nearly 2-fold favorable effect on both dysplasia regression (39% vs 21%; relative difference of 82%) and dysplasia progression (19% vs 36%; relative difference of 48%) compared with placebo selenomethionine ($P = .02$ for the combined primary end point). Among the 123 subjects who started the trial with moderate dysplasia, active selenomethionine had no apparent chemopreventive effect, as evidenced by increased dysplasia regression (47% vs 42%) and increased dysplasia progression (9% vs 3%) compared with placebo selenomethionine ($P = 1.00$ for the combined primary end point). Stratified analyses by baseline histology grade did not show further effects from celecoxib treatment status.

Discussion

In this randomized, double-blind, factorial placebo-controlled clinical trial, we evaluated the effects of a 10-month intervention with selenomethionine or celecoxib on the natural history of esophageal squamous carcinogenesis among subjects with mild or moderate dysplasia. The primary analysis of the entire cohort did not show an overall benefit from either candidate chemoprevention agent. However, among the subcohort of patients who began the trial with mild dysplasia, selenomethionine significantly increased regression and decreased progression compared with placebo. Although it is based on unplanned stratified analyses, this finding is the first report of a possible beneficial effect for any candidate ESCC chemopreventive agent in a randomized controlled trial.

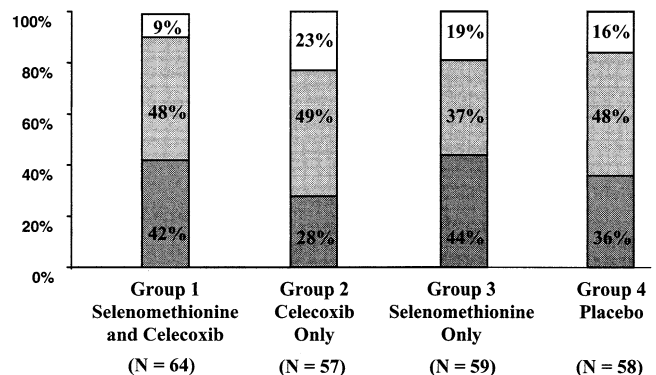


Figure 3. Change in severity of esophageal squamous dysplasia by intervention group. □, Progression; ▨, stable; ■, regression.

When defining our final analytic cohort, we excluded 29 study participants (11% of the randomized, eligible cohort, including 14 assigned to receive active selenomethionine and 15 assigned to receive placebo selenomethionine) because of missing postintervention endoscopy data. The reasons why these subjects did not complete their end-of-trial evaluations are unknown; however, we do know that they were seen by their village doctors on a daily basis while receiving the intervention agents. Any changes in health status would have been reported to the investigators by the village doctors according to comprehensive procedures outlined in the study protocol. Therefore, it seems unlikely that undetected intercurrent illnesses or agent-related toxicities precipitated these early withdrawals. Without the end-of-trial endoscopy data, it is impossible to know if or how the exclusion of these subjects might have influenced our final trial results. However, we have no reason to suspect that this relatively small subgroup experienced different effects from the intervention agents compared with subjects who completed the end-of-trial evaluations.

Selenium compounds have shown a variety of anticarcinogenic properties in cell culture and animal model systems, including reduced cellular proliferation, decreased angiogenesis, increased apoptosis, enhanced immune function, antioxidant effects, and altered carcinogen metabolism.²⁹ Epidemiological data further suggest that low selenium status may be a risk factor for both ESCC and esophageal adenocarcinoma. Case-control and cohort studies have shown that selenium status (as measured in either peripheral blood samples or toenail clippings) is inversely associated with incident esophageal malignancies of both histological subtypes.^{11–15} Low serum selenium levels have also been associated with advanced dysplasia among subjects at risk for ESCC¹¹ and adenocarcinoma.¹⁶

Three randomized, placebo-controlled clinical trials have examined the effects of selenium supplementation, alone or in combination with other nutritional elements, on esophageal cancer risk.^{17–20} In the US Nutritional Prevention of Cancer study,^{17,18} 1312 subjects with non-melanoma skin cancer were treated with 200 $\mu\text{g}/\text{day}$ of high-selenium yeast vs placebo. Through the end of the blinded treatment period (a mean of 7.4 years of follow-up), the relative risk (RR) for nonmelanoma skin cancer was statistically significantly increased (RR, 1.17; 95% confidence interval [CI], 1.02–1.34), although the total (RR, 0.75; 95% CI, 0.58–0.97) and prostate (RR, 0.48; 95% CI, 0.28–0.80) cancer incidences were statistically significantly reduced. The number of observed esophageal cancer cases was too low ($n = 7$) to afford a precise

risk estimate (RR, 0.40; 95% CI, 0.08–2.07) for this target organ.¹⁸

Two previous trials were also conducted in Linxian, PRC. In the Dysplasia Trial,¹⁹ 3318 subjects with premalignant esophageal lesions, as detected by balloon cytology, were randomized to receive a daily multivitamin (26 vitamins and minerals, including 50 μg of sodium selenate) vs placebo for 6 years. The active agent was associated with a modest (but not significant) reduction in risk for fatal ESCC (RR, 0.84; 95% CI, 0.54–1.29), particularly among subjects who began the trial with cytologically defined low-grade dysplasia (RR, 0.75; 95% CI, 0.44–1.31; unpublished data). No appreciable association was observed with incident ESCC (RR, 0.94; 95% CI, 0.73–1.20). In the General Population Trial,²⁰ 29,584 asymptomatic adults were randomly assigned to receive 1 of 8 combinations of 4 vitamin/mineral intervention groups (designated factors A–D). Factor D included a combination of selenium (50 $\mu\text{g}/\text{day}$ as selenium yeast), β -carotene (15 mg/day), and α -tocopherol (30 mg/day). After an intervention period of 5.25 years, subjects who received factor D experienced significantly lower all-cause mortality (9%), total cancer mortality (13%), and gastric cancer mortality (21%) rates compared with other trial participants. ESCC incidence (RR, 1.02; 95% CI, 0.87–1.19) and mortality (RR, 0.96; 95% CI, 0.78–1.18) rates were not altered by this intervention regimen, but prevalent ESCCs were less common (odds ratio, 0.58; 95% CI, 0.19–1.76) among subjects who received factor D in a small subset of trial participants who underwent postintervention EGDs.³⁰

Although results from the initial Linxian clinical trials cannot be analyzed with respect to individual micronutrients, others have proposed that the observed cancer-prevention benefits were likely due to selenium.³¹ The form and/or dose of selenium applied in these earlier trials may have been suboptimal, however, for ESCC chemoprevention. Selenium from selenomethionine (ie, organic selenium) is better absorbed and retained than selenium from selenite (ie, inorganic selenium) and results in substantially greater overall body stores.³² Because the purity and stability of selenium yeast can vary from lot to lot, synthetic selenomethionine may be the preferred supplemental form of organic selenium. On the basis of extended data from the US Nutritional Prevention of Cancer Trial,¹⁸ as well as the present study, 200 $\mu\text{g}/\text{day}$ of selenomethionine seems safe and well tolerated and may be associated with greater chemopreventive effects than lower doses. Also, a recent case-cohort study nested within the General Population Trial found that low preintervention serum selenium levels were strongly associated with an increased ESCC risk (RR, 0.56; 95%

CI, 0.44–0.71 for the highest vs lowest quartiles),¹⁵ thus indirectly supporting the possibility that a higher and/or more bioavailable dose of selenium may provide additional chemopreventive benefits in this subject population.

COX-2 inhibitors are being actively investigated as candidate chemoprevention agents for several malignancies, including esophageal adenocarcinoma, in recent clinical trials.³³ Experimental data suggest that these compounds may prevent carcinogenesis by favorably affecting proliferation, apoptosis, angiogenesis, or other as yet unidentified growth-regulating processes.^{34,35} Completed intervention trials have shown efficacy from selective³⁶ and nonselective COX-2 inhibitors^{37,38} against colorectal neoplasia end points. With respect to esophageal cancer, epidemiological studies have reported wide-ranging risk reductions (20%–90%) among regular NSAID users compared with nonusers,^{21–24} although most individual studies have lacked sufficient power to provide separate risk estimates by histological subtype. Recently, Corley et al²⁵ performed a meta-analysis of existing observational data and found protective associations for both ESCC (odds ratio, 0.58; 95% CI, 0.43–0.78) and esophageal adenocarcinoma (odds ratio, 0.67; 95% CI, 0.51–0.87) in the pooled analyses.

Despite these encouraging observational data, we found no association between celecoxib treatment and changes in the histological grade of squamous dysplasia in our subject cohort. Although this observation could signify that COX-2 plays a limited role in esophageal squamous carcinogenesis, several other factors might have contributed to this result. First, the timing of the intervention within the carcinogenic spectrum may have been less than ideal. Laboratory studies have shown that COX-2 protein expression increases progressively with advancing grades of squamous dysplasia.^{39,40} By including only subjects with mild or moderate dysplasia in this trial, our ability to discern an effect from COX-2 inhibition may have been reduced. Second, the intervention period may have been too short. Observational studies have reported direct associations between chronicity of NSAID use and degree of esophageal cancer risk reduction.^{23,24} Indeed, 1 study reported a statistically significant decrease in cancer risk only after ≥ 5 years of regular use.²⁴ Third, the intervention dose may have been too low, because 400 mg twice daily was required to show a statistically significant effect on colorectal neoplasia among genetically predisposed subjects.³⁶

The change in each subject's worst histological grade of squamous dysplasia was defined a priori as the primary outcome for our study. Because cancer events are relatively infrequent even among high-risk patient popula-

tions, modulation of intermediate end points such as intraepithelial neoplasia has been proposed as an appropriate surrogate end point for phase II chemoprevention trials.^{41,42} Two key features of suitable surrogate end points for chemoprevention trials are the degree of association between the surrogate biomarker and the cancer outcome (ie, the degree to which the biomarker is on the causal pathway for disease) and the sensitivity of the biomarker measurement technique.³³ A previous study by our group in Linxian, which followed up 682 participants of an endoscopic survey for 13.5 years, found significant and dramatically increasing risks of ESCC among subjects with initial biopsy diagnoses of mild, moderate, and severe squamous dysplasia (cumulative incidences of 24%, 50%, and 74% and RRs of 2.9, 9.8, and 28.3, respectively).^{8,9} Another study by our group in 225 Linxian patients showed that mucosal staining with Lugol's iodine solution had an 84% sensitivity and a 69% specificity for detecting esophageal squamous dysplasia, including sensitivities of 93% and 63% for detecting moderate and mild dysplasia, respectively.¹⁰ We believe that the strong association of squamous dysplasia with ESCC risk and its endoscopic visibility after mucosal iodine staining make it an excellent surrogate end point for the initial assessment of candidate ESCC chemoprevention agents. These previous Linxian studies also have other implications that are important for the interpretation of the current trial results. The close relationship between histological grade of dysplasia and ESCC risk in the follow-up study suggests that a shift in grade over time (with or without an intervention) may correspond to a real change in cancer risk. The relatively low sensitivity of Lugol's staining for identifying mild dysplasia implies that some of these lesions were probably missed at each of the EGDs in this trial. However, such misclassification should bias the intervention result toward the null and cause an underestimation of the true effect of selenomethionine.

Despite probable (and unavoidable) overlap between histologically defined categories of intraepithelial neoplasia, mild dysplasia and moderate dysplasia are thought to represent distinct stages of disease progression within the esophagus. In our earlier Linxian follow-up study, subjects in the latter dysplasia subgroup were found to be at markedly higher ESCC risk.^{8,9} In addition, we have shown that molecular profiles differ by degree of esophageal squamous dysplasia.^{43,44} Thus, it seems biologically plausible that the potential chemopreventive benefits we observed among subjects with mild dysplasia at baseline may imply a stronger effect from selenomethionine during the early stages of esophageal squamous carcinogenesis.

The population of Linxian has very low levels of serum selenium, and observational data from the General Population Trial suggest that selenium insufficiency is a significant contributor to the development of ESCC in this area. In a recent case-cohort study nested within the General Population Trial, baseline measurements on 2141 Linxian subjects showed a median serum selenium level of 71 $\mu\text{g/L}$ (compared with a median level of 124 $\mu\text{g/L}$ in the United States). Low preintervention serum selenium levels were strongly associated with ESCC risk (RR, 0.56; 95% CI, 0.44–0.71 for the highest vs lowest quartiles),¹⁵ and it was estimated that increasing the selenium level of the entire cohort to that of the highest quartile ($\geq 82 \mu\text{g/L}$) would prevent 26% of the esophageal cancer cases in the group. Thus, the current trial findings may be most pertinent to low-selenium populations.

In summary, our data support further pursuit of selenium compounds as potential ESCC chemopreventive agents, particularly among subjects with early premalignant disease. However, because the key genetic, epigenetic, and environmental factors associated with ESCC risk among Linxian residents remain incompletely defined, extrapolation of these data to other high-risk populations should be made with appropriate caution. The absence of any appreciable effects from celecoxib on changes in dysplasia grade among our study subjects, coupled with emerging reports of increased cardiovascular toxicity associated with prolonged use of selective COX-2 inhibitors,⁴⁵ tempers enthusiasm for additional investigation of this agent class in the context of ESCC chemoprevention.

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Address requests for reprints to: Paul J. Limburg, MD, MPH, Division of Gastroenterology and Hepatology, Mayo Clinic, 200 First Street Southwest, Rochester, Minnesota 55905. e-mail: limburg.paul@mayo.edu; fax: (507) 266-0350.

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