

Short CommunicationPhase I/Pharmacodynamic Study of *N*-Acetylcysteine/Oltipraz in Smokers: Early Termination Due to Excessive Toxicity<sup>1</sup>

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**Abstract**

An *N*-acetylcysteine (NAC)/oltipraz (OLZ) combination was studied in healthy volunteer smokers who received daily NAC (1200 mg/day) and were randomized to weekly placebo (Arm A), OLZ 200 mg (Arm B), or 400 mg (Arm C). Treatment was for 12 weeks with follow-up at 16 weeks. The objective was to study toxicity and the modulation of pharmacodynamic end points. After treatment of 19 of a planned 60 subjects, (Arm A, six; Arm B, four; and Arm C, nine), the study was closed because of toxicity. Eight subjects failed to complete 12 weeks of drug administration, (Arm A, two, and Arm C, six). The most frequent side effects were gastrointestinal, fatigue, conjunctival irritation, and skin rash. Pharmacodynamic end points were measured pretreatment and 48 h after the dose of OLZ at weeks 1, 5, and 12 and 4 weeks after the end of treatment. Glutathione (GSH) was measured in plasma and in peripheral blood lymphocytes (PBLs). Other end points measured in PBLs were the enzyme activities of total glutathione-*S*-transferase (GST), GST $\pi$ , and NAD(P)H:quinone oxidoreductase; and the mRNA expression of  $\gamma$ -glutamylcysteine synthetase ( $\gamma$ GCS), GST $\pi$ , and NAD(P)H:quinone oxidoreductase. GSH in PBLs, GST (total), and the mRNA of  $\gamma$ GCS showed increases at some time points in some subjects. Most consistent was the mRNA of  $\gamma$ GCS, which showed a  $\geq 30\%$  increase at one or more time points in 11 of 19 subjects. Other end points were unchanged. We concluded that NAC/OLZ modulates some end points related to GSH but is too toxic for chemoprevention at the doses used.

**Introduction**

NAC<sup>3</sup> has been shown to have chemopreventive activity in a number of preclinical systems, including lung, trachea, breast,

and colon malignancies (1, 2). It is thought to act by enhancing intracellular levels of thiols, especially GSH (3). In a previous Phase I study of NAC as a chemopreventive agent in normal volunteers (4), we found a significant increase in GSH in PBLs at daily doses  $\geq 800$  mg/m<sup>2</sup>/day. The predominant toxicities were GI disturbance and fatigue. In the escalation phase of the study, 13 of 13 subjects tolerated 800, 11 of 13 tolerated 1600, and 4 of 7 tolerated 6400 mg/m<sup>2</sup>/day ( $\approx 11$  g/day) for 1 month without side effects. When 800 mg/m<sup>2</sup> (median dose, 1750 mg/day; range, 1300–2000 mg) was given daily to 13 subjects, 1 developed an idiosyncratic reaction to the drug, and 1 withdrew after 1 month. The remaining subjects tolerated 3 months of daily drug administration without significant side effects.

In a European randomized trial (Euroscan) in patients with head and neck and lung cancer, after therapy with curative intent, 2592 patients were randomized to NAC (600 mg/day), retinyl palmitate, a combination of both drugs, or placebo (5). Treatment was for 2 years. The NAC arm was the least toxic, with 18.1% of patients failing to complete 2 years of treatment versus 25.8% in the retinyl palmitate arm and 25.2% in the combined-treatment arm. Some toxicity was seen in 45% of patients in the retinyl palmitate arm versus 24% for NAC.

OLZ is a dithiolthione originally developed for the treatment of schistosomiasis. It showed chemopreventive activity against tumors of the lung, liver, and GI tract (6). Mechanistic studies indicate that OLZ increases GSH levels in several target organs in rodents and induces Phase II enzymes (7). It has undergone a number of Phase I studies as a chemopreventive agent and is currently undergoing extensive evaluation in randomized trials, for the prevention of hepatoma, in the Qidong Province of China (8). A number of side effects have been reported. However, in the chemoprevention studies in China (8), in which 195 subjects received placebo, 125 mg daily, or 500 mg weekly for 8 weeks in a randomized trial, only one side effect occurred in the treated group significantly more frequently than in the control group. This was a syndrome of numbness, tingling, and sometimes pain in the extremities noted in 11.5% of the study participants. Both GSH and GST levels were noted to be elevated in the PBLs of subjects receiving 100–500 mg of OLZ by Gupta, *et al.* (9); O'Dwyer, *et al.* (10) observed an elevation of mRNA for  $\gamma$ GCS and DT-diaphorase in PBLs and colon biopsies after 250–1000 mg/m<sup>2</sup> of the drug.

In view of the nonoverlapping nature of their reported major toxicities and the potential for augmentation of their biochemical action, we initiated a randomized Phase I study to test the combination of NAC and OLZ. Doses near the upper limit of the nontoxic dose range of the individual drugs were used to maximize the potential for observing augmentation of PD modulation. All subjects received NAC, and subjects were randomized to two doses of OLZ and a placebo.

The objectives of the study were: (a) to establish the safety and side effects of daily NAC at a dose of 1200 mg/day alone or with OLZ given as a weekly dose at 200 or 400 mg/week for 12 weeks; (b) to evaluate the effects of the combination of NAC

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<sup>3</sup> The abbreviations used are: NAC, *N*-acetylcysteine; GSH, glutathione, OLZ, oltipraz; PD, pharmacodynamic; GST, glutathione *S*-transferase; DT-diaphorase, NAD(P)H:quinone oxidoreductase; GI, gastrointestinal; PBL, peripheral blood lymphocyte; GCS, glutamylcysteine synthase.

Table 1 Commonly Reported Symptoms

Symptom	Arm <sup>a</sup>			Total
	A (n = 6)	B (n = 4)	C (n = 9)	
Increased intestinal gas/bloating/cramps	4	3	8	15
Fatigue/malaise	3	2	4	9
Nausea	1	2	4	7
Diarrhea	2	0	3	5
Tingling of extremities	3	0	1	4
Altered taste	0	0	3	3
Skin rash/palmar erythema	2	0	1	3
Increased lacrimation	2	0	1	3
Anorexia	1	0	1	2

<sup>a</sup> Arm A NAC 1200 mg/d + placebo; arm B NAC 1200 mg/d + OLZ 200 mg/week; and arm C NAC 1200 mg/d + OLZ 400 mg/week.

and OLZ on PD end points; and (c) to explore the correlations between doses of NAC and OLZ and alterations in PD end points.

### Materials and Methods

**Subjects.** Subjects were normal volunteers free of overt malignant disease. They were smokers, smoking at least one pack/day of cigarettes and with a minimum smoking history of 10 pack-years. They were required to be free of clinically unstable intercurrent disease; to have a normal performance status; to have a serum bilirubin  $\leq 1.4$  mg/dl, a serum creatinine  $\leq 1.5$  mg/dl, an aspartate aminotransferase  $\leq 75$  IU/liter, WBC  $\geq 4.0 \times 10^9$ /liter, and a platelet count  $\geq 10^{11}$ /liter. Exclusions included pregnancy or lactation (women of childbearing potential were required to have a negative pregnancy test before entering into the study), a history of alcohol and/or drug abuse, and daily use of nonsteroidal anti-inflammatory drugs. Subjects or their partners, male or female, were required to be on adequate birth control. All subjects were required to give written informed consent to enter into the study.

### Conduct of the Study

**Drug Administration.** NAC, OLZ, and placebo were supplied by the National Cancer Institute, Chemoprevention Branch repository, McKesson Bioservices. NAC was supplied as capsules containing 200 and 500 mg. OLZ was supplied as capsules containing 50, 100, and 250 mg. Placebo was supplied as capsules identical to the low dose (50 mg) and high dose (100 and 250 mg) capsules of OLZ. A 4-week supply of drug for each subject was received from the pharmacy at Roswell Park Cancer Institute in blister packs of identical appearance. The returned blister packs were used to monitor compliance.

All subjects received NAC 1200 mg daily p.o. In addition, subjects were randomly assigned to receive placebo (Arm A), OLZ 200 mg (Arm B), or 400 mg (Arm C) once weekly. Because of possible diurnal variation in PD end points, drugs were taken at the same time each day. Drugs were given for 12 weeks. The study was double-blind. There was no dose modification for toxicity. Subjects exhibiting toxicity greater than grade I were taken off-study (see below).

**Monitoring of the Study.** Subjects were contacted weekly by telephone, and a symptom questionnaire was completed by the interviewer. Pretreatment, monthly during treatment, and at 1 month after treatment cessation, the following were performed: (a) a physical examination including detailed neurologic evaluation with emphasis on the known toxicity of OLZ; and (b) complete blood count with differential, blood chemistry profile,

Table 2 Grade II and III Toxicity<sup>a</sup>

Toxicity	Arm A (n = 6)	Arm B (n = 4)	Arm C (n = 9)	Total
GI <sup>b</sup>	1	0	4	5
Fatigue	3	0	2 (1)	5 (1)
Skin rash	1 (1)	0	0	1 (1)
Bronchospasm	0	0	1 (1)	1 (1)
Neuropathy <sup>c</sup>	0	0	1	1
Total	5 (1)	0	8 (2)	13 (3)

<sup>a</sup> Numbers in parentheses indicate grade III toxicity.

<sup>b</sup> Nausea, vomiting, diarrhea, anorexia, bloating, and abdominal pain.

<sup>c</sup> Numbness and tingling of hands and arms.

and urinalysis. An electrocardiogram was performed pretreatment and at the final visit.

**Study End Point.** Occurrence of any toxicity greater than grade I resulted in discontinuation of the drugs. In cases where doubt existed as to the relationship of symptoms to drug administration, the drug was reintroduced after symptoms had cleared. If symptoms recurred, the subject was taken off-study.

**Pharmacodynamic Measurements.** Total GSH in plasma, free and total GSH in PBLs, the enzyme activities of GST (total), GST $\pi$ , and DT-diaphorase, and the mRNA levels of  $\gamma$ GCS, GST $\pi$ , and DT-diaphorase were measured at baseline and at weeks 1, 5, and 12 and at 1 month after the end of the study. Blood was collected 48 h post-dosing at the same time of day. On the basis of previous reports, a  $\geq 30\%$  increase in the PD end points was chosen as the cutoff value for a consistent change (4, 9, 10).

**Measurement of GSH in Plasma and PBLs.** GSH was measured by high performance liquid chromatography as has been described earlier (11).

**Enzyme Assays.** The activities of GST total and GST $\pi$  in PBL extracts were measured according to Habig *et al.* (12), and Yellin *et al.* (13), respectively. DT-diaphorase activity was assayed by Gordon *et al.* (14).

**mRNA Levels of  $\gamma$ GCS, GST $\pi$  and DT-diaphorase.** The measurement of the mRNA levels of  $\gamma$ GCS, GST $\pi$  and DT-diaphorase was carried out with real-time quantitative reverse transcription-PCR (Taqman assay) using the PE-ABI Prism 7700 Sequence Detection System.  $\beta$ -actin was used as the endogenous standard from the same cDNA preparations. Total RNA from PBLs was isolated using the phenol guanidinium isothiocyanate procedure (Tri Reagent, Molecular Research Center, Inc.), according to the manufacturer's directions. cDNA synthesis was carried out using Superscript II (Life Technologies Inc., Grand Island, NY) in an oligodeoxythymidylate-primed reaction. The comparative C<sub>T</sub> method was used for quantitation of the target gene expression relative to the expression of the endogenous standard (15). All the pre- and posttreatment samples for the three genes of interest and the  $\beta$ -actin for any given subject were analyzed simultaneously. The primers and probes for these assays were synthesized at the Roswell Park Cancer Institute Biopolymer Core Facility.

**Statistical Analyses.** Statistical analyses included: (a) testing for an effect among the three treatment groups; (b) testing for an effect from baseline to posttreatment; and (c) evaluating adverse effects. Statistical tests were nonparametric and included the Fisher exact test, the Sign test, the Kruskal-Wallis test, and the Spearman rank correlation. Testing was performed at a significance level of 0.05.

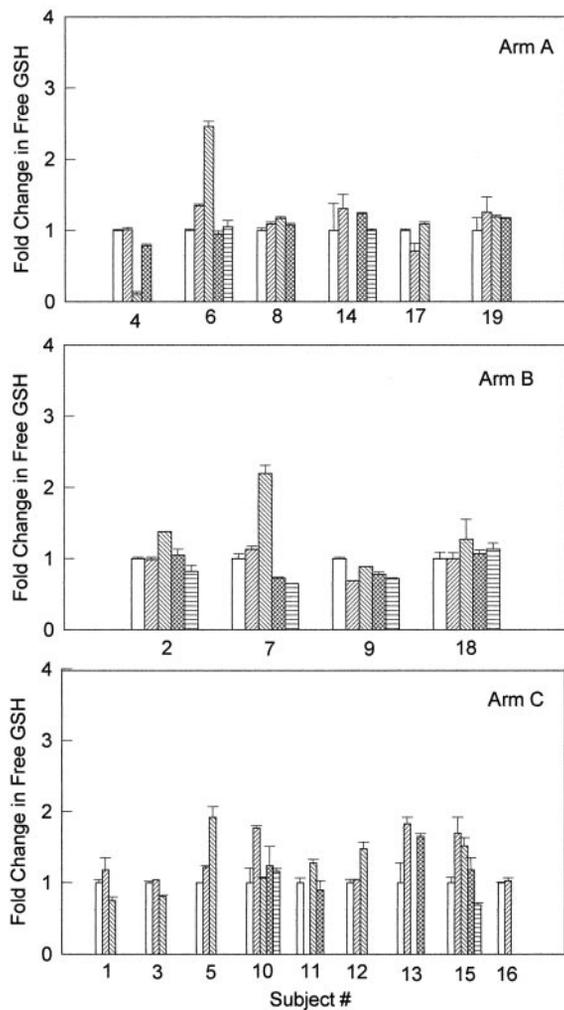


Fig. 1. Free GSH in the PBLs of subjects in the three Arms. The pretreatment values are normalized to 1, and posttreatment levels are expressed as a fold change relative to the pretreatment. □, pretreatment; ▨, wk 1; ▩, wk 5; ▪, wk 12; ▫, wk 16.

## RESULTS

**Tolerance and Toxicity.** The study was planned for 60 subjects, 20 in each arm. After the entry of 19 subjects, recruitment to the study was discontinued and the code broken because of toxicity, which was considered to be excessive for a chemoprevention combination. At that time, six subjects had been entered into Arm A (three male, three female; median age, 49.5 years; age range, 31–67 years), four into Arm B (three male, one female; median age, 60 years; age range, 34–69 years), and nine into Arm C (three male, six female; median age, 48 years; age range, 31–60 years). Eight subjects failed to complete 12 weeks of drug administration, two in Arm A and six in Arm C. In Arm C, the six subjects failing to complete 12 weeks of drug administration were on study for a median of 6 weeks (range, 1 day–8 weeks). The most frequent drug-related toxicities were GI (excessive gas, nausea, vomiting, diarrhea, anorexia, bloating, abdominal cramping, and epigastric discomfort), fatigue or malaise, conjunctival irritation and lacrimation, and a nonirritating skin rash or palmar-plantar erythrodysesthesia. Tingling of the extremities was reported by a number of subjects, but its

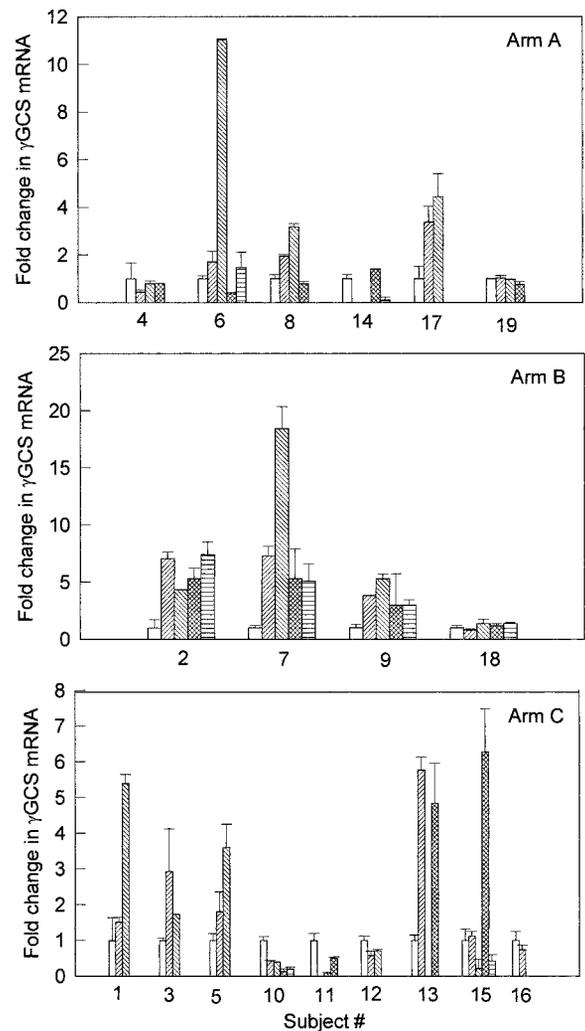


Fig. 2.  $\gamma$ -GCS/ $\beta$ -actin mRNA levels in the PBLs of subjects in the three Arms. The pretreatment values are normalized to 1, and posttreatment levels are expressed as a fold change relative to the pretreatment. □, pretreatment; ▨, wk 1; ▩, wk 5; ▪, wk 12; ▫, wk 16.

relationship to drug administration was difficult to evaluate. One subject had an allergic reaction (bronchospasm) and one had paresthesias and numbness of the upper extremities. The commonly reported symptoms with their frequencies are shown in Table 1. The grade II and grade III toxicities are shown in Table 2. Some subjects showed more than one toxicity. All toxicity was reversible without sequelae on discontinuation of the drug.

**GSH Levels in Plasma and PBL.** There were no apparent changes in the total GSH levels in plasma. The free (Fig. 1) and total (data not shown) GSH levels in PBLs increased in some subjects after NAC/OLZ. A  $\geq 30\%$  increase over pretreatment was noted in weeks 1, 5, or 12 in seven of nine in the 400-mg OLZ group (subjects 1, 5, 10, 11, 12, 13, and 15) for both free and total GSH in PBLs. In subjects 13 and 15, this increase was seen at more than one time point. Increases were also seen in one of four subjects (Subject 7) receiving the 200-mg OLZ. In the placebo group, subject 6 showed a  $>30\%$  increase in GSH in PBLs. The difference in free and total GSH levels between pretreatment and week 1 were significant with the combined

data from all three arms (Sign test;  $P = 0.01$  and  $0.03$ , respectively).

**Enzyme Activities.** No consistent changes were seen in total GST activity after NAC/OLZ. A  $\geq 30\%$  increase at one or more time points was seen in three of six subjects after NAC alone, in two of four subjects after NAC + 200 mg of OLZ, and in three of nine subjects after NAC + 400 mg of OLZ (data not shown). Tests for differences at each sampling time between pooled data of Arms B and C against the data from Arm A showed significant differences (Kruskal-Wallis test;  $P = 0.03$ ) at week 5.

The GST $\pi$  and DT-diaphorase activities at baseline were near detection limits and did not show consistent increases after treatment with NAC/OLZ.

**mRNA Expression for  $\gamma$ GCS, GST $\pi$  and DT-diaphorase.** No increase in mRNA expression was observed for either DT-diaphorase or GST $\pi$ . On the other hand, 11 of 19 subjects showed a  $\geq 30\%$  increase in the mRNA expression of  $\gamma$ GCS at one or more time points (Fig. 2). There were five of nine in the 400-mg OLZ group (subjects 1, 3, 5, 13, and 15), three of four in the 200-mg OLZ group (subjects 2, 7, and 9), and three of six in the NAC-alone group (subjects 6, 8, and 17). The increase in  $\gamma$ GCS mRNA expression seems to relate to an increase in free and total GSH in the PBLs in some subjects (2, 5, 6, 7, 13, and 15) but not in all.

## Discussion

This trial of the combination of daily NAC with weekly OLZ was designed to give the optimal chance of demonstrating a greater modulation of biochemical end points than could be achieved with either drug alone while keeping toxicity within acceptable limits. From our Phase I studies of NAC in a limited number of subjects, it appeared that 1700 mg/day was well tolerated for 3 months, and thus the dose of 1200 mg/day seemed appropriate for this study. The dose-limiting toxicity was predominantly GI. Both doses of OLZ were within the limits of those tolerated previously, and the dose-limiting toxicity of OLZ from the large-scale studies in China was peripheral neuropathy, although a variety of other toxicities, including GI toxicity, were reported from other trials. Of the first 10 subjects entered, 8 completed the trial; of the next 9 subjects, only 3 were able to do so. For this reason, accrual was halted and the code broken. Although a high dose of OLZ contributed to the toxicity, two of six subjects on NAC alone failed to complete the trial. In spite of the small numbers, it was clear from this experience that the doses were too high for a chemoprevention combination.

The randomization of the initial 19 subjects resulted in six in Arm A, four in Arm B, and nine in Arm C. The small numbers, the large percentage of incomplete studies, and the unequal distribution between the groups lowered the power of statistical analysis to detect differences between the treatment groups. Thus any conclusions drawn are necessarily tentative. The choice of biochemical and molecular parameters tested was based on previous studies of NAC (4) and OLZ (9, 10). Gupta *et al.* (9) reported an increase in GSH and total GST activity 6–24 h after OLZ in six subjects receiving doses of 100–125 mg. O'Dwyer *et al.* (10) reported an increase in mRNA expression of both  $\gamma$ GCS and DT-diaphorase in PBLs 2–4 days after OLZ at doses of 250–1000 mg/m<sup>2</sup>. A maximum increase of 5.75-fold for  $\gamma$ GCS in colon mucosa after OLZ was observed

by these investigators. Doses  $>250$  mg/m<sup>2</sup> were not more effective in inducing  $\gamma$ GCS or DT-diaphorase. In the present study, sample collection was carried out 48 h after OLZ dosing. Total GST activity showed a difference in our study between the groups at one time point only (week 5), and only when pooled data from Arm B and Arm C were tested against that from Arm A. We found no changes in DT-diaphorase mRNA expression. It is unclear whether the addition of NAC or the sampling schedule resulted in our failure to observe significant changes in GST activity and DT-diaphorase mRNA expression.

## Conclusion

At the doses used in this study, the combination of NAC/OLZ is too toxic for use in a chemoprevention trial. However, at lower tolerable doses, the modulation of PD endpoints is unlikely to be detectable.

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