Pharmacokinetic and Pharmacodynamic Studies of N-Acetylcysteine, a Potential Chemopreventive Agent during a Phase I Trial

L. Pendyala2 and P. J. Creaven
Department of Investigational Therapeutics, Division of Medicine, Roswell Park Cancer Institute, Buffalo, New York, 14263

Abstract
A Phase I, pharmacokinetic and pharmacodynamic study of N-acetylcysteine (NAC), a potential chemopreventive agent, given daily p.o. for 6 months was carried out in 26 volunteers at higher than normal risk of malignancy. The goals of the study were to define the highest nontoxic dose, the toxicity profile, and the pharmacokinetics and pharmacodynamics of NAC. The pharmacodynamic end points studied included glutathione (GSH) in plasma, RBC and peripheral blood lymphocytes (PBL), cysteine in plasma, and two GSH-metabolizing enzymes glutathione S-transferase and oxidized glutathione reductase in PBL. The study was carried out in 2 stages. The first stage consisted of an inter- and intrasubject dose escalation; the second, an assessment of a single daily dose. Starting doses for the first 4 cohorts of 3 subjects were 400, 800, 1600, and 3200 mg/m²/day in divided doses doubled at the end of each month in the absence of toxicity to a final dose of 6400 mg/m²/day. The total planned period on NAC for each subject was 6 months. Pharmacokinetic and pharmacodynamic measurements were carried out at the beginning of the study and at the end of each month. The second stage of the study consisted of a daily dose of 800 mg/m²/day. During this part of the study, NAC in plasma and GSH and oxidized glutathione reductase (GRD) in PBL were measured on day 1 and again at the end of first, second, and sixth month on NAC. Major toxicities were bad taste and gastrointestinal disturbances. The highest nontoxic dose was 800 mg/m²/day in most of the subjects. NAC was absorbed rapidly (median tmax, 1.0 h) and showed a monoexponential plasma decay (median t1/2, 2.4 h). Pharmacokinetics were linear with dose. A statistically significant increase in GSH in PBL was seen following doses of NAC of 800 mg/m² and higher; GRD in PBL showed a trend toward statistical significance. No changes were found in the other pharmacodynamic end points. The second part of the study with daily dosing of 800 mg/m²/day suggested that chronic dosing of NAC may result in reduced absorption and/or increased metabolism. The administration of NAC did not elevate either the base-line GSH, or the area under the curve t1/2 to 6 h of GSH in PBL. Following the NAC dose however, at least a 20% increase in GSH in PBL was seen in 30% or more of the subjects at all the time points studied. GRD in PBL varied extensively and showed little increase. No correlations were observed between the cmax and area under the curve of NAC and the percentage of change of GSH in PBL. It is concluded that p.o. NAC at a dose of 800 mg/m² will modulate pools of intracellular NAC in some subjects but that this increase is of brief duration. Other relevant pharmacodynamic end points are relatively little affected by this dose of the drug.

Introduction
NAC has been used clinically as a mucolytic agent (1, 2) and as an antidote against acetaminophen-induced hepatotoxicity (3). It has also been used to prevent hemorrhagic cystitis caused by cyclophosphamide and ifosfamide (4).

NAC is readily deacetylated in the body to form CYS, which efficiently supports GSH biosynthesis because CYS availability can be the rate-limiting step in GSH formation (5). GSH conjugates with and detoxifies many electrophiles thus providing a protective effect against many promoters and carcinogens. Therefore, raising the intracellular level of GSH is an attractive cancer chemoprevention strategy. NAC counteracts the mutagenicity of direct acting carcinogens and precarcinogens (6). Evidence for the chemopreventive effect of NAC comes from the studies of DeFlora et al. (7) and Wilpart et al. (8) which indicate that NAC prevents the formation of ethyl carbamate (urethane)-induced lung tumors in mice and colonic carcinogenesis in the rat.

On the basis of these data, NAC is considered an appropriate compound to evaluate clinically as a potential chemopreventive agent. Serving as the initial stage of this evaluation, a Phase I toxicity, pharmacokinetic, and pharmacodynamic trial was conducted in 2 stages, a dose escalation stage and a constant dose stage. The clinical and biochemical results of this study are presented here.

1 The abbreviations used are: CYS, cysteine; NAC, N-acetylcysteine; GSH, glutathione; GST, glutathione-S-transferase; GRD, oxidized glutathione reductase; SSA, sulfosalicylic acid; PBL, peripheral blood lymphocytes; AUC, area under the curve; CBC, complete blood count; EKG, electrocardiogram; tmax, time to achieve maximum concentration.
Materials and Methods

Subjects
Subjects were adult volunteers at high risk of recurrence from a previous malignancy or who were considered to belong to a high risk group for the development of cancer (Table 1). They were required to have normal renal, hepatic, and bone marrow function and to be free of acute medical problems. Each subject gave written informed consent for entry into the study, which was approved by the Institutional Review Board of the Roswell Park Cancer Institute.

Drug Administration
NAC was supplied by the Chemoprevention Branch of the National Cancer Institute. It was administered in capsules containing 50, 200, or 500 mg or sachets containing 1 g of NAC. Monitoring of compliance was by measuring unused amounts of the drug.

In the initial stage of the study, the starting dose was 400 mg/m²/day, given in 2 divided doses. This was given for 1 month to 3 subjects. In the absence of side effects, the dose was doubled to 800 mg/m²/day in these 3 subjects and 3 new subjects were entered at 800 mg/m². This procedure was repeated with a new cohort of 3 subjects being entered every month at the escalated dose until 4 cohorts had been added. Each subject was treated for 6 months, the final dose being given for 2 or more months. The highest dose explored was 6400 mg/m²/day. In the event of toxicity, the dose was reduced to the previous (nontoxic) dose level and continued for the remainder of the 6 months of treatment. In the second stage of the study subjects were entered at 800 mg/m²/day and treated for 6 months without dose escalation provided there was no or only minimal toxicity and subjects were compliant.

Study Monitoring
Pretreatment, monthly during treatment and 3 months after the last dose, a CBC, blood chemistry profile (Serum Na⁺, K⁺, Cl⁻, HCO₃⁻, CO₂, Ca²⁺, PO₄³⁻, BUN, glucose, creatinine, uric acid, total protein, albumin, total bilirubin, alkaline phosphatase, lactate dehydrogenase, aspartate transcarbamylase, γ-glutamyl transferase) and urinalysis were performed. An EKG was done during pretreatment and was repeated at the end of the study.

Pharmacokinetic and Pharmacodynamic Studies
Sample Collection, Processing and Analytical Methodology. Blood (8 ml) was collected pretreatment and at 0.5, 1, 2, 3, 4, 6, and 8 h after the first dose and again at the end of each month prior to dose escalation, during the 6-month duration of the study. The method of Burgunder et al. (9), with minor modifications, was used for processing and derivatization of thiols in plasma. The method of Anderson (10) was followed for processing and extraction of thiols in RBC and PBL. All the processing was carried out at 4°C. Blood was brought to the laboratory on ice and centrifuged immediately. Much of the plasma was removed, and 0.9-ml aliquots were transferred to tubes containing 100 µl of L-serine sodium/borate [to inhibit γ-glutamyl transpeptidase which would otherwise degrade the GSH (9)]. The RBC were removed with a long needle and 0.5-ml aliquots were added to an equal volume of 5% SSA. The buffy coat along with the remaining plasma and RBC were layered on Ficoll-hypaque and the PBL were separated, counted with a hemocytometer, and stored in 0.5 ml 5% SSA. Samples were stored at -70°C until extraction and derivatization for HPLC.

To 1 ml of plasma (containing L-serine/sodium borate) or RBC in 5% SSA (diluted 200 times) 100 µl penicillamine (8 mg/100 ml) were added as an internal standard. Plasma or RBC extract (220 µl; prepared after sonication and centrifugation) was derivatized with 40 µl thiole (monobromo-bimane; 5 mg/0.7 ml acetonitrile), in the dark, at pH 8.0 for 20 min. The reaction was stopped and protein was precipitated with 40 µl of 50% ice-cold SSA. After removing the precipitate, 10 µl of the supernatant was injected into the HPLC column. This procedure measured free (reduced) GSH. Total thiols (mixed disulfides, including protein disulfides) were determined by treating the samples with DTT (2.5 mM final) prior to derivatization.

PBL (0.5–1.5 x 10⁶ cells) were extracted into 0.5 ml of 5% SSA by sonication. The derivatization of GSH in the extract was identical to that for RBC and plasma. An external standard method was used for the quantitation of GSH in PBL because of the recovery differences in penicillamine if the standards were not prepared in the same biological matrix.

The HPLC procedure was performed by the method of Fahey and Newton (11). The thiol separation was carried out on an Altech C18 column with a 0.25% acetic acid to methanol gradient. Analytical standards prepared in the same biological matrix were used for quantitation of the different thiols, except for GSH in PBL where standards were prepared in 5% sulfosalicylic acid.

The determinations of day-to-day precision and accuracy of measurements for the different thiols were carried out based on 9 standard curves generated on 3 different days for each thiol. The day-to-day precision as determined by the coefficients of variation for the NAC in plasma, CYS in plasma, GSH in plasma, GSH in RBC and GSH in PBL at 1 µg/ml were 20.4, 20.1, 9.8, 23.0, and 19.7%, respectively, and at 5 µg/ml they were 14.7, 7.3, 8.3, 13.5, and 16%, respectively. Using the data from 2 standard curves at a time and back calculating the concentrations for the remaining values, estimated accuracy rates of 99% for NAC in plasma, 91% for CYS in plasma, 93% for GSH in plasma, and 91% for GSH in RBC and PBL were calculated for the determination of the unknowns.

The activity of GST in PBL was measured by the method of Habig et al. (12) using 1-chloro-2,4-dinitroben-
enzene as the substrate. The GST activity is expressed as nmol of product formed/min/mg protein.

The GRD assay in PBL extracts was carried out by the method of Racker (13) with the modifications of Ansher et al. (14) with NADPH and oxidized glutathione as the substrates. Enzyme activity is expressed as units/min/mg protein.

Both enzyme assays were linear with respect to protein concentration and time for up to 10 min; the assays were typically carried out for 5 min.

Data Analysis. Calculation of the pharmacokinetic parameters $AUC_{0-\infty}$ and $t_{1/2}$ was carried out using the noncompartmental pharmacokinetic data analysis program LA-GRAN (15). For $C_{max}$ versus time data, the computer program calculates the terminal $AUC$ as the ratio of the last concentration value and $\lambda_2$ as the slope of the terminal disposition phase (15). The correlations between pharmacokinetics and pharmacodynamics and the statistical significance of the GSH variations were evaluated using the computer program EPISTAT.

Results
Twenty-six subjects were entered, 13 on the dose escalation and 13 on the constant daily dose stage of the study. Details of the subjects with their risk factors are given in Table 1.

In the dose escalation part of the study all 13 subjects tolerated 800 mg/m²/day, 11 tolerated 1,600; 7, 3,200; and 4, 6,400 mg/m²/day without side effects. The side effects were minor and consisted mainly of gastrointestinal symptoms of which the most frequently reported were the effects of excessive intestinal gas. These side effects are detailed in Table 2.

A dose of 800 mg/m²/day did not cause side effects but did produce measurable pharmacodynamic effects (see below). This dose was, therefore, selected for daily administration in the constant dose part of the study. A single daily dose is most suitable for long-term administration in chemoprevention studies, so this schedule was selected for the remainder of the study. Thirteen subjects were entered on this part of the study. Eight completed 6 months of treatment without significant side effects. Three subjects withdrew because of gastrointestinal side effects (nausea, bloating, gas with foul odor). One subject had persistent leucopenia and was later found to have hairy cell leukemia; one subject had a syndrome of rhabdomyolysis. The subject with hairy cell leukemia was a 58-year-old white male with an 80 pack-year smoking history and a strong family history of malignancy but no other relevant past history. His CBC on screening work-up were declined by the subject who became totally symptom free. Apart from this subject and the subject who was subsequently shown to have hairy cell leukemia, there were no abnormalities noted in any of the monthly CBC or serum chemistry profiles. No changes were noted on EKG.

Pharmacokinetics and Pharmacodynamics
The total NAC in plasma was measured for pharmacokinetic evaluation. As pharmacodynamic end points, which are often referred to as intermediate biomarkers, total GSH in plasma, free and total CYS in plasma, and free and total GSH in RBC and PBL were measured. Additional pharmacodynamic end points included the measurement of GST and GRD activities in PBL.

During the dose escalation part of the study, the plasma and RBC thiol measurements were carried out in individual samples during a 6–8 h period following NAC administration. The GSH levels and enzyme activities in the PBL were carried out in pooled lymphocytes in a 6–8 h period after NAC. Data obtained for 7 subjects (3 at the 400 mg/m² dose), at the beginning of the study and again at the end of each month, monitored for a total of 6 months at the administered doses of 400–3,200 mg/m² NAC is presented for the dose escalation part.

During the constant dosing part of the study, NAC in plasma and GSH and GRD in PBL were measured pretreatment, 1 and 4 h after NAC for each subject after the first dose and after the end of month 1, 2, and 6. Data presented are for the 13 subjects entered into this part of the study, except for the final visit at which time only 8 subjects

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Side effects noted in more than one subject (n = 13)</td>
<td></td>
</tr>
<tr>
<td>Intestinal gas*</td>
<td>7</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>6</td>
</tr>
<tr>
<td>Heartburn</td>
<td>5</td>
</tr>
<tr>
<td>Bad taste</td>
<td>5</td>
</tr>
<tr>
<td>Nausea</td>
<td>4</td>
</tr>
<tr>
<td>Fatigue</td>
<td>2</td>
</tr>
<tr>
<td>Nontoxic dose (mg/m²/day, n = 13)</td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>13</td>
</tr>
<tr>
<td>1,600</td>
<td>11</td>
</tr>
<tr>
<td>3,200</td>
<td>7</td>
</tr>
<tr>
<td>6,400</td>
<td>4</td>
</tr>
</tbody>
</table>

*Bloating, flatulence, foul odor, cramps.
remained on the study; a complete set of samples was available for 6 of these subjects.

**Pharmacokinetics of NAC.** The plasma absorption and decay profile for total NAC measured in plasma at 5 dose levels is shown for a representative subject in Fig. 1. In all the subjects studied, absorption of NAC was rapid $t_{\text{max}}$: median, 1.0 h; range, 0.5–3 h; mean, 1.4 ± 0.7 h (SD) with a monoeponential decay $t_{1/2}$: median, 2.4 h; range, 1.4–3.9 h; mean, 2.5 ± 0.6 h. Pharmacokinetics were linear with the administered dose in the range of 200–3200 mg/m² (AUC versus dose $r$, 0.887; $P < 10^{-6}$; $C_{\text{max}}$ versus dose $r$, 0.867; $P < 10^{-6}$).

**Pharmacodynamics during Dose Escalation.** The basal levels of total GSH in plasma averaged 3.8 ± 1.0 μM; that of free CYS 40 ± 30 μM and total CYS 250 ± 60 μM. Free GSH in RBC constituted 72% of the total and averaged 2.1 ± 0.5 μM. The thiols (CYS and GSH), measured as pharmacodynamic end points in plasma, and RBC fluctuated in the 6–8 h period after NAC with no specific pattern; none of these thiols in plasma and RBC showed an increase in their mean concentration after NAC, at doses of 200–3200 mg/m². The basal activity of GST in PBL was 40 ± 23 nmol/min/mg protein and also showed no change on any NAC dose.

The free GSH in PBL like that in RBC, accounted for approximately 70% of the total. The basal level of free GSH was 0.8 ± 0.4 nmol/10⁶ cells. In contrast to GSH in plasma and RBC, the GSH (both free and total) in PBL showed a statistically significant ($P < 0.05$) increase after NAC at doses of 800 mg/m² and above (Fig. 2). At the highest doses, a slight reduction in this increase was observed and the reason for this is not known. A trend toward an increase in the activity of GRD in the PBL was also seen at doses of 800 mg/m² and higher, although the elevations did not reach statistical significance (Fig. 2). The basal activity of GRD in PBL was 135 ± 69 units/min/mg protein.

**Constant Daily Dosing**

Since the dose escalation part of the study indicated that only the PBL GSH, and possibly the oxidized glutathione reductase were showing elevations after NAC, the pharmacodynamic aspects of the second part of the study focused on these, along with the pharmacokinetics of NAC. Pretreatment and 1 and 4 h posttreatment sampling times were selected for this part of the study based on the GSH profile obtained for 2 patients studied in detail (Fig. 3).

**NAC Pharmacokinetics.** At a dose of 800 mg/m²/day, there was extensive intersubject variation in plasma NAC levels. At the first visit the $C_{\text{max}}$ varied from 1.7 to 20.8 μg/ml ($n = 13$). At the end of the first month it was 2.8–18.5 ($n = 12$), at the end of the second month, 2.2–15.1 ($n = 9$) and after the final dosing 1.6–7.8 ($n = 7$). Similarly, the AUC varied from 9.2 to 83.9 μg/ml-h ($n = 13$) at the first visit, from 21.2 to 76.6 ($n = 12$) at the end of the first month, from 14 to 62.8 ($n = 9$) at the end of the second
month, and from 16.7 to 42.7 (n = 6) after the final dose. A summary of the results presented in Table 3 shows that the mean Cmax and the AUC for NAC decrease on chronic administration, indicating a change in pharmacokinetics.

**GSH and GRD.** Free GSH in PBL constituted 78% of the total and had a mean value of 1.4 ± 0.5 nmol/10⁶ cells (n = 13) pretreatment. The changes in free and total GSH following NAC are shown in Figs. 4 and 5. Not all subjects showed an elevation in GSH following NAC; some showed a decline. Where an increase was observed it did not always show a maximum at 1 h with a decline thereafter as had been predicted from initial studies in 2 patients (Fig. 3). However, taking an arbitrary 20% increase of GSH in PBL as a significant positive change after NAC, 29–67% of the individuals showed the positive change during the different monthly visits (Table 4). However, as seen in Figs. 4 and 5, the observed increases were not confined to the same subjects through this trial. A comparison of the AUC(0.4) for GSH from the initial visit and the remaining visits using a t test indicated no statistically significant differences. Similarly a paired t test between the pretreatment GSH values and those obtained at the end of 1, 2, and 6 months of chronic NAC administration indicates no statistically significant changes. No statistically significant correlations could be found between the Cmax and AUC of NAC and between the percentage of change of GSH in PBL at 1 and 4 h after NAC during any of the visits.

The base-line GRD levels in this group of subjects was quite variable ranging from 187 to 640 units/min/mg protein [mean, 375 ± 115.3 (SD)]. The profile of changes at 1 and 4 h after NAC indicated that the enzyme activity varied extensively (data not shown). Increases of greater than 20% in GRD in PBL were observed for a much smaller number of subjects than those for GSH (Table 4); a significant proportion of the changes in GRD were negative. A statistical evaluation (paired t test) of pretreatment GRD activity between day 1 and the end of the first and the second months showed no statistically significant differences (Table 5). However, the same between day 1 and that at the end of the final month, showed a statistically significant decline (P = 0.003; n = 6) in the activity for GRD (Table 5).

**Discussion**

NAC has been shown to be chemopreventive in experimental systems, and this effect is thought to be related to its acting as a precursor of CYS, the rate-limiting substrate in the biosynthesis of GSH, an intracellular scavenger of free radicals, and electrophilic metabolites of carcinogens. The present study was designed to identify a dose of NAC which would be well tolerated on chronic p.o. administration but that would produce measurable modulation of the pharmacodynamic end points, namely GSH and possibly GSH-metabolizing enzymes. The starting dose of 400 mg/m²/day was chosen for the dose escalation part of the study based on a previous report (16) that 5.6 g/day (≈3.3 g/m²/day) chronic p.o. administration of NAC for 6 months was well tolerated, with vomiting (1 of 12) and diarrhea (4 of 12) as the only side effects. Six of these subjects received 11.2 g/day for a further 3 months without vomiting or diarrhea. We chose approximately one-tenth of the lower chronic dose used in that study (16) as the safe starting dose for our trial.

Side effects in the dose escalation study were mainly gastrointestinal and minor. A dose of 800 mg/m²/day was chosen for the constant dose part of the study as a dose which was tolerated without side effect by all subjects. However, this dose proved to be at the upper limit of tolerability for continuous administration as 3 of 13 subjects withdrew because of gastrointestinal side effects. For a longer study in larger populations, a slightly lower dose might be required. However in the present study the next lowest dose (400 mg/m²) did not demonstrate any modulation of intracellular GSH (Fig. 2), which might indicate
Pharmacodynamics of N-Acetylcysteine
dosing of the study. Data presented are the percentage change in the level of
total GSH over the pretreatment. The description of each of the bars and the
total GSH in PBL at 1 and 4 h after NAC during the constant daily
Figure 5. Total GSH in PBL at 1 and 4 h after NAC during the constant daily
closing.

Table 4 Numbers of subjects showing greater than 20% increase in free and total GSH and GRD in PBL following NAC at the different visits during the constant daily dosing of NAC:

<table>
<thead>
<tr>
<th>Pharmacodynamic parameters</th>
<th>Subject Day 1</th>
<th>End of 1 month</th>
<th>End of 2 months</th>
<th>End of 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>After the first dose (n = 13)</td>
<td>7 (54%)*</td>
<td>8 (62%)</td>
<td>4 (33%)</td>
<td>5 (42%)</td>
</tr>
<tr>
<td>End of 1 month (n = 12)</td>
<td>7 (54%)*</td>
<td>8 (62%)</td>
<td>6 (50%)</td>
<td>5 (42%)</td>
</tr>
<tr>
<td>End of 2 months (n = 10)</td>
<td>2 (15%)</td>
<td>3 (25%)</td>
<td>2 (17%)</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>End of 6 months (n = 6)</td>
<td>1 (16%)</td>
<td>2 (20%)</td>
<td>1 (16%)</td>
<td>1 (16%)</td>
</tr>
</tbody>
</table>

*Activity, units/mg protein/min.

that it may be too low. In the ongoing EUROSCAN trial being conducted by the European Organization for Research on Treatment of Cancer, 600 mg/day (~350/mg/m²/day) is being given for chemoprevention. In this trial, randomization is between retinol, NAC, and retinol plus NAC. More than 2000 subjects have been randomized onto this trial in which treatment is given daily for 2 years. Data from this trial are not available at this time.

Two additional subjects were removed from the trial. One of these proved to have hairy cell leukemia which presumably antedated his treatment with NAC although review of the peripheral blood smears done at the time of accession onto the trial did not show any hairy cells. The other developed a syndrome of muscle pain and weakness, secondary to acute rhabdomyolysis during his first month on the trial. Because he developed this while on NAC and because it resolved rapidly when he was taken off the drug, this must be assumed to be an unusual idiosyncratic reaction to the drug. Because the subject declined a muscle biopsy, a more definitive statement cannot be made.

The NAC pharmacokinetics in general are consistent with those in the literature showing rapid absorption and short half-life (17, 18). The data from the dose escalation part of the study indicate linear kinetics for NAC. However, chronic administration at the dose of 800 mg/m² indicates that there is either a lowered absorption or an increased metabolism of NAC on prolonged administration. The NAC pharmacokinetics were highly variable between subjects, indicating variations in absorption and/or metabolism of this agent.

As possible pharmacodynamic end points, we measured GSH in plasma and RBC, PBL, and CYS in plasma, during the dose escalation part of the study. Of these, only GSH in PBL was elevated after NAC. The lack of an effect of NAC on plasma GSH is not surprising since plasma lacks GSH biosynthetic enzymes, liver being an important source for plasma GSH (19); elevations in plasma GSH have been observed only in circumstances where cellular GSH requirements are increased such as after paracetamol ingestion (9). The failure of NAC to augment GSH pools in RBC is more difficult to explain. NAC raises the intracellular levels of GSH in RBC of rodents (20) an observation that formed the basis of our decision to monitor GSH in RBC in the present study. The reason for this species difference is unclear. There may be differences in the deacetylation of NAC and in the uptake of NAC and/or CYS.

In this study we also examined the possibility that GSH-metabolizing enzymes might be induced by NAC, as has been reported by De Flora et al. (20) in rodent tissues. While we saw no increase in the activity of GST in PBL, consistent with the findings of De Flora et al. (20), a trend toward an increase in GRD was found with escalating doses of NAC, which did not, however, show the same trend during the constant daily dosing of the study. The pretreatment (basal) GRD activity itself showed a decline from the beginning of the study to that at the last dosing of NAC; the reason for this is unclear.
The elevation in PBL GSH following NAC observed in the second part of the study was transient and did not elevate basal (pretreatment) levels of GSH in PBL. In addition this elevation was not seen in all of the subjects entered into the study. In some subjects there was even a lowering of GSH in post NAC samples. It is perhaps not altogether surprising that this was seen in a few cases since the size of the intracellular GSH pool is the net result of biosynthesis and depletion by a variety of factors, which we did not attempt to control in this study, including food additives and tobacco smoke. Additional poorly understood factors, such as possible efflux of GSH from the cell and factors which regulate this, may also contribute to the net GSH level in PBL. Efflux of GSH has been demonstrated in cultured lymphoid cells under conditions when the salvage pathway activity was inhibited (21).

The modulation of GSH is an attractive objective. However, it is clearly difficult to increase the pools indefinitely because of the tight feedback regulation of the biosynthesis by the end product GSH. Moreover, we have no information on how GSH modulation in PBL in humans reflects the situation in the other organs such as the liver and lung, which are important in the detoxification activity of GSH. Studies in rats have indicated that NAC produces significant elevations of GSH in lung and liver tissue (20).

The significant toxicity seen in this trial at the doses of NAC required to modulate the intermediate markers is of concern and should be evaluated in more extensive blind-placebo controlled Phase II trials. A lower dose of NAC is being tolerated in the ongoing EUROSCAN trial as discussed above, but this dose may be insufficient to produce a significant modulation of the intermediate end points. The final results from the EUROSCAN trial will not be available for some years.

In summary, the present study has demonstrated a short-term modulation in GSH pools in PBL in some subjects at doses of NAC which are tolerated by most. The impact of this on the chemopreventive potential of NAC in the clinical situation must be determined in larger and more extensive trials.

Acknowledgments

We acknowledge with gratitude the contributions of Joseph R. Zdanowicz, Michael I. Murphy, and Martha V. Molnar for assistance with the laboratory assays; Elmer Berghorn, R.N., April Proefrock, R.N., and Kimberly Wilkes, R.N., for monitoring of the study subjects; Dr. William Greco for statistical validation of the HPLC assays; Marilyn Kuroski for data entry; Marty Courtney for manuscript preparation; and Dr. Derek Raghavan for reviewing the manuscript and offering many helpful suggestions.

References

Pharmacokinetic and pharmacodynamic studies of N-acetylcysteine, a potential chemopreventive agent during a phase I trial.

L Pendyala and P J Creaven