CYP2E1 and Carboxylesterase Enzymes in Vinyl Carbamate Metabolism in Human Lung Microsomes

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ABSTRACT:

Previous studies have shown that CYP2E1 and carboxylesterase enzymes contributed to vinyl carbamate (VC) metabolism in murine lung. Moreover, these studies have implicated CYP2E1 and the carboxylesterases in bioactivation and detoxication, respectively. Here we have tested the hypothesis that CYP2E1 and carboxylesterase enzymes are involved also in VC metabolism in human lung. Demethylation of N-nitrosodimethylamine (NDMA) is an enzyme activity associated with CYP2E1, and was used as a catalytic marker for this P450 in human lung microsomes. NDMA demethylation activity in lung microsomes from 10 patients ranged from 36.9 ± 1.0 to 82.4 ± 2.4 pmol/mg protein/min. Significant decreases (40–65%) in demethylation activity were detected in lung microsomes incubated with VC and NADPH, compared with the controls in which incubations were performed with only VC or only NADPH. Preincubation with the CYP2E1 inhibitor diallyl sulfone also significantly decreased demethylase activity, and abrogated the VC-induced effect. Similarly, preincubation of lung microsomes with a human CYP2E1 inhibitory monoclonal antibody ameliorated the VC-induced reduction in demethylase activity. Microsomal carboxylesterase activity in lung microsomes from 10 patients ranged from 19.02 ± 2.28 to 48.18 ± 4.34 nmol/mg protein/min, and was significantly decreased (25–45%) in microsomes incubated with phenylmethylsulfonyl fluoride, an inhibitor of the carboxylesterase enzyme. Preincubation of lung microsomes with phenylmethylsulfonyl fluoride and subsequent incubation with VC and NADPH exacerbated the reduction (60–80%) in demethylase activity evoked by reaction with VC and NADPH. These results are consistent with a role for the CYP2E1 enzyme and microsomal carboxylesterases in VC metabolism.

Vinyl carbamate (VC) is derived from metabolism of ethyl carbamate (EC), a by-product that is formed during the process of fermentation and that is present in alcoholic beverages and a wide variety of fermented foods, including soya sauce, bread, cheeses, and yogurt (Battaglia et al., 1990; Zimmerli and Schlatter, 1991). EC is found also as a natural constituent in tobacco leaves and tobacco smoke (Schmeltz et al., 1978). Although both of these carbamates possess carcinogenic properties and induce the same spectra of tumors in mice and rats, VC is a much more potent carcinogen than EC (Dahl et al., 1978, 1980). VC, but not EC, is mutagenic to Salmonella typhimurium, and is more active in inducing sister chromatid exchange in mammalian cells (Dahl et al., 1978, 1980; Allen et al., 1982; Leithauser et al., 1990). Both EC and VC formed DNA adducts in livers and lungs of mice, but formation of adducts induced by VC was produced at levels that were 3-fold greater than those for EC (Fernando et al., 1996). Moreover, VC generated numbers of lung tumors that were 20- to 50-fold greater than those produced by EC (Dahl et al., 1978, 1980). Interestingly, although EC induces tumors in a variety of tissues, including lung, skin, liver, and mammary gland (Salaman and Roe, 1953; Tannenbaum, 1964), the lung appears to be the most susceptible to tumor formation (Mirvish, 1968; Shimkin and Stoner, 1975).

Neither EC nor VC is carcinogenic in the parent form but requires bioactivation to a metabolite to produce a carcinogenic effect. The proposed mechanism and pathway of metabolism involve cytochrome P450-mediated oxidation of EC to VC, followed by an additional P450-catalyzed oxidation to the electrophilic metabolite VC epoxide (Dahl et al., 1978) (Fig. 1). This epoxide is believed to be the ultimate carcinogenic metabolite that binds to nucleic acids, leading to formation of RNA and DNA adducts (Dahl et al., 1978; Leithauser et al., 1990; Park et al., 1990, 1993). Studies in human liver microsomes implicated the P450 enzyme CYP2E1 as being involved in the two-step oxidation of EC to VC epoxide (Guengerich et al., 1991; Guengerich and Kim, 1991). Data from our recent studies in murine lung and liver supported a role for CYP2E1 in activation of EC to a reactive metabolite that binds covalently to microsomal proteins (Forkert and Lee, 1997; Lee et al., 1998). This binding is significantly decreased by treatment of microsomes with the CYP2E1 inhibitor diallyl sulfone (DASO2). Conversely, binding levels are augmented by inhibition of carboxylesterase enzymes with agents such as paraoxon or phenylmethylsulfonyl fluoride (PMSF), thus implicating this enzyme system in detoxication of EC. More recently, our studies in murine lung demonstrated that VC is metabolized also through a mechanism similar to that of EC (Lee and Forkert, 1999). Taken together, these findings suggested that the final balance resulting from...
activation and detoxication may determine the carcinogenic outcome resulting from carbamate exposure.

Concern has been raised regarding the potential risk of EC and VC to humans, particularly in view of high levels of EC used as a cosolvent in analgesic and sedative drugs in Japan between 1950 and 1975 (Nomura, 1975; Miller, 1991). There is also concern about the continuous long-term exposure of humans to low concentrations of EC that are present naturally in foods and alcoholic beverages (Ough, 1976; Zimmerli and Schlatter, 1991). We were interested in identifying the mechanisms leading to the toxic and/or carcinogenic effects of VC in human lung. As an initial step to address this issue, we have tested the hypothesis that the P450 enzyme CYP2E1 and microsomal carboxylesterases contribute to VC metabolism in human lung microsomal incubations. Our approach was to determine the extent to which the CYP2E1 enzyme is inactivated by VC, as assessed by N-nitrosodimethylamine (NDMA) demethylation, a catalytic activity associated with CYP2E1. We have determined also the effects of VC on carboxylesterase activity in human lung microsomes. We have additionally investigated the relationship between NDMA demethylase and carboxylesterase activities, as assessed by alterations in one enzyme activity to modify the other. It should be emphasized that the experiments were performed in lung microsomes from the same patients to obviate inherent metabolic differences that may be present in different individuals. Although not directly an objective, these studies were undertaken also with the anticipation that the levels of CYP2E1 and carboxylesterase activities detected in the lung tissue of these patients would be helpful to other studies involving these enzyme systems in human lung.

Experimental Procedures

Materials. Chemicals and reagents were obtained from suppliers as detailed in the following: phenylmethylsulfonyl fluoride, p-nitrophenol and p-nitrophenyl acetate from Sigma Chemical Co. (St. Louis, MO); [14C]formaldehyde (>95% radiochemical purity, specific activity 10 mCi/mmole) from NEN Life Science Products (Boston, MA); dialyl sulfone (>97% purity) from Parish Chemical Co. (Orem, UT); and Eco-Lite scintillation fluid from ICN Chemical Co. (Costa Mesa, CA). [14C]N-nitrosodimethylamine, formaldehyde-free, specific activity 40 mCi/mmol, was kindly donated by Dr. C. S. Yang (Laboratory for Cancer Research, Rutgers University, Piscataway, NJ). Vinyl carbamate was a generous contribution from Dr. J. A. Miller (McArde Laboratory for Cancer Research, University of Wisconsin, Madison, WI). An inhibitory human CYP2E1 (mAb 1-73-18) monoclonal antibody was a generous donation from Dr. H. V. Gelboin (Laboratory of Molecular Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, MD). All other chemicals were of reagent grade and were purchased from standard commercial suppliers.

Preparation of Microsomes. Samples of human lung tissue were obtained from Kingston General Hospital (Kingston, Ontario, Canada) from consenting patients undergoing surgical lobectomies. Tissue distant from primary lesions was surgically excised and placed immediately on ice. Samples were transferred to a biohazard facility and submerged in cold 100 mM K2HPO4 buffer containing 1.5 mM EDTA, pH 7.0. Tissue samples were minced with scissors and individual pieces were thoroughly rinsed in cold buffer to remove excess blood from the tissue. This rinsing step was repeated several times to minimize sample contamination with blood. After clearing the tissue of blood, a small tissue sample was removed and fixed with 4% paraformaldehyde in 0.1 M Sorenson’s phosphate buffer for histopathological analysis to confirm normal structure. The remaining tissue was used for preparation of microsomes, using differential centrifugation techniques described in our previous studies (Lee and Forkert, 1995). Microsomal pellets were resuspended in 4 volumes of 100 mM K2HPO4 buffer containing 1.5 mM EDTA, pH 7.0, and aliquots (250 μl) were dispensed into Eppendorf tubes. Microsomes were layered over argon, frozen in liquid nitrogen, and stored at −70°C. Protein concentrations were determined by the method of Lowry et al. (1951), using bovine serum albumin as the standard.

Microsomal Incubations. Human lung microsomes at a protein concentration of 5 mg were suspended in 1 ml of 100 mM K2HPO4 buffer containing 1.5 mM EDTA, pH 7.0. Components of a NADPH-generating system (7.5 mM glucose 6-phosphate, 2 units of glucose-6-phosphate dehydrogenase, 5.0 mM MgCl2, and 0.4 mM NADP+) were added and reaction mixtures were preincubated for 3 min at 25°C in a shaking water bath. The reaction was initiated by addition of VC dissolved in water. The reaction was continued for an additional 30 min, and was terminated by cooling on ice. The microsomes were then washed in 100 mM K2HPO4 buffer containing 1.5 mM EDTA, pH 7.0, and centrifuged for 30 min at 105,000g. The resulting pellet was resuspended in buffer (1 ml), and was used for enzyme assays.

Dialyl sulfone was used to inhibit CYP2E1-selective catalytic activity in lung microsomes. This chemical has been shown to be an efficacious inhibitor of lung CYP2E1 in mice (Forkert et al., 1996). The reaction mixtures, containing microsomes and components of the NADPH-generating system, were preincubated for 3 min at 37°C in a shaking water bath. The reaction was initiated by addition of DASO2 in water, and incubation was continued for an additional 30 min. The samples were then placed on ice to terminate the reaction, and the microsomes were washed, recovered, and incubation with VC and/or enzyme assays performed.

For the immunoinhibition experiments, the microsomal suspensions were preincubated for 3 min at 25°C. An inhibitory human CYP2E1 mAb (Gelboin et al., 1996) was added, using a microsomal protein:antibody protein ratio of 0.5, and the reaction mixtures were incubated for an additional 30 min at 25°C with gentle agitation. The reaction was terminated by placing the samples on ice, and subsequently incubated with VC, as described previously. Phenylmethylsulfonyl fluoride was used to inhibit microsomal carboxylesterase activity. Our previous studies showed that PMSF significantly inhibits lung microsomal carboxylesterase activity in mice (Forkert and Lee, 1997). The reaction mixtures were preincubated for 3 min at 25°C. The reaction was initiated by addition of 25 μM PMSF in dimethyl sulfoxide, and the incubation was continued for an additional 20 min. The samples were cooled on ice; the microsomes were then washed and recovered as described. The microsomes were resuspended in 100 mM K2HPO4 buffer, pH 7.0, and incubation with VC and/or enzyme assays performed.

Enzyme Assays. N-Nitrosodimethylamine demethylase activity was determined by using a radiometric assay, and was performed as described in our previous studies (Forkert et al., 1996). The reaction mixtures contained 5 mg of microsomal protein suspended in 100 mM K2HPO4 buffer containing 1.5 mM EDTA, pH 7.0, and components of the NADPH-generating system as detailed previously. The final incubation volume was 1 ml. The microsomal samples were preincubated for 3 min at 37°C after which the reaction was initiated by addition of 40 μl of [14C]NDMA (specific activity 40 mCi/mmol). The [14C]NDMA was purified before performing the enzyme assay by using a Dowex-1-bisulfite column, and following procedures described in our previous studies (Forkert et al., 1996). The reaction mixtures were incubated for 10 min at 37°C in a shaking water bath; the reaction was terminated by cooling of the
samples on ice and addition of a solution containing 1 M sodium acetate, pH 4.5, 100 mM HCHO, and 400 mM dimethone. The incubation vessels were capped, and the samples were vortexed and boiled for 5 min. The [14C]formaldehyde was then extracted into hexane (4 ml). After an additional extraction with H2O, the hexane layer (2 ml) was dispensed into scintillation fluid, and levels of radioactivity were determined. Demethylase activity was determined by measuring the amounts of [14C]HCHO formed. Triplicate determinations from microsomal preparations of individual patients were performed in simultaneous incubations. Preliminary experiments were performed to assess linearity of reactions with VC. NDMA demethylase activity declined progressively in microsomal incubations containing an NADPH-generating system and VC concentrations ranging from 10 μM to 1 mM, with no further alterations at VC concentrations ranging from 1 to 10 mM. Time course experiments revealed decreases in demethylase activity at incubation times ranging from 0 to 30 min, with no further reduction at times ranging from 30 to 90 min. Based on these data, a concentration of 0.5 mM and an incubation time of 30 min were used in all experiments with VC.

Microsomal carboxylesterase activity was determined by using procedures described in our previous studies (Forkert and Lee, 1997). The reaction mixture in a final volume of 1 ml contained 100 mM K2HPO4 buffer, pH 7.0, 1.5 mM EDTA, and 500 μg of microsomal protein. This reaction mixture was placed in a 1.5-ml sample cuvette, and the reaction was initiated by addition of 1 mM p-nitrophenyl acetate in dimethyl sulfoxide. The hydrolysis of p-nitrophenyl acetate to p-nitrophenol was determined spectrophotometrically by measuring changes in absorbance at 410 nm. The reference cuvette contained only the buffer and substrate to correct for any nonenzymatic hydrolysis. Formation of p-nitrophenol was determined by comparison with a standard calibration curve relating absorbance levels to known amounts of p-nitrophenol. Triplicate determinations from microsomal preparations of individual patients were performed in the same incubations.

Statistical Analysis. Data are expressed as mean ± S.D., and were analyzed by one-way analysis of variance, followed by the Student-Newman-Keuls test to identify significant differences between experiments. The level of significance was set at p < 0.05.

Results

Effects of VC and/or PMSF on NDMA Demethylase Activity. Results of the microsomal incubations with VC and/or PMSF on NDMA demethylase activity in 10 patients are summarized in Table 1. Control experiments consisted of lung microsomal incubations that were performed with only VC (0.5 mM) or only an NADPH-generating system. The control levels of demethylase activity detected in 10 patients ranged from 36.91 ± 1.00 to 82.36 ± 2.40 pmol/mg protein/min, and were similar in microsomes incubated with VC alone or NADPH alone. Similar levels of demethylase activity were observed in lung microsomes incubated with the carboxylesterase inhibitor PMSF and an NADPH-generating system. These demethylase activities represented the constitutive levels of CYP2E1 present in the human lung microsomes. However, incubations of microsomes with VC in the presence of an NADPH-generating system produced significant decreases of 40 to 65% in NDMA demethylation in microsomes from all 10 patients examined. This decrease in demethylase activity was exacerbated and was elevated to 60 to 80% in microsomes preincubated with PMSF and incubated subsequently with VC and an NADPH-generating system (Table 1).

Effects of DASO2 or a CYP2E1 Inhibitory mAb on NDMA Demethylase Activity. The results of microsomal incubations with DASO2 and/or VC on NDMA demethylase activity are summarized in Fig. 2. Control levels of demethylase activity in the five patients studied were similar to those obtained in the same patients in separate microsomal incubations, and ranged from 53.49 ± 1.95 to 81.08 ± 3.56 pmol/mg protein/min (Table 1; Fig. 2). Significant decreases (35–45%) in demethylase activity were detected in microsomes incubated with VC and an NADPH-generating system, compared with levels in the controls. Similar decreases (40–50%) were obtained in lung microsomes reacted with only DASO2. No additional diminution in demethylase activity was found in microsomes reacted with both DASO2 and VC, compared with the amounts detected in incubations with DASO2 alone.

Immunoinhibition studies with an inhibitory human CYP2E1 mAb were performed to confirm further the contribution of CYP2E1 to VC metabolism in human lung microsomal incubations. Figure 3 summarizes the results of these immunoinhibition experiments. Levels of NDMA demethylase activity in the controls were consistent with those detected in microsomal preincubations from the same five patients in separate incubations, and ranged from 59.12 ± 1.61 to 80.54 ± 2.39 pmol/mg protein/min (Table 1; Fig. 3). The levels were significantly decreased (35–50%) in lung microsomes reacted with only DASO2. No additional diminution in demethylase activity was found in microsomes reacted with both DASO2 and VC, compared with the amounts detected in incubations with DASO2 alone.

Effects of PMSF and/or VC on Microsomal Carboxylesterase Activity. Results of reactions with PMSF and VC on microsomal carboxylesterase activity are summarized in Table 2. Substantial amounts of carboxylesterase activity, as assessed by hydrolysis of
carboxylesterase activity were similar in control incubations in which microsomes were incubated with only the vehicle or only an NADPH-generating system (Table 2). However, the levels were significantly decreased (25–45%) when microsomes were incubated with PMSF, relative to control levels. Carboxylesterase activity detected in microsomal incubations containing only PMSF was analogous to those detected in incubations containing only VC or PMSF and VC (Table 2).

Discussion

Impetus for this investigation on VC emanated from issues raised by findings from studies of exposures to carbamate compounds and their potential carcinogenic effects. In a letter published in 1975, Nomura provided details on the medical use of EC in Japan between 1950 and 1975 (Nomura, 1975). EC was used as a cosolvent for analgesic and sedative drugs and it was estimated that the total dose of EC administered to a 60-kg patient was about 0.6 to 3.0 g. These EC doses produced a high frequency of lung tumors in mice (Nomura, 1975). This disclosure prompted Miller (1991) to urge in the strongest terms that epidemiological studies be performed to determine incidences of tumors in the patients given EC, compared with those in the normal population. It has been 50 years since the start of the 25-year period during which millions of humans were administered "the largest doses of a pure carcinogen that is on record" (Miller, 1991). To the best of our knowledge, no such studies were initiated (Dr. J. A. Miller, personal communication). Also of concern is the potential carcinogenic risk to humans posed by long-term continuous exposure to relatively low levels of EC from dietary sources, including a wide variety of fermented food products and alcoholic beverages (Zimmerli and Schlatter, 1991). The risk associated with EC is presumably related to the capacity for metabolism of EC to VC and the subsequent oxidation of VC to the VC epoxide, an electrophilic species believed to be the ultimate carcinogen (Dahl et al., 1978, 1980; Park et al., 1990, 1993). Of interest in this respect is the high susceptibility of the lung to tumor formation in mice (Mirkish and Stoner, 1975), and the high numbers of lung tumors induced by VC. A long latent period of 1 year is required for tumor development in a variety of tissues; in contrast, lung tumors developed more rapidly and were manifested about 2 to 6 months after EC treatment. In an initial effort to address questions that were raised by the available data, we have investigated herein the potential of human lung microsomes to metabolize VC and to inactivate enzymes implicated in its metabolism. Also of importance is the issue of whether the mouse model is relevant for studying the effects of carbamate exposure in the human.

Previous studies have identified the metabolite generated from EC and VC as the VC epoxide (Dahl et al., 1978; Guengerich et al., 1991). The metabolic pathway by which this epoxide is generated has been investigated in human liver microsomes, and the findings implicated the CYP2E1 enzyme in EC and VC oxidation (Guengerich et al., 1991; Guengerich and Kim, 1991). A similar CYP2E1-mediated pathway has also been identified in murine lung and liver for the metabolism of EC and VC (Forkert and Lee, 1997; Lee et al., 1998; Lee and Forkert, 1999). Our results described herein showed that NDMA demethylase activity was significantly inhibited (40–65%) in human lung microsomes incubated with VC and NADPH (Table 1). These data implicating CYP2E1 in VC oxidation are supported by findings from experiments with DASO₂, a garlic derivative that has been shown to be an efficacious inhibitor of lung CYP2E1 in mice (Forkert et al., 1996b, 2000). Incubation of lung microsomes with DASO₂ and NADPH resulted in significant reduction in NDMA demethylase activity, producing levels similar to those in incubations with VC and NADPH or DASO₂, VC, and NADPH.
Our results from the immunoinhibition experiments showed also that reaction of microsomes with a CYP2E1 inhibitory mAb significantly decreased demethylase activity (~65%), and there was no further reduction in activity upon subsequent reaction with VC (Fig. 3). These findings from the inhibitory studies suggested that all of the CYP2E1 enzyme that is available for VC metabolism has been inhibited by DASO₂ or the CYP2E1 mAb, suggesting that once inhibited and/or inactivated, no further metabolism of VC by CYP2E1 can proceed. Interestingly, the decrease in demethylase activity produced by the mAb was greater than that elicited by VC, suggesting that metabolism of VC by CYP2E1 had achieved saturation. Taken together, these data derived from three separate sets of experiments exhibited consistency in all the patients examined, and supported a mechanism in which VC metabolism is mediated by lung microsomal CYP2E1.

Previous studies indicated that more than 90% of a dose of EC is converted to CO₂ and ethanol (Boyland and Rhoden, 1949; Bryan et al., 1949) (Fig. 1). Moreover, studies in liver homogenates showed that the microsomal carboxylesterases were involved in the hydrolysis of EC to CO₂ (Mivris, 1968; Yamamoto et al., 1990). More recently, our studies have shown that the microsomal carboxylesterases played an important role in detoxication of VC in murine lung, inasmuch as levels of covalent binding to microsomal proteins increased when the carboxylesterase pathway was inhibited (Lee and Forkert, 1999). In the present study, levels of carboxylesterase activity in human lung tissue from 10 patients ranged from 19.02 ± 2.28 to 48.18 ± 4.34 nmol/mg protein/min, and were significantly decreased in microsomal incubations containing VC (Table 2). Inhibition of the carboxylesterases with PMSF significantly decreased enzyme activity, so that no further reduction occurred upon subsequent incubation with VC (Table 2). However, preincubation of microsomes with PMSF exacerbated the diminution in NDMA demethylation evoked by VC (Table 1). This decrease was not due to the direct action of PMSF on demethylase activity because incubation of microsomes with PMSF alone produced no alterations in levels of demethylation (Table 1). These findings suggested that VC is metabolized by the carboxylesterases in human lung microsomes, and are consistent with a role of detoxication inasmuch as metabolism by the carboxylesterase pathway exacerbates VC bioactivation by CYP2E1 (Table 1). In regard to PMSF, previous studies in rats have reported that although paraxox in particular by hydrolase A (Forkert and Lee, 1997; Lee et al., 1998). A similar pathway of metabolism by the carboxylesterases and hydrolase A has also been shown for VC in murine lung (Lee and Forkert, 1999). These results supported a pertinent role for the microsomal carboxylesterases and hydrolase A in VC metabolism in mice, but this isozyme-selective pathway has not been identified in human lung.

A question arises regarding the extent to which the lung metabolism of VC in mice reflects that in the human lung, and the relevance of the murine model for investigating the metabolic and toxic effects of the carbamate compounds in humans. The comparisons, although restricted, are nonetheless of interest. There are similarities in the metabolic disposition of VC in human and murine lung microsomes in at least some respects. Our results showed that levels of NDMA demethylase activity were detectable in microsomes from the lungs of all 10 patients studied, and this activity was inhibitable by both DASO₂ and a human CYP2E1 mAb (Tables 1; Figs. 2 and 3). Levels of demethylase activity in human lung described herein were markedly lower than those found in murine lung (Forkert and Lee, 1997; Lee and Forkert, 1999). Comparative studies with lung microsomes performed under identical incubation conditions showed that the mean rate of CYP2E1-dependent p-nitrophenol hydroxylation was about 7-fold higher in murine lung than in human lung (Dowsley et al., 1999). In the context of enzyme activities, our previous studies confirmed that the catalytic activities associated with CYP2E1, as estimated by p-nitrophenol hydroxylation and NDMA demethylation, yielded levels that are surprisingly similar (Forkert et al., 1996a). Hence, both of these enzyme activities are comparable for estimates of the catalytic function of CYP2E1, and the available data indicated that levels of CYP2E1 activity are markedly higher in murine than in human lung. On the other hand, levels of carboxylesterase activity are markedly higher in human lung (Table 2) than in murine lung (Lee and Forkert, 1999) in terms of absolute amounts. However, metabolism of VC in human and murine lung is abrogated by prior reaction with PMSF, suggesting that hydrolyase A is involved in VC metabolism in both tissues. Taken together, the findings indicated that, although CYP2E1 and carboxylesterase activities differ quantitatively in human and murine lung, the data obtained are consistent with involvement of both CYP2E1 and carboxylesterase enzymes in VC metabolism in both these species. Hence, the murine model is a relevant one for investigating mechanistic aspects of VC exposures in the human. However, it might be speculated that the relative risk of lung exposure to VC is less in humans than in mice due to relatively lower CYP2E1 and higher carboxylesterase levels in the former.

### Table 2

<table>
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<tr>
<th>Patient</th>
<th>Control (nmol/mg protein/min)</th>
<th>+NADPH</th>
<th>+PMSF</th>
<th>+VC</th>
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<tr>
<td>1</td>
<td>47.13 ± 7.07</td>
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<td>30.51 ± 1.94&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>38.20 ± 4.58</td>
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<td>21.88 ± 3.69&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>24.10 ± 4.05&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>27.82 ± 2.76&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>a</sup> Experimental incubations were performed with PMSF alone (+PMSF), with VC alone (+VC), or with PMSF and VC (+PMSF + VC). Control incubations were performed with only the vehicle or with only an NADPH-generating system (+ NADPH). Values are mean ± S.D. of triplicate determinations in simultaneous incubations from the same microsomal preparations of individual patients.

<sup>b</sup> p < 0.05 compared with levels in the controls (vehicle and + NADPH).
investigation has set the stage for studies to elucidate the metabolic outcome of the enzymatic maneuvers described in this report.

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References
Leithauser MT, Liem A, Stewart BC, Miller EC and Miller JA (1990) 1,3cis-Ethenoxysadine formation, mutagenicity and murine tumor induction as indicators of the generation of an electrophilic epoxide metabolite of the closely related carcinogens ethyl carbamate (urethane) and vinyl carbamate. Carcinogenesis 11:463–473.