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Human peripheral lymphocytes as indicators of microsomal epoxide hydrolase activity in liver and lung

Curtis J. Omiecinski[1]*, Lauri Aicher[1], Richard Holubkov[2] and Harvey Checkoway[1]

[1]*Department of Environmental Health, SC-34, and* [2]*Department of Biostatistics, SC-32, University of Washington, School of Public Health and Community Medicine, Seattle, WA 98195, USA*

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In this study, we have applied an improved assay for the determination of microsomal epoxide hydrolase activity to assess enzymatic levels in human lung, liver, and blood lymphocytes. The assay is fluorescence-based and monitors the epoxide hydrolase-mediated conversion of (±)-benzo[a]pyrene-4,5-epoxide to (±)-trans-benzo[a]pyrene-4,5-dihydrodiol, using a high pressure liquid chromatography separation system. Approximately a 40-fold range in microsomal epoxide hydrolase activities was detected in blood lymphocytes collected from 70 individual donors. In 38 individuals who were sampled twice after a 3-month interval, the repeatability of an individual's lymphocyte epoxide hydrolase activity was highly correlated ($r = 0.80$, $p < 0.02$). In addition, within the same individual there appeared to be a strong correlation between lymphocyte and liver epoxide hydrolase activity ($r = 0.92$, $p = 0.02$), and some correlation between liver and lung activity ($r = 0.58$, $p = 0.05$). Activities were assessed in lymphocytes from a styrene-exposed worker population but no significant associations between blood concentrations of styrene and epoxide hydrolase activity levels were observed. Neither were any correlations detected in these workers between epoxide hydrolase activities and age, years on the job, alcohol consumption, sex, or smoking status. The results of our study suggest that blood lymphocytes are a useful sentinel cell for epoxide hydrolase activity determinations in individuals, as these measures are relatively stable over time and appear to reflect activity levels in other target organs.

Introduction

Several forms of epoxide hydrolase exist in mammalian tissues that are distinguished by primary structure, substrate specificity, and intracellular location. The microsomal form of epoxide hydrolase (mEH) is a key biotransformation enzyme that is active in the metabolism of numerous xenobiotics, and is expressed at varying levels in most tissue and cell types (Oesch *et al.*, 1977). Substrates for mEH include the procarcinogenic epoxide metabolites of polyaromatic hydrocarbons, neurotoxic epoxide metabolites of certain solvents, e.g. styrene, as well as endogenous steroid epoxides, such as estroxide (Oesch, 1972; DePierre *et al.*, 1983). The primary sequences of human, rat, and rabbit mEH have been reported, and exhibit a high degree of structural similarity – both at the cDNA (>75%) and amino acid (>83%) levels (Porter *et al.*, 1986; Skoda *et al.*, 1988; Hassett *et al.*, 1989). This high degree of sequence conservation suggests an important functional role for this enzyme in the cell.

Several lines of evidence indicate that population heterogeneity exists in humans for mEH, and that inheritance of low activity mEH alleles may be associated with a predisposition to hepatic toxicity and birth defects subsequent to exposures to antiepileptic compounds, especially diphenylhydantoin (Martz *et al.*, 1977; Strickler *et al.*, 1985; Beuhler *et al.*, 1990). Furthermore, since mEH is an inducible enzyme in the liver (DePierre *et al.*, 1983; Hassett *et al.*, 1989), it is plausible that occupational or iatrogenic exposures to certain agents may produce alterations in cellular mEH expression levels and potentially modulate toxicological outcomes involving the mEH metabolic pathway.

Worker exposure to the industrial solvent styrene is a potentially important occupational example that may relate to the mEH pathway. Styrene has been demonstrated consistently to be a neurotoxicant (Lilis *et al.*, 1978; Flodin *et al.*, 1989). Styrene oxide,

*To whom correspondence should be addressed.

a cytochrome P450-mediated metabolite, is the initial and principal product of styrene metabolism (Oesch, 1972), and has been postulated as the active neurotoxic agent (Bond, 1989). Styrene oxide is a good substrate for mEH and appears to play a central role in its detoxication (Oesch, 1972).

Although mEH activity is expressed at relatively high levels in liver, low activity levels also are detected in blood lymphocytes (Glatt *et al.*, 1980). At present, it is not known whether lymphocyte mEH activity measures are useful predictors of mEH levels in liver or other organs. There is a great potential benefit of developing reliable bioindicators of mEH activity in accessible tissues, such as peripheral blood cells, because of the difficulties of obtaining activity measurements in many human target tissues. Despite the documented role for this enzyme in xenobiotic metabolism, only limited data are available from human population studies of mEH activity.

Quantitation of metabolic rates for (±)-benzo[a]pyrene-4,5-epoxide (BP-4,5-oxide), to its corresponding dihydrodiol derivative (BP-4,5-diol), has been used previously to assess mEH activity, and this measure was shown to be specific for the microsomal form of the enzyme (Schmassmann *et al.*, 1976; Gill *et al.*, 1983a; Eaton & Stapleton, 1989). However, in our experience these existing assays exhibit several limitations, including relatively high backgrounds, variable recoveries of product and substrate upon organic extraction, low sensitivities, and in two of the assays (Schmassmann *et al.*, 1976; Gill *et al.*, 1983a), the requirement of radiolabelled BP-4,5-oxide at high specific activity.

Utilizing BP-4,5-oxide as substrate, we report the development of a modified analytical approach for mEH activity determination. The high pressure liquid chromatography (HPLC) assay we utilized is fluorescence-based and relatively simple, highly sensitive and well-suited for routine analysis of blood lymphocyte activities. We have used this method to investigate the capacity of blood lymphocytes to serve as biomarkers of liver and lung metabolism, respectively. We also took advantage of the availability of venous blood from a styrene-exposed worker population to assess our HPLC-based assay for measuring relatively low mEH activity in circulating lymphocytes.

Materials and methods

Materials

(±)-Benzo[a]pyrene-4,5-epoxide was purchased from the National Cancer Institute Chemical Carcinogen Standard Reference Repository (Midwest Research Institute, Kansas City, MO). (±)-trans-Benzo[a]pyrene-4,5-dihydrodiol was generously provided by M. Namkung and Dr M. Juchau, University of Washington. 1-Chloro-2,4-dinitrobenzene (1-chloro-2,4-dinitrobenzene) was purchased from Aldrich (Milwaukee, WI). HPLC grade acetone, hexane, and ethyl acetate were obtained from JT Baker (Phillipsburg, NJ). Leucoprep™ cell separation tubes were obtained from Becton Dickinson (Lincoln Park, NJ).

Study subjects

Full details of the study population have been reported previously (Checkoway *et al.*, 1992). The occupational study group consisted of 59 workers exposed to styrene and 11 non-exposed reference workers. The absence of styrene exposure in the reference workers was confirmed by air monitoring and from determinations of blood styrene concentrations (Dills *et al.*, 1991). The exposed group included 39 males and 20 females, ranging in age from 20 to 47 years. The reference group included eight males and three females, age range 22 to 51 years. Informed consent forms explaining the purposes and procedures of the study were given to the workers. Data on demographic variables, height, weight, employment history, smoking, alcohol use, and medical history, including prevalence of nervous system symptoms, were obtained by means of a self-administered questionnaire. Three months after the initial survey, we obtained repeated measurements of mEH and blood styrene from 38 workers in an effort to determine intra-individual variability over time.

Styrene exposure assessment

Full (8 h) shift monitoring of styrene exposure was conducted with personal breathing zone passive dosimeters (SKC Anasorb, model No 530-11). Exposure concentrations ranged from <1 to 160 ppm in air as an 8 h time-weighted average. Styrene concentrations in venous blood were determined by headspace gas chromatography using automatic cryogenic focusing and high resolution capillary chromatography (Dills *et al.*, 1991).

In addition, human tissue samples (liver, lung, blood) from surgical and autopsy procedures were obtained from the National Cancer Institute's National Human Tissue Network, Northwestern Division, Columbus, OH, and from the Northwest Tissue Center, Seattle, WA. Liver and lung samples were flash frozen in liquid nitrogen at the time of acquisition and kept on dry ice or at $-80°$ C until use. Blood samples were stored on wet ice until lymphocyte purification (see below). Twelve matched sets of lung and liver from the same individuals, and five sets each of lung and blood,

and liver and blood, also from the same individuals, were acquired from these sources. All protocols involving humans or human tissues were conducted in accordance with approval from the University of Washington's human subjects review committee.

Blood cell fractionation

Blood samples were obtained by venipuncture, collected in EDTA anticoagulant tubes, and stored on wet ice until cell fractionations were performed. For separation of white cells, 8–10 ml of whole blood was applied to a Leucoprep™ tube and fractionated according to the manufacturer's protocol. Isolated white cells were washed in phosphate-buffered saline and cell pellets were stored in plastic 1.5 ml microfuge tubes at −80° C until use. Upon thawing, 250 µl of solution A (0.15 M KCl, 0.05 M KH_2PO_4, pH 7.35) was added, and each sample was sonicated on ice for three 10 s bursts using a Cole Palmer 4710 ultrasonicator. Samples were allowed to cool for 30 s between bursts. Following disruption, cell homogenates were microfuged for 10 min at 9000 × **g** at 4° C. Resulting supernatants (S9 fractions) were transferred to clean tubes; 15 µl aliquots were removed for protein determinations, and the remaining sample was stored at −80° C until use. Protein concentrations were determined with the Pierce BCA reagent system (Rockford, IL).

HPLC enzymatic determinations

For analytical determination of microsomal epoxide hydrolase activities, a fluorometric-based high pressure liquid chromatography (HPLC) assay was developed using BP-4,5-oxide as substrate and monitoring the production of the highly fluorescent product, BP-4,5-diol. Incubations were performed in siliconized plastic microfuge tubes containing solution A buffer supplemented with 0.1 mM EDTA, 0.24 mM BP-4,5-oxide (10 µl of a 3.7 mM BP-4,5-oxide stock in acetone), 10.0 mM 1-chloro-2,4-dinitrobenzene (5 µl of a 0.3 M 1-chloro-2,4-dinitrobenzene stock in acetonitrile) and 100 µg of lymphocyte S9 protein in solution A (100 µg of lymphocyte S9 protein was typically derived from approximately 1 ml of whole blood and consisted of approximately 1×10^6 cells). Assays with lung or liver S9 fractions were performed identically except that 5–10 µg of S9 protein was added to incubation tubes. Blanks were routinely included that contained all reaction components except for S9 fractions. Reactions were initiated by addition of substrate and allowed to proceed for 45 min at 37° C. Reaction rates were linear with respect to time and protein concentration under these conditions. All reactions were run in duplicate. 1-Chloro-2,4-dinitrobenzene was added to the S9 reactions as a competitive inhibitor of cytosolic glutathione S-epoxide transferases to prevent depletion of the BP-4,5-oxide substrate via this route of metabolism (Jansen *et al.*, 1986; Eaton & Stapleton, 1989). In separate experiments (data not shown), using liver and lung microsomal fractions, additions of 10 mM 1-chloro-2,4-dinitrobenzene had no measurable effect on mEH activities.

Immediately following the incubation, samples were placed on ice, and 750 µl of an acetone:hexane mixture (1:4, v:v) was added. The tubes were vortexed vigorously for 60 s, placed back on ice for 5 min, then microfuged for 1 min at 12 000 × **g**. The upper organic phase was transferred to a clean tube, and the original sample was re-extracted as above but with 750 µl of ethyl acetate; the organic phases from each extraction were then pooled. Extraction efficiencies for both BP-4,5-oxide and BP-4,5-diol consistently exceeded 99% with this method. The pooled extracts were evaporated for 1 h to dryness in a Savant Speed-Vac centrifuge at −60° C. For lymphocyte extracts, 50 µl of acetone was added to reconstitute the dried samples. For liver and lung extracts, the samples were reconstituted in 500 µl of acetone. Two successive 5 µl aliquots from each sample were injected into a Rainin (Woburn, MA) Dynamax 5 µM Microsorb C18 1.5 × 25 cm reverse-phase column, using a Rainin 5 µm C18 1.5 × 5 cm guard column. Flow rates were set at 1 ml min^{-1}. A linear 75–100% methanol/water gradient over 7.5 min was employed. The column was integrated into a Rainin Rabbit HPX HPLC system, complete with an ICI LC1600 autoinjector and a Shimadzu RF-535T fluorescence detector. The detector was tuned to 266 nm excitation and 384 nm emission wavelengths. System control and data acquisition were performed with Rainin Dynamax HPLC software operating on a Macintosh SE/30 computer. Fluorescence intensities of products, measured in arbitrary units, were converted to pmol of BP-4,5-diol product by regression analysis to standard curves generated with authentic BP-4,5-diol standard. The limit of detection of the BP-4,5-diol product per HPLC injection was 0.1 pmol (0.025 ng).

Data analysis

For HPLC data, the fluorescence intensity obtained for each sample was corrected for any background fluorescence, then averaged between values obtained from duplicate HPLC injections of each sample, and between values obtained from the duplicate reactions. The total variation observed between parallel incubations and duplicate HPLC injections was typically $< 10\%$ for each sample. Regression analyses

were performed using linear least-squares regression analysis. Statistical determinations and graphics were performed using Axum computer software (Trimetrix Inc, Seattle, WA).

Results

HPLC analysis of mEH enzymatic activity

Figure 1 displays representative HPLC tracings of the detector output from the assay conducted with lymphocyte S9 (panel A), lung S9 (panel B), and liver S9 (panel C). This particular set of tissues was obtained from the same donor. The early peak exhibiting a retention time of approximately 3.1 min was generated by the acetone solvent front; acetone was used to reconstitute the dried sample extracts. The prominent peak eluting at approximately 6.0 min co-chromatographed with authentic BP-4,5-diol standard. To increase sensitivity of detection for the inherent low levels of activity present in lymphocytes, 1/10 of the total lymphocyte extract was chromatographed. For liver and lung, 1/100 of the total organic extract was chromatographed. At the higher level injections, several other small peaks also were apparent that migrated between BP-4,5-diol and BP-4,5-oxide. These materials were identified as impurities contained within the BP-4,5-oxide standard itself, presumably quinone derivatives. However, due to their altered retention times and low levels, these background peaks did not interfere with detection or quantitation of the mEH product, BP-4,5-diol.

It is noteworthy that we have observed significant but variable residual mEH activity in the 100 000 × g supernatant fraction of tissue preparations. Consequently, we prepared and analysed S9 fractions throughout this study.

Correlation of mEH activity between matched sets of liver, lung, and lymphocytes

Initially, we utilized the HPLC assay to examine mEH activities in human blood lymphocytes, lung, and liver. Tissue specimens were collected during surgical or autopsy procedures as matched sets, such that at least two separate tissues could be sampled from each individual. The majority of the tissue sets available ($n = 12$) were matched sets of liver and lung; however, in several cases we also obtained blood and liver, or blood and lung from the same individuals ($n = 5$). One aim of our analyses was to evaluate how well mEH activity levels correlated between tissues from a given individual. The data derived from these experiments are presented in the regression plots of Fig. 2.

As shown in panel A of Fig. 2, there was a reasonably strong correlation between liver and lung activity ($r = 0.58$, $p = 0.05$). The activity correlations between lymphocyte and lung (panel B), as well as lymphocyte and liver (panel C), were based on only five data points, yet suggested consistent associations, especially for lymphocyte *versus* liver activity ($r = 0.92$, $p = 0.02$).

mEH Activities in lymphocytes from styrene-exposed workers

From the results demonstrated in Fig. 2, we concluded that mEH activities in lymphocytes were readily measurable using this analytical method, and that lymphocyte activities appeared to reflect mEH activity profiles in other organs. We therefore undertook an analysis to assess mEH activities in lymphocytes isolated from the subset of 38 styrene-exposed workers with repeated measurements.

Results presented in Fig. 3 show the lack of significant correlation between styrene exposure and mEH activity level. Each of the 38 individuals in this cohort was sampled on two separate occasions; the second sampling occurred 3 months after the first. Blood styrene concentrations were not available for three subjects at the second sampling. An overall lack of correlation between mEH level and blood styrene concentration was apparent at each sampling time ($r = 0.12$ and $r = 0.05$, respectively; Fig. 3). We also failed to detect any correlation between mEH activity and age ($r = 0.14$); years on the job ($r = 0.10$); mEH level and level of alcohol consumption (drinks per week; $r = 0.01$); or, with sex or smoking status of the individual. Furthermore, in attempts to assess whether ΔmEH activity (change in mEH activity measured on the first *versus* second samplings) was associated with Δ blood styrene concentrations, no correlation was apparent ($r = 0.06$).

The repeatability of an individual's lymphocyte mEH activity with successive sampling was highly correlated ($r = 0.80$, $p < 0.02$). These data are presented in Fig. 4. This latter result indicates that, in general, lymphocyte mEH levels are relatively consistent over time within the same individual.

Distribution of mEH activities in human blood lymphocytes

As there was no apparent effect of styrene on mEH activity in exposed workers, it was of interest to assess the range of lymphocyte activities determined from the entire population of subjects available. These data are presented in the histogram of Fig. 5. Lymphocytes from all 70 subjects, including 59 styrene-exposed workers and the 11 selected non-exposed reference workers, were assayed for lymphocyte mEH activities. Averages of the two assays were used to represent

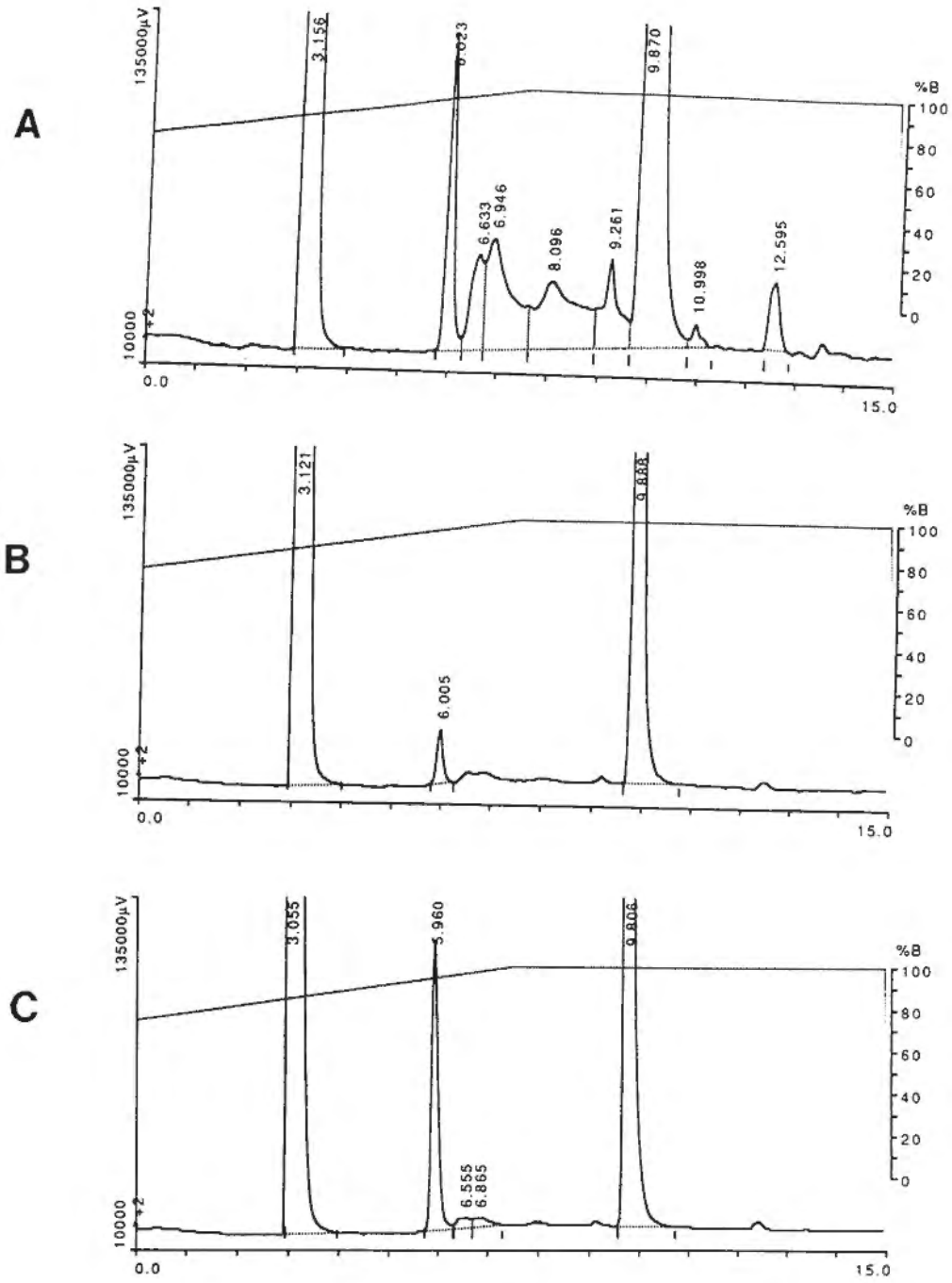

Fig. 1. Fluorometric detection of HPLC-separated benzo[a]pyrene-4,5-dihydrodiol. For each panel, the x-axis indicates the time base in min and the y-axis indicates the detector sensitivity, in μV. Retention times of each peak are indicated, as is the separation gradient, expressed as % methanol/water. The peak eluting at 6.0 min co-chromatographed with authentic BP-4,5-diol standard. The major peak eluting at 9.8 min co-chromatographed with the BP-4,5-oxide substrate. All tissues were processed and assayed as 9000 × g supernatant fractions (S9) and all tissues were obtained from the same individual. Panel A: Typical detector profile obtained with human blood lymphocyte activity determination. 1/10 of the total organic extract was injected for each HPLC run. Panel B: Human lung S9 HPLC profile injecting 1/100 of the total organic extract. Panel C: Human liver S9 HPLC profile injecting 1/100 of the total extract.

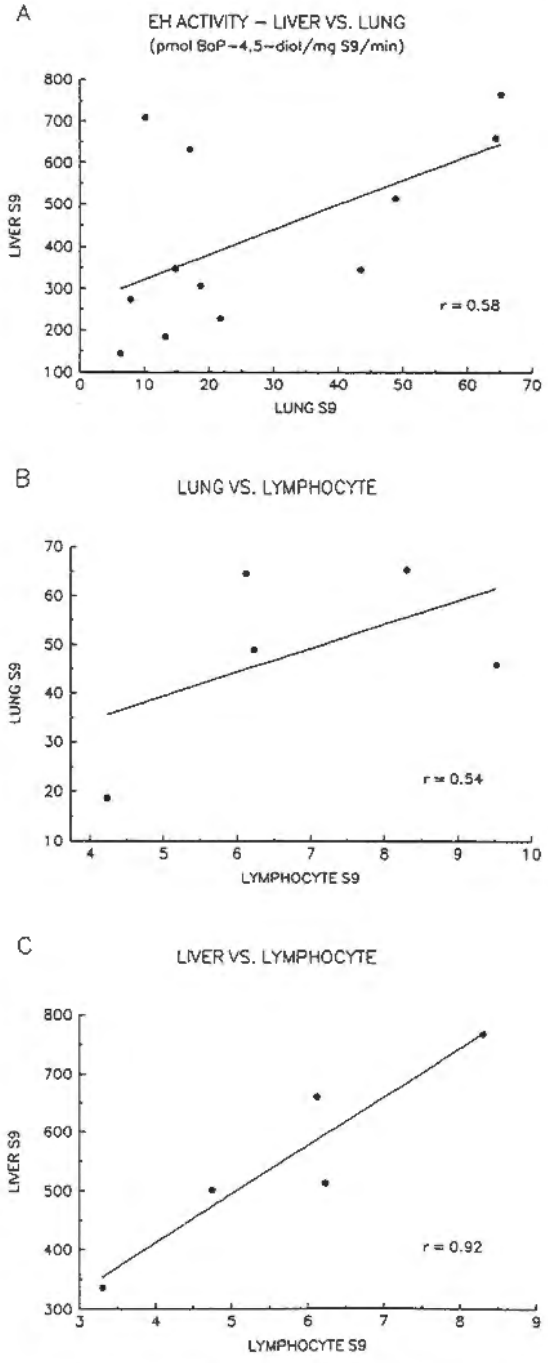

Fig. 2. Correlation of mEH activity levels between tissues of the same individual. Activity assays were conducted for mEH on matched sets of liver, lung, and blood lymphocyte tissues collected from individual donors. For liver and lung comparisons, matched sets of tissue were available from 12 individuals and these data are presented in panel A. Panel B, mEH activity data from matched sets of lung and blood lymphocyte activity determinations from five individuals. Panel C, mEH activity data from matched sets of liver and blood lymphocytes obtained from five individuals.

mEH values for the subset of 38 subjects with repeat measurements. As can be seen from Fig. 5, the mEH activity from blood lymphocytes obtained from the entire sample ranged from 0.22 to 8.9 pmol BP-4,5-diol product mg^{-1} S9 protein min^{-1} of reaction time, i.e. spanning a 40-fold range. The distribution appeared to be skewed toward the higher end of the activity range.

Discussion

The enzyme, mEH, is an important component of the xenobiotic biotransformation process, participating in the metabolism of a diverse array of environmentally-prominent epoxides. Relevant substrates include the carcinogenic polyaromatic hydrocarbon epoxides; commercial solvents, such as epoxides of benzene and styrene; and pharmaceuticals, such as the epoxide intermediates of phenytoin and carbamazepine (Oesch, 1972; Martz *et al.*, 1977; DePierre *et al.*, 1983; Kerr & Levy, 1989). Although the role of the enzyme in the metabolism of endogenous substances is not well defined, mEH is active in the metabolism of certain steroidal epoxides, such as estroxide (Seidegard *et al.*, 1986). In humans, several reports have indicated a genetic heterogeneity in mEH expression (Strickler *et al.*, 1985; Buehler *et al.*, 1990). Although comparisons of high *versus* low activity status may ultimately reveal individual risk to chemically-initiated toxicity, little is known concerning the distribution of mEH activity in the human population, effects of chemical exposures on mEH expression, correlation of mEH activity levels across tissue sets within an individual, or the genetic basis for any potential diversity in mEH expression.

One aim of our laboratory is to identify potential polymorphisms within the human mEH gene locus. Only minimal population data are currently available with respect to human mEH expression. Due to the relative accessibility of blood samples and their potential use in the biomonitoring of human populations, we initiated this study to establish baseline data for the enzyme and to assess whether isolated blood lymphocyte activities reflect levels of mEH activities in other organs. We began by developing an HPLC-based assay for determination of mEH metabolism, based on the detection of the highly fluorescent and specific mEH metabolite BP-4,5-dihydrodiol, using BP-4,5-oxide as substrate. In comparison to previous methods (Schmassmann *et al.*, 1976; Gill *et al.*, 1983a; Eaton & Stapleton, 1989), the present assay was relatively simple to conduct and proved highly sensitive and reproducible. The latter considerations are especially important for measuring mEH activity in samples

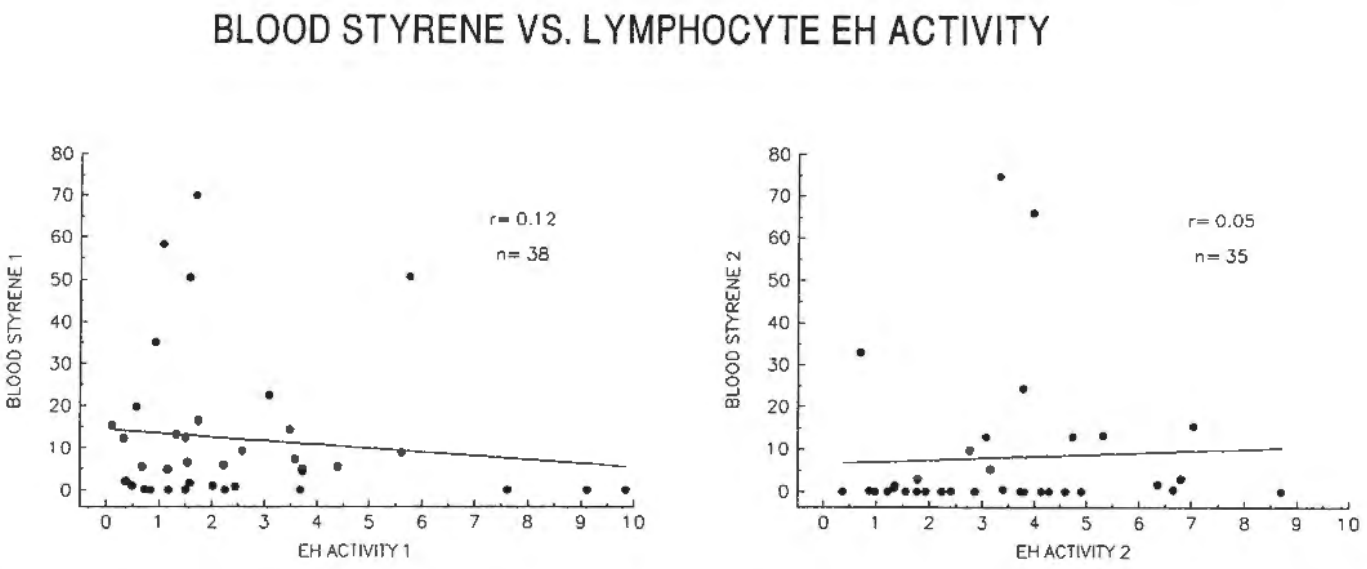

Fig. 3. Blood styrene concentrations relative to mEH activities in blood lymphocytes. Regression plots of styrene concentrations in blood *versus* the corresponding mEH activity determinations in blood lymphocytes are presented. For both panels, the ordinates indicate blood styrene concentrations expressed as μg ml^{-1}, and the abscissa indicate mEH activity expressed as pmol BP-4,5-diol formed mg^{-1} S9 protein min^{-1} of reaction time. The panel on the left depicts the correlation data obtained from a sampling of 38 individuals from the styrene-exposed cohort. Both mEH activity and styrene blood concentration data from 35 of these individuals were available from a repeated blood analysis 3 months later and these data are presented in the panel on the right.

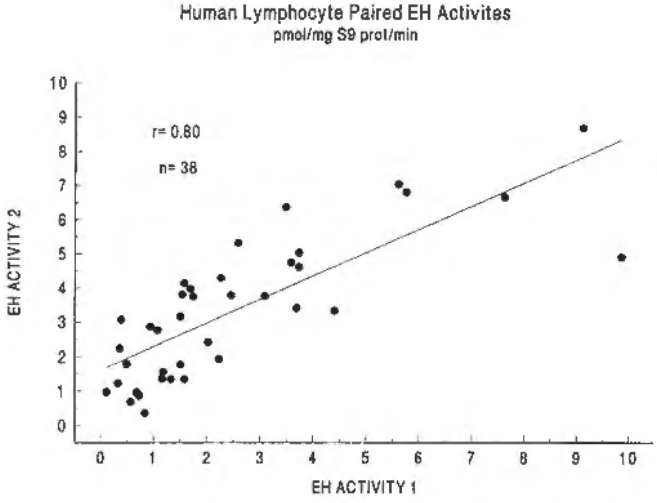

Fig. 4. Regression plot of lymphocyte mEH activities determined from individuals sampled successively on different days. Activity data are expressed as pmol mg^{-1} S9 protein min^{-1} reaction time. Data obtained from two separate mEH activity analyses repeated on the same individual are shown; data from the initial sampling are given on the x-axis and data obtained from the second sampling are on the y-axis.

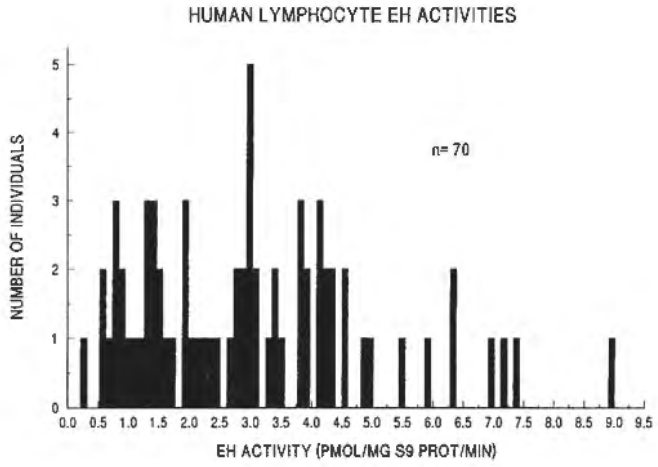

Fig. 5. Frequency distribution of lymphocyte mEH activities in a human population. A total of 70 individuals were evaluated for lymphocyte mEH activity. Thirty eight of these individuals were sampled successively 3 months apart. Since the values obtained from these latter individuals were highly correlated, an average activity was ascertained for each of these individuals and this average value was used as the corresponding data point in the plot.

possessing only very low levels of activity, such as in lymphocytes. Additionally, we have demonstrated the utility of the assay for other tissues, including liver, possessing high mEH activity.

The localization of the enzyme has been established as an integral smooth endoplasmic reticulum component, and appears to be anchored in the reticulum by a hydrophobic membrane-spanning domain at the amino-terminus of the protein (Hassett *et al.*, 1989). However, during preparation of cellular and tissue homogenates we routinely detected variable but significant levels of mEH activity remaining in 100 000 × **g** cytosolic fractions. For this reason, we utilized only 9000 × **g** supernatant fractions (S9) for enzymatic activity analysis in the current study. Several other investigators also have detected mEH activity in 100 000 × **g** cytosolic fractions (Gill *et al.*, 1983b; Kizer *et al.*, 1985; Jansen *et al.*, 1986). It appears reasonable to conclude that, although microsomal in origin, human mEH activity is not tightly associated with the reticulum membrane, and that caution should be exercised in extrapolating from mEH experiments directed exclusively at microsomal preparations.

Of particular interest were the data generated in experiments conducted on matched tissue sets from single individuals (Fig. 2). Although limited with respect to the number of samples, the results suggest a strong association between the levels of mEH activity in lymphocytes and that in liver; similarly, there was also a trend toward a positive association between liver and lung activities. The correlation between liver and lymphocyte activities may be particularly important because the liver is the main organ contributing to whole body metabolism of many xenobiotics, including epoxide compounds. These findings suggest that lymphocytes may indeed be a useful sentinel cell for mEH activity evaluations.

The poorer correlation observed for lung *versus* lymphocyte mEH activities is noteworthy. It is possible that the highly heterogeneous cellular composition of the lung, relative to the liver or the isolated lymphocyte population, may be reflected in our results. Conclusions from earlier immunohistochemical studies examining the distribution of mEH in rabbit lung indicated a heterogeneous expression pattern of mEH among the various cell types in this organ (Devereaux *et al.*, 1986). Assuming a similar histological distribution in human lung, it might be inherently more difficult to extrapolate lung activities from lymphocyte analysis (compared to liver). However, our sample size for this comparison was quite limited, i.e. only five individuals were compared with matched samples of lymphocytes and lung, therefore additional studies will be required to test these associations more rigorously.

In view of the role of mEH in styrene metabolism, specifically, in the hydrolysis of styrene oxide (Oesch, 1972), a cytochrome P450-mediated product, we examined the association of mEH activities in lymphocytes from workers occupationally exposed to styrene. The results of these analyses indicated that blood styrene levels were not correlated with mEH activity. These data suggest that styrene exposures in humans do not induce or otherwise modulate mEH activity levels.

Currently it is not known whether human lymphocyte mEH levels, *in vivo*, are subject to modulation by chemical exposures. One study (Glatt *et al.*, 1983) has reported that both α-naphthoflavone and β-naphthoflavone activate mEH activity in human lymphocytes *in vitro*; however, we have not been able to repeat these observations in our laboratory (L.A., C.J.O., unpublished data). It is apparent that hepatic mEH levels can be enhanced by exposure to certain agents, including phenobarbital (Hassett *et al.*, 1989) and aromatic amines (Gonzalez *et al.*, 1982). Our observations in the present study lead us to propose that lymphocyte-derived mEH levels are not subjected to marked modulation by chemical inducers *in vivo*. This suggestion is based on the findings that: (1) lymphocyte mEH activity appeared to be highly correlated within individuals over a 3-month period (Fig. 4); (2) lymphocyte mEH activities did not correlate with blood styrene levels (Fig. 3); and (3) no clear associations were apparent with lymphocyte mEH activity and smoking behaviour (data not shown). A previous report also indicated that several prototypic agents were ineffective in inducing mEH activities in cultured lymphocytes (Glatt *et al.*, 1980).

The observed 40-fold range in lymphocyte activity within the 70 individuals assayed, may suggest the presence of genetic variability in enzyme expression and potential biotransformation capacity. Although no clear antimodes were distinguished, the mEH frequency profile plotted in Fig. 5 may be consistent with a bimodal or trimodal distribution of two or more mEH alleles. Previous reports examining human mEH activities in lymphocytes, utilizing different assay schemes and conducted on smaller numbers of individuals, indicated only a 2–3.6-fold range in activity levels (Glatt *et al.*, 1980; Seidegard *et al.*, 1984). More extensive population surveys, combined with molecular genetic approaches and family studies, will be required to substantiate conclusively the existence of genetic polymorphism for human mEH.

In summary, the results obtained from these studies suggest that mEH activities can readily be determined

and reproduced in isolated fractions of human blood lymphocytes, and that such measures may prove useful in further studies assessing genetic diversity of the enzyme in humans. Lymphocyte mEH activity may also be a reliable indicator of metabolic rates and toxic outcomes in other target tissues of an individual.

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