Measurement and Characterization of Membrane-bound and Soluble Epoxide Hydrolase Activities in Resting Mononuclear Leukocytes from Human Blood

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ABSTRACT

Membrane-bound and soluble epoxide hydrolase activities in the mononuclear cell fraction from human blood have been characterized using cis- and trans-stilbene oxides as substrates, respectively. Because of the low activities in these cells, it was necessary to modify assay procedures developed for rat and mouse liver in the following ways: (a) the substrates were relatively highly labeled (2 Ci/mmol) and carefully purified; (b) the incubation time was extended to 45 to 60 min, during which period the activities were linear; (c) as many as 6 million cells were used for a single assay, which was also within the linear range of the procedure.

The membrane-bound epoxide hydrolase characterized in this manner has an apparent $V_{\text{max}}$ of 7.26 pmol product formed per min per $10^7$ cells and an apparent $K_m$ of 9.96 $\mu$M. The pH optimum was observed to be around 9.8. The dependence of this activity on temperature showed its optimum at 40°. The soluble epoxide hydrolase activity has an apparent $V_{\text{max}}$ of about 8.26 pmol product formed per min per $10^7$ cells, an apparent $K_m$ of 1.63 $\mu$M, a pH optimum of 6.2 to 6.8, and a temperature optimum at 60°.

Using these techniques, these activities have also been determined in other blood components, i.e., lymphocytes, monocytes, granulocytes, erythrocytes, platelets, and plasma. Lymphocytes account for most of the epoxide hydrolase activity towards cis-stilbene oxide, and all of the activity towards trans-stilbene oxide is in the human mononuclear cell fractions.

Different substances known to affect rodent epoxide hydrolases were tested for their effects on the human mononuclear blood cell activities. Interestingly, 1,1,1-trichloropropane 2,3-epoxide, a potent inhibitor of liver microsomal epoxide hydrolase in different species including rat, mouse, and human, had little or no effect on the membrane-bound activity measured here. However, cyclohexene oxide inhibits this membrane-bound activity 60%. The soluble epoxide hydrolase is inhibited to 90% of control levels by chalcone epoxide. The membrane-bound and soluble epoxide hydrolase activities determined in 27 subjects varied from 8.2 to 18.5 and from 3.5 to 17.0 pmol product formed per min per $10^7$ cells, respectively. The mean coefficient of intraindividual variation, determined with three subjects measured four times each over the course of 18 days, was approximately 10% for both enzyme activities.

INTRODUCTION

Because of the central importance of drug-metabolizing systems to the toxic and genotoxic effects of many xenobiotics, there is at present a rapidly growing interest in characterizing these systems in human tissues. Since circulating mononuclear leukocytes are one of the few human tissues which can be obtained without undue stress or damage to the individual, these cells have received much attention in this respect. Indeed, after the pioneering work of Kellerman, et al. (8, 9, 11) and Whitlock et al. (26) in the early 1970s, a large number of studies have measured the level and inducibility of benzo(a)pyrene monooxygenase activity in human lymphocytes and in relationship to lung cancer. Most of these studies have involved incubating the lymphocytes for at least 4 days in the presence of a mitogen and with or without an AHH inducer in order to obtain a more measurable enzymatic activity. However, the effects of mitogens themselves on the drug-metabolizing systems in human lymphocytes remain unclear.

At the same time, relatively little is known about other drug-metabolizing systems in human lymphocytes. In our opinion, this is a serious oversight, since the detoxication of reactive xenobiotic metabolites, especially by epoxide hydrolases and glutathione transferases, can have equally profound effects on the toxicity and genotoxicity of these compounds, as does the actual formation of reactive intermediates via the cytochrome P-450 system. In addition, since there are many different isozymes of cytochrome P-450, benzo(a)pyrene monooxygenase activity may not reflect the rate of metabolism of other xenobiotics via the cytochrome P-450 system. Finally, the levels of cofactors and of the enzymes responsible for producing these cofactors (e.g., glutathione reductase and glucose-6-phosphate dehydrogenase) may also have importance in determining the rate and nature of xenobiotic metabolism by human lymphocytes.

For these reasons, we have begun a series of characterizations of several different drug-metabolizing systems in human lymphocytes. In the present study, we have examined both membrane-bound and soluble epoxide hydrolase activities in the mononuclear leukocyte fraction from human blood. Only a few reports on the level of microsomal epoxide hydrolase in human lymphocytes have appeared (6, 7, 10), whereas the corresponding cytosolic enzyme has not yet been studied in these cells. In order to avoid the possible influence of mitogens and tissue culture conditions, we have modified published assay procedures for microsomal and cytosolic epoxide hydrolase activities (5) so that they are sensitive enough for the measurement and characterization in subcellular fractions of mononuclear leukocytes prepared directly from human blood and without any mitogenic treatment. In addition, since the mononuclear fraction from human blood is heterogeneous (containing 75 to 80% lymphocytes, 20 to 25% monocytes, and other blood components as well),
we have further purified individual blood cell components in order to determine their contribution to the total activity of this fraction. Finally, we present data on intra- and interindividual variations in these 2 epoxide hydrolase activities in freshly isolated resting human mononuclear leukocytes.

**MATERIALS AND METHODS**

**Chemicals.** cis- and trans-[3H]stilbene oxides of high radiospecificity (2 Ci/mmol) were synthesized in collaboration with Dr. Åke Pilotti, Department of Organic Chemistry, University of Stockholm, according to a published procedure (5). Chalcone epoxide (27) and unlabeled cis-stilbene oxide (5) were also synthesized using methods reported in the literature. These compounds, which we synthesized ourselves, were purified by recrystallization, and their identities were confirmed by nuclear magnetic resonance spectroscopy and melting point determinations.

4,7-Diphenyl-1,10-phenanthroline, trans-stilbene oxide, cyclohexene oxide, 1,1,1-trichloropropane-2,3-epoxide, metyrapone, benzil, isocoumarone, 5,6-benzoflavone, and 7,8-benzoflavone were obtained from EGA-Chemie, Steinheim/Albuch, Federal Republic of Germany, and mit-conalone, clotrimazole, cholesterol 5α,6α-epoxide, 16α,17α-epoxypregn-nenolone, and ellipticine were obtained from Sigma Chemical Co., St. Louis, MO. Other chemicals used were of reagent grade and were commonly supplied by commercial sources.

**Isolation of Blood Components.** Routinely, the mononuclear fraction was isolated from the whole blood of normal individuals by the standard procedure of Isopaque:Ficoll gradient centrifugation (2). However, in order to determine the contributions of individual blood cell components to the total activities of this fraction, individual blood cell types were also isolated in several experiments.

The mononuclear leukocyte fraction was rapidly depleted of monocytes by passage through a column of gelatin beads in the presence of fresh plasma and heparin (24). Lymphocytes could be eluted from this column simply by washing with the elution buffer (Hanks' Eagle plus 10% plasma plus 50 U.S.P. units of heparin/ml), whereas monocytes were subsequently eluted using 50 mM EDTA in phosphate-buffered saline. The first fraction obtained in this manner contained 95% lymphocytes, whereas the second fraction was 50 to 70% monocytes.

Granulocytes were isolated from the pellet obtained after Isopaque:Ficoll gradient centrifugation by adding an equal volume of 2% Dextran T-500 in 0.15 M NaCl. After standing at room temperature for 30 min, the erythrocytes sedimented from this solution. The granulocytes remaining in suspension were about 90% pure.

Finally, platelets were isolated from platelet-rich plasma by differential centrifugation at 400 × g for 20 min.

**Preparation of Membrane and Soluble Fractions.** The mononuclear leukocyte fraction or isolated blood components were sonicated for 5 to 10 sec at 0–4°C with a Branson sonifier at a setting of 1.5 amp. This procedure was chosen because it was found to disrupt all of the cells without denaturing the activities under investigation. In the case of mononuclear leukocytes, centrifugation at 105,000 × g for 60 min was subsequently used to obtain a membrane and a soluble fraction which were used to characterize epoxide hydrolase activities. For the isolated blood components, whole sonicates were used.

**Enzyme Assays.** The assay procedures utilized here are essentially those reported by Gill et al. (5), in which highly labeled cis- and trans-stilbene oxides were used as the substrates (see above). In addition, these substrates were also carefully purified from the stock solution (toluene) using thin-layer chromatography plates (Silica Gel 60 F254) developed in a solvent system of hexane:ethyl acetate (95:5), shortly before addition to the assay medium in ethanol. This was done in order to keep the background as low as possible. Routinely, membrane or soluble fractions from 2 to 3 million cells in a total incubation volume of 100 µl were used to characterize the epoxide hydrolase activities.

Optimal membrane-bound epoxide hydrolase activity could be measured in 250 mM glycine, pH 9.8, with 60 µM cis-stilbene oxide (at a final radiospecificity of 10 to 15 mCi/mmol), whereas optimal soluble epoxide hydrolase activity was obtained in 250 mM sodium phosphate, pH 6.8, with 25 µM trans-stilbene oxide (at a final radiospecificity of 20 to 30 mCi/mmol) (see also "Results"). After addition of the substrate in 1 µl ethanol, the reaction mixtures were incubated for 45 to 60 min at 37°C. The assay was terminated by adding 200 µl dodecane and then shaking vigorously before centrifuging in a desk centrifuge to obtain phase separation.

In both cases, 99.9% of the remaining substrate was thus extracted into the dodecane phase, while 92% of the dihydriodiol product formed remained in the aqueous phase. Unfortunately, glutathione conjugates of cis- and trans-stilbene oxides also remained in the aqueous phase after this extraction. The presence of such conjugates can be tested for by subsequent extraction with 200 µl hexanol, which removes the dihydriodiol but leaves the glutathione conjugates in the aqueous phase. This test demonstrated that glutathione conjugates are formed with trans-stilbene oxide in the presence of the soluble fraction but not with cis-stilbene oxide in the presence of the membrane fraction. Consequently, extraction with hexanol was used routinely when measuring the soluble epoxide hydrolase activity, but not for the membrane-bound enzyme.

Aliquots of the aqueous phases were taken after extraction with either dodecane or hexanol and scintillation counted and corrected for quenching. The enzyme activities obtained were also routinely corrected for the incomplete recovery of product in the aqueous phase after extraction with dodecane. These assays were routinely performed in duplicate; and in one experiment involving 30 sets of duplicates, the duplicates were found to differ from one another by averages of 7.1% in the case of membrane-bound epoxide hydrolase activity and 4.2% in the case of the soluble enzyme.

**Measurements of Protein.** This was achieved using the method of Lowry et al. (12) with bovine serum albumin as standard. The membrane fraction from 10 million cells was found to contain approximately 0.36 mg protein, whereas the corresponding soluble fraction contained 0.44 mg protein.

**RESULTS**

**Assay Procedures for Membrane-bound and Soluble Epoxide Hydrolase Activities.** One of the advantages of using cis- and trans-stilbene oxides as substrates for membrane-bound and soluble epoxide hydrolases, respectively, is that the nonenzymatic conversion of these compounds to dihydriodiol and other substances with similar solubility properties (i.e., the background) is relatively low. The backgrounds obtained by incubating cis- or trans-stilbene oxides in buffer alone or in combination with boiled membrane or soluble fractions for 1 hr were about 0.9 and 0.3%, respectively. Consequently, as little as 100 pmol dihydriodiol formed from cis-stilbene oxide and 20 pmol dihydriodiol formed from trans-stilbene oxide during 1 hr incubation could be detected at the concentrations of these substrates used. Thus, by using these substrates at a high radiospecificity and in highly purified form, together with long incubation times (both membrane-bound and soluble epoxide hydrolase activities were linear for at least 1 hr under the conditions described), it proved possible to measure both epoxide hydrolase activities with relatively small numbers of freshly isolated human mononuclear leukocytes. The activities were linear with membrane and soluble fractions prepared from up to at least 6 million cells (which corresponds to approximately 6 ml blood).

Another important advantage of using cis-stilbene oxide as substrate for the membrane-bound epoxide hydrolase activity and trans-stilbene oxide for the soluble activity is that these substrates are relatively specific for the indicated hydrolase

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activities. Gill et al. (5) have shown that microsomes from mouse or rat liver hydrate cis-stilbene oxide much faster than the cytosolic fraction from the same organ; whereas trans-stilbene oxide is hydrated effectively by the cytosolic fraction, but not at all by the microsomes. We have demonstrated similar selectivities in the case of the lung, testis, and kidney from the mouse and the trunk and head kidneys from the pike (3). However, this does not seem to be the case for human liver (25). In the present study, we found that the membrane fraction from human mononuclear leukocytes does not hydrate trans-stilbene oxide at a detectable rate, while the soluble fraction does not hydrate cis-stilbene oxide.

It has been reported (17) that microsomal epoxide hydrolase activity in rat liver preparations can be enhanced by the presence of different detergents. Here the use of Triton X-100 in concentrations from 0.1 to 2% did not affect the activity of the membrane-bound enzyme. Consequently, since we were unable to increase this activity by treatment with detergent, it was eliminated from the assay system.

The epoxide hydrolase activity of both membrane and soluble fractions seems to be reasonably stable during storage at -20°C. During 1 week of such storage, the membrane-bound activity decreased 15.4% (mean of 9 determinations), whereas the soluble activity increased 4.6% (mean of 5 determinations). Little additional change in these activities could be detected after 1 month of storage in the freezer.

In order to test the possibility that the activity of the membrane-bound and/or soluble epoxide hydrolase is affected by sonication of the mononuclear leukocytes and subsequent centrifugation to prepare the membrane and soluble fractions, these enzyme activities were measured in intact cells in one experiment. The activities measurable in this case were very similar to those obtained with the isolated fractions.

**Kinetic Parameters.** The dependence of the membrane-bound epoxide hydrolase activity in human mononuclear leukocytes on substrate concentration is shown in Chart 1. An apparent $K_m$ of 9.96 ± 1.07 μM and an apparent $V_{max}$ of 7.26 ± 0.78 pmol threo-1,2-diphenylethane-1,2-diol formed per min per 10^7 cells can be calculated from the Eadie-Hofstee plot. Thus, the 60 μM concentration of cis-stilbene oxide used routinely in the present investigation seems to be appropriate.

The dependence of the soluble epoxide hydrolase in human mononuclear leukocytes on substrate concentration is shown in Chart 2. This curve is broader than that seen for the membrane-bound enzyme, demonstrating a significantly lower apparent $K_m$ of 1.63 ± 0.10 μM but approximately the same apparent $V_{max}$ of 8.26 ± 0.52 pmol erythro-1,2-diphenylethane-1,2-diol formed per min per 10^7 cells. The 25 μM concentration of trans-stilbene oxide used throughout this investigation seems also to be appropriate.

**Dependence of Epoxide Hydrolase Activity on pH.** Membrane-bound and soluble epoxide hydrolase activities were examined over a wide range of pH (Charts 3 and 4, respectively). Three different buffers, i.e., citrate, sodium phosphate, and sodium glycine, were used, and no buffer effects were observed. As in other tissues (1, 13, 16, 21), the membrane-bound epoxide hydrolase activity is most pronounced at higher pH values, demonstrating a marked peak at 9.8. The activity of this enzyme at the “physiological” pH of 7.5 is about one-half of that found at the optimal pH. The soluble activity, on the other hand, demonstrates a broader optimal pH between 6.2 and 6.8. It is interesting to note that at pH 9.8, where the membrane-bound epoxide hydrolase activity is maximal, there is virtually no soluble activity.

**Dependence of Epoxide Hydrolase Activity on Temperature.** The optimal temperature for microsomal epoxide hydrolase activity has been reported to be about 50°C in rat liver (4) and 47°C in pike liver (1). The optimal temperature for the membrane-bound activity in human mononuclear leukocytes is close to 40°C, i.e., not much higher than the physiological temperature of 37°C (Chart 5).

On the other hand, the temperature optimum for the soluble epoxide hydrolase activity has not been measured in other species, but in human mononuclear leukocytes the optimal temperature was determined to be about 60°C (Chart 6). At this optimal temperature, the soluble activity is approximately twice that observed at 37°C. The membrane-bound activity is virtually zero at 60°C. At present, we have no explanation for this high temperature optimum of the soluble epoxide hydrolase.

**Influence of Low-Molecular-Weight Effectors.** Microsomal
Epoxide Hydrolases in Human Mononuclear Leukocytes

Chart 2. Dependence of the soluble epoxide hydrolase activity of human mononuclear leukocytes on substrate concentration. The units for the specific activity are pmol erythro-1,2-diphenylethane-1,2-diol formed per min per 10⁷ cells. Points, means of 3 different determinations; bars, average deviations.

Epoxide hydrolase activity in rat liver and in a number of other tissues is known to be strongly activated or inhibited by a number of low-molecular-weight effectors (20). The cytosolic epoxide hydrolase is known to be strongly inhibited by chalcone epoxides (14), but much less is known about the effect of other substances on this enzyme. In order to compare the epoxide hydrolase activities of human mononuclear leukocytes to those in other tissues, as well as to obtain clues concerning the mechanism by which these enzymes function, the influence of a number of different low-molecular-weight effectors on the mononuclear leukocyte enzymes was tested (Table 1).

Interestingly, 1,1,1-trichloropropane 2,3-oxide, a potent inhibitor of microsomal epoxide hydrolase in other species (20), had little or no effect (18% decrease) on the membrane-bound activity in these cells, whereas cyclohexene oxide was more effective (57% decrease). There might also be a small stimulatory effect of 5,6-naphthoflavone. The soluble epoxide hydrolase was effected by chalcone epoxide and clotrimazole, where this activity was decreased 88 and 24%, respectively.

Level of Epoxide Hydrolase Activities in Individual Blood Components. It is often assumed that the properties of mononuclear leukocytes isolated from human blood are also those of circulating human lymphocytes. However, as can be seen in Table 2, this fraction also contains appreciable amounts of monocytes. Consequently, we measured the membrane-bound and soluble epoxide hydrolase activities in individual blood components, partially because these data are themselves of interest and partially to ascertain to what extent the epoxide hydrolase activities of the human mononuclear leukocyte fraction can be taken to reflect these same activities in circulating human lymphocytes.
Table 1

<table>
<thead>
<tr>
<th>Epoxide hydrolase(^6) ((% \text{ of control activity}))</th>
<th>Membrane-bound(^6)</th>
<th>Soluble(^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,1,1-Trichloropropane 2,3-oxide</td>
<td>82</td>
<td>95</td>
</tr>
<tr>
<td>Cyclohexane oxide</td>
<td>43</td>
<td>91</td>
</tr>
<tr>
<td>cis-Stilbene oxide</td>
<td>95</td>
<td>92</td>
</tr>
<tr>
<td>trans-Stilbene oxide</td>
<td>92</td>
<td>12</td>
</tr>
<tr>
<td>Chalcone epoxide</td>
<td>104</td>
<td>102</td>
</tr>
<tr>
<td>Cholesterol 5α,6α-epoxide</td>
<td>109</td>
<td>101</td>
</tr>
<tr>
<td>16α,17α-Epoxyprogesterone</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>Metyrapone</td>
<td>94</td>
<td>98</td>
</tr>
<tr>
<td>Benzil</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td>Miconazole</td>
<td>81</td>
<td>76</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>104</td>
<td>103</td>
</tr>
<tr>
<td>Isoquinoline</td>
<td>109</td>
<td>95</td>
</tr>
<tr>
<td>Ellipticine</td>
<td>116</td>
<td>99</td>
</tr>
<tr>
<td>5,6-Benzoflavone</td>
<td>96</td>
<td>97</td>
</tr>
</tbody>
</table>

\(^6\) In another experiment, membrane and supernatant fractions were used, and similar results were obtained.

On the other hand, only lymphocytes and granulocytes exhibit significant levels of epoxide hydrolase activity towards trans-stilbene oxide (Table 2). Consequently, lymphocytes account for virtually all of this activity in the mononuclear fraction.

Inter- and Intraindividual Variation in the Epoxide Hydrolase Activities of Human Mononuclear Leukocytes. Measurements of the benzo(a)pyrene monooxygenase activity in circulating lymphocytes from different individuals have demonstrated as much as a 50-fold variation (8, 9). Such interindividual differences may result from both hereditary and environmental factors and may have profound influences on the metabolism and thereby on the toxicity and genotoxicity of different xenobiotics. More problematic is the observation that the benzo(a)pyrene monooxygenase activity in circulating lymphocytes from the same individual at different points of time also demonstrates large differences (8). Whether such intraindividual differences result from environmental, hormonal, or other factors or whether they are at least partially due to methodological difficulties is not yet clear.

Membrane-bound epoxide hydrolase activity could be detected in all the blood components examined, with the exception of plasma. Indeed, both monocytes and granulocytes exhibit more epoxide hydrolase activity towards cis-stilbene oxide than do lymphocytes.

As can also be seen from the Table 2, lymphocytes account for most of this activity in the mononuclear fraction, but the monocytes present also make a major contribution.
**DISCUSSION**

The importance of oxidative metabolism to the carcinogenic process via activation of carcinogens-mutagens to DNA-damaging epoxide intermediates is now well established (18, 23). AHH is one of these oxidizing enzymes that has been quantified in the lymphocytes of the human population (8, 9, 26). AHH inducibility was shown to be under genetic control (11) and in human mononuclear leukocytes are influenced by small effects in a somewhat different manner than are the rat liver enzymes. For example, rat epoxide hydrolase is nearly completely inhibited by 1,1,1-trichloropropane 2,3-oxide (21), whereas in human leukocytes this enzyme was essentially unaffected (Table 1). The fact that epoxide hydrolase activity is affected differently in human cells warrants further characterization into its role in regulation of chemically induced DNA.

*Epoxide Hydrolases in Human Mononuclear Leukocytes*

Table 2

<table>
<thead>
<tr>
<th>Component</th>
<th>Membrane-bound</th>
<th>Soluble</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific activity of epoxide hydrolase (pmol product/min/10^7 cells)</td>
<td>% present in total mononuclear cell fraction</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>6.27 ± 1.01</td>
<td>0.3</td>
</tr>
<tr>
<td>Monocytes</td>
<td>13.50 ± 2.34</td>
<td>ND</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>22.36 ± 0.26</td>
<td>0.3</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>0.17</td>
<td>ND</td>
</tr>
<tr>
<td>Platelets</td>
<td>&lt;0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>Plasma</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Mean ± S.D.

Table 3

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Membrane-bound</th>
<th>Soluble</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subject 1</td>
<td>Subject 2</td>
</tr>
<tr>
<td>Day 0</td>
<td>16.10</td>
<td>14.08</td>
</tr>
<tr>
<td>Day 6</td>
<td>13.70</td>
<td>17.49</td>
</tr>
<tr>
<td>Day 12</td>
<td>16.13</td>
<td>13.33</td>
</tr>
<tr>
<td>Day 18</td>
<td>15.77</td>
<td>16.44</td>
</tr>
<tr>
<td></td>
<td>16.93 ± 1.61</td>
<td>15.34 ± 1.69</td>
</tr>
<tr>
<td>Coefficient of variation (%)</td>
<td>9.5</td>
<td>11.0</td>
</tr>
<tr>
<td>Mean of coefficient of variation (%)</td>
<td>11.7</td>
<td>11.7</td>
</tr>
</tbody>
</table>

* Mean ± S.D.

We have demonstrated in the present study that membrane-bound and soluble epoxide hydrolase activities can be measured easily and routinely in freshly isolated circulating human mononuclear leukocytes. The use of cis- and trans-stilbene oxides as substrates, together with long incubation times, make such measurements possible. The cis- and trans-stilbene oxides are optimal substrates for epoxide hydrolases because they are hydrolyzed nonenzymatically at a very slow rate and because they can be highly purified in a simple thin-layer chromatographic system.

We have also defined the optimal conditions with regard to substrate concentration, pH, and temperature which should be used in assaying membrane-bound and soluble epoxide hydrolase activities in human mononuclear leukocytes. With few exceptions, most notably the high temperature optimum of the soluble enzyme, these properties are similar to those observed for microsomal and cytosolic epoxide hydrolases in other cell types (1, 16). On the other hand, the epoxide hydrolase activities in human mononuclear leukocytes are influenced by small effectors in a somewhat different manner than are the rat liver enzymes. For example, rat epoxide hydrolase is nearly completely inhibited by 1,1,1-trichloropropane 2,3-oxide (21), whereas in human leukocytes this enzyme was essentially unaffected (Table 1). The fact that epoxide hydrolase activity is affected differently in human cells warrants further characterization into its role in regulation of chemically induced DNA.
damage and the consequences thereof (i.e., risk estimation).

Other studies of microsomal epoxide hydrolase activity in human lymphocytes, utilizing both styrene 7,8-oxide (10) and benzo(a)pyrene 4,5-oxide as substrate (5, 6), have appeared previously. Because of the relatively high background obtained with styrene 7,8-oxide, epoxide hydrolase could not be measured directly in human lymphocytes with this substrate, but measurement could be made after culturing the cells for 3 to 4 days in the presence of mitogens. Because the background obtained with benzo(a)pyrene 4,5-oxide is much lower, microsomal epoxide hydrolase activity towards this substrate could be measured in fresh whole human mononuclear cells (7) or in sonicates prepared from these cells (6). The activity obtained was approximately 5 pmol product formed per min per mg protein, which is 1000-fold less than the corresponding activities in human and rat liver microsomes and approximately the same as the activity obtained here with cis-stilbene oxide as substrate (see Table 2).

We have also shown that most of the epoxide hydrolase activity in human mononuclear cell fractions towards cis-stilbene oxide is catalyzed by lymphocytes, but monocytes also make a significant contribution to this activity, because of their high activity per cell (2 times that of the lymphocytes). Granulocytes exhibit even higher epoxide hydrolase activities per cell (4 times that of the lymphocytes), but because they are present only at low levels in mononuclear cell fractions, they do not contribute very much to the total activity of this fraction. All of the epoxide hydrolase activity towards trans-stilbene oxide in human mononuclear cell fractions is catalyzed by the lymphocytes, which exhibit this activity per cell at a level which is at least 20-fold greater than the activity of any of the other blood components examined.

The interindividual variations in the membrane-bound and soluble epoxide hydrolases in the mononuclear cell fraction from 27 different subjects were 2.5-fold and 5-fold, respectively. This is similar to the interindividual variations reported for microsomal epoxide hydrolase activity toward styrene 7,8-oxide (10) and benzo(a)pyrene 4,5-oxide (6) in these same cells. Glatt et al. (6) reported that differences in sex or smoking habits could not account for these individual variations. However, in this study, we did show that mitogenic stimulation reduced interindividual variation. In the future, it will be of great interest to determine whether genetic, environmental, or methodological factors are the major determinants of these variations. During the course of an 18-day period, we found an intraindividual variation of only about 10% for both enzymes which indicates that the observed interindividual variation was not due solely to methodological factors.

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We are grateful to Dr. Åke Pilotti and Winni Birberg for their help in synthesizing the substrates.

REFERENCES

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