COMPARISON OF THE FORMATION OF N-ALKYLPROTOPORPHYRIN IX AFTER INTERACTION OF PORPHYRINOGENIC XENOBIOTICS WITH SINGLE cDNA-EXPRESSED HUMAN P450 ENZYMES IN MICROSOMES PREPARED FROM BACULOVIRUS-INFECTED INSECT CELLS AND HUMAN LYMPHOBLASTOID CELL LINES

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ABSTRACT:

In a previous study using microsomes from human lymphoblastoid cell lines (HLCL) containing single cDNA-expressed human cytochrome P450 (P450) enzymes, human P450 enzymes were identified that are susceptible to mechanism-based inactivation by the porphyrinogenic xenobiotics, 3-[arylthio]ethyl)sydnone (TTMS), 3,5-diethoxycarbonyl-1,4-dihydro-2,6-dimethyl-4-ethylpyridine (4-ethylDDC) and allylisopropylacetamide (AIA). In this study, we tested the hypothesis that N-alkylprotoporphyrin IX (N-alkylPP) formation following interaction of porphyrinogenic xenobiotics with single cDNA-expressed human P450 enzymes in microsomes from HLCL would occur only with P450 enzymes that had undergone mechanism-based inactivation. In a previous study, when 4-ethylDDC and NADPH interacted with human liver microsomes containing CYP1A2 and CYP2C9, N-ethylprotoporphyrin IX (N-ethylPP) was not formed despite the fact that it was formed in microsomes from baculovirus-infected insect cell lines (BIICL) containing either CYP1A2 or CYP2C9. In this study, we tested the hypothesis that 4-ethylDDC underwent biotransformation by CYP3A4 present in human liver microsomes, diverting the xenobiotic from CYP1A2 and CYP2C9. Fluorometry was used to measure N-alkylPP formation following interaction of porphyrinogenic xenobiotics and NADPH with cDNA-expressed human P450 enzymes in microsomes from HLCL or BIICL. With TTMS and 4-ethylDDC but not with AIA, N-alkylPP formation was observed only with human P450 enzymes CYP2D6, 1A2, 3A4, or 2C9 in microsomes from HLCL, which had undergone mechanism-based inactivation. Microsomes from BIICL containing CYP3A4 were added to a mixture of NADPH, 4-ethylDDC, and microsomes from BIICL containing CYP1A2 and 2C9. The addition of CYP3A4 to CYP1A2 and 2C9 did not decrease N-ethylPP formation, providing no support for the hypothesis.

Mechanism-based inactivation of cytochrome P450 (P450) resulting in porphyrinogenic effects involves the biotransformation of xenobiotics to reactive species, which bind to one of the four pyrrole nitrogens of the heme moiety of P450 to form a N-alkylprotoporphyrin IX (N-alkylPP) (Marks et al., 1988; Ortiz de Montellano and Correia, 1995). TTMS, 4-ethylDDC, and AIA (Fig. 1) are porphyrinogenic xenobiotics that undergo mechanism-based inactivation by P450 to form N-vinylPP, N-ethylPP, and N-AIAPP, respectively (Tephy et al., 1979; De Matteis et al., 1980; Sutherland et al., 1986; Ortiz de Montellano et al., 1986; Riddick et al., 1990). This is followed by depletion of heme and increased activity of the rate controlling enzyme, aminolevulinic acid synthase (E.C. 2.3.1.37; aminolevulinic acid synthase), porphyrin accumulation, and porphyrinia (De Matteis and Marks, 1996).

When a xenobiotic owes its porphyrinogenicity to mechanism-based inactivation of selective P450 enzymes with formation of N-alkylPPs, differences in P450 enzymes between humans and animals can lead to difficulties extrapolating results gained in test animals to humans. Thus, it is important to determine which P450 enzymes in both rats and humans are targets for mechanism-based inactivation, resulting in N-alkylPP formation.

Using microsomes from human lymphoblastoid cell lines (HLCL) containing single cDNA-expressed human P450 enzymes, McNamee et al. (1997) used selective enzyme activities to determine which human P450 enzymes are susceptible to mechanism-based inactivation after administration of TTMS, 4-ethylDDC, and AIA. There are several different mechanisms by which xenobiotics can cause mechanism-based inactivation of P450, namely, N-alkylPP formation, covalent binding to the apoprotein, degradation of the heme prosthetic group to products that may modify the apoprotein, and coordination to the prosthetic heme iron (Ortiz de Montellano and Correia, 1995).

Since hepatic porphyrin accumulation only occurs if N-alkylPP formation accompanies mechanism-based inactivation (Marks et al., 1988), it was necessary to determine which human P450 enzymes...
targeted for mechanism-based inactivation also elicited N-alkylPP formation. Since the studies of McNamee et al. (1997) were performed in HLCL, microsomes from baculovirus-infected insect cell lines (BIICL) possessing single cDNA-expressed P450 enzymes (Supersomes) became available. In view of the fact that these microsomes contained considerably higher amounts of P450 than the microsomes from the lymphoblastoid cells, we elected to use Supersomes to enhance the potential for formation of N-alkylPP from the heme moiety of P450. When Supersomes (containing CYP1A2, 2C9, 2D6, or 3A4) were incubated with TTMS, 4-ethylIDDC, and AIA, plus NADPH, N-alkylPP formation was quantitated using the sensitive technique of fluorometry (Lavigne et al., 2002). Some of the results obtained were unexpected. Thus, while mechanism-based inactivation of CYP2D6, 2C9, and 3A4 was not observed after interaction of AIA and NADPH, N-alkylPP formation was quantitated using the sensitive technique of fluorometry (Lavigne et al., 2002). Some of the results obtained were unexpected. Thus, while mechanism-based inactivation of CYP2D6, 2C9, and 3A4 was not observed after interaction of AIA and NADPH with microsomes prepared from HLCL (McNamee et al., 1997), considerable amounts of N-AIAPP were formed after interaction of AIA and NADPH with Supersomes (Lavigne et al., 2002). A possible explanation for these unexpected results was the difference between the microsomes obtained from HLCL and BIICL (e.g., a difference in the P450 to 450 reductase ratio). The first objective of this study was to determine by fluorometry the formation of N-alkylPPs after mechanism-based inactivation by TTMS, 4-ethylIDDC, and AIA of single cDNA-expressed human P450 enzymes in microsomes prepared from HLCL and to compare the results with those previously obtained in microsomes from BIICL. The hypothesis to be tested was that N-alkylPP formation following interaction of porphyrinogenic xenobiotics with single cDNA-expressed human P450 enzymes in microsomes from HLCL would occur only with P450 enzymes that had undergone mechanism-based inactivation.

In a previous study, 4-ethylIDDC and NADPH were allowed to interact with human liver microsomes possessing elevated levels of CYP1A2 and 2C9 (P450:NADPH-P450 reductase, 1:0.34; BD Gentest Donor HG56). It was anticipated that N-ethylPP would be formed since N-ethylPP formation was observed with Supersomes containing either CYP1A2 (ratio of P450:NADPH-P450 reductase, 1:10.4) or 2C9 (ratio of P450:NADPH-P450 reductase, 1:0.7) after 4-ethylIDDC treatment. However, following incubation less than 0.04 nmol of N-ethylPP was formed where 0.04 nmol is the lower limit of detection (LLD) (Lavigne et al., 2002), and this unexpected result was explained as follows: although human liver microsomes (HG56) contained elevated levels of CYP1A2 and 2C9, CYP3A4 also constitutes 12.5% of the total P450. Correia et al. (1987) have shown that 4-ethylIDDC caused mechanism-based inactivation of rat CYP3A2, which is not accompanied by N-ethylPP formation. This finding suggests that the major portion of 4-ethylIDDC may have undergone biotransformation by CYP3A4 diverting the xenobiotic from CYP1A2 and 2C9. The second objective of this study was to test this hypothesis by comparing N-ethylPP formation in a mixture containing NADPH-P450 reductase and human cDNA-expressed CYP1A2 and 2C9 with N-ethylPP formation in a mixture of NADPH-P450 reductase and human cDNA-expressed CYP1A2, 2C9, and 3A4.

Materials and Methods

Source of Chemicals. TTMS and 4-ethylIDDC were obtained from Color Your Enzyme (Bath, Ontario, Canada). AIA was obtained as a gift from F. Hoffman-La Roche (Vaudreuil, Quebec, Canada). Solvents were purchased from VWR Canada (Mississauga, Ontario, Canada). NADPH was purchased from Sigma-Aldrich (St. Louis, MO). Microsomes containing human lymphoblast-expressed P450 enzymes and Supersomes were purchased from BD Gentest Corporation (Woburn, MA).

Mechanism-based Inactivation of Single Human P450 Enzymes Expressed in Microsomes from Human Lymphoblastoid Cell Lines with Porphyrinogenic Xenobiotics. Microsomes containing single cDNA-expressed P450 enzymes (TTMS, 4-ethylIDDC, or AIA) were added to a preincubation mixture containing the porphyrinogenic xenobiotic, NADPH, and P450 (0.66 nmol CYP1A2, 0.43 nmol CYP2C9, 0.90 nmol CYP2D6, 0.58 nmol CYP3A4). The P450:NADPH-P450 reductase ratios were 1:0.11 (CYP1A2), 1:4.30 (CYP2C9), 1:0.60 (CYP2D6), and 1:0.77 (CYP3A4) as provided by BD Gentest Corporation. Controls were carried out in which the experiments were repeated with the omission of NADPH or the porphyrinogenic xenobiotic.

Partial Purification and Measurement of N-AlkylPP Formation. After the incubation period, the sample mixture was combined with five volumes of ice-cold 5% H2SO4/methanol (v/v) and stored in the dark at 4°C for 18 h. This mixture was then filtered, diluted with an equal volume of deionized water, and the N-alkylPPs were extracted twice with the addition of dichloromethane (DCM) (3 ml) in a separatory funnel. The DCM extract was washed with 5% sodium bicarbonate (1 ml), and zinc acetate (12 μmol) in methanol (1 ml) was added to form the Zn-N-alkylPP dimethyl ester. The DCM solution was evaporated to dryness under a stream of nitrogen, and the Zn-N-alkylPP dimethyl ester was redissolved in DCM (2.0 ml). The amount of Zn-N-alkylPP dimethyl ester formed was subsequently estimated via fluorometry using an excitation wavelength of 432 nm and measuring the peak height of the major emission band at 650 to 660 nm (Lavigne et al., 2002). Peak heights obtained from control experiments in which NADPH was omitted were subtracted.

Mechanism-based Inactivation of Human P450 Enzymes in Supersomes with Porphyrinogenic Xenobiotics and Partial Purification and Measurement of N-AlkylPP Formation in the Supersomes. A procedure similar to that described above for microsomes from HLCL was used for the incubation of the Supersomes and partial purification and measurement of N-alkylPP formation. CYP1A2 (0.5 nmol), CYP2C9 (0.5 nmol), and CYP3A4 (0.25 nmol) were combined in a ratio corresponding approximately to the ratio of these enzymes in human microsomes from Donor HG56 (BD Gentest Corporation). The mixture of enzymes was incubated with NADPH (2.0 mM) and 4-ethylIDDC (1.0 mM) for 30 min at 37°C. The experiment was repeated with the omission of CYP3A4 or with the omission of 4-ethylIDDC. The experiments were carried out in duplicate except for the control experiment in which 4-ethylIDDC was omitted, which was carried out once.

Results and Discussion

The first objective of this study was to determine by fluorometry the formation of N-alkylPPs after mechanism-based inactivation by TTMS, 4-ethylIDDC, or AIA of single cDNA-expressed human P450 enzymes in microsomes prepared from HLCL and to compare the results with those previously obtained in microsomes from BIICL. The hypothesis to be tested was that N-alkylPP formation following interaction of porphyrinogenic xenobiotics with single cDNA-expressed human P450 enzymes in microsomes from HLCL would
occur only with P450 enzymes that had undergone mechanism-based inactivation. When TTMS (0.5 mM), 4-ethylDDC (1.0 mM), or AIA (1.0 mM) were incubated in the presence of NADPH with microsomes from HLCL possessing cDNA-expressed human CYP2D6, no significant amount of N-alkylPP was detected (Table 1). The LLD for N-alkylPP detection by fluorometry was found previously in our laboratory to be 0.04 nmol/2 ml of DCM (Lavigne et al., 2002). This LLD would apply equally to studies of N-alkylPP formation determined by fluorometry in microsomes from HLCL and BIICL. It was also shown that omission of a thin-layer chromatography step for N-alkylPP purification, which was required for UV detection but was not required for fluorometric detection, resulted in good recovery of N-alkylPP (Lavigne et al., 2002). These results accord with the hypothesis to be tested since in previous studies neither TTMS, 4-ethylDDC, nor AIA caused mechanism-based inactivation of CYP2D6 (Table 1) (McNamee et al., 1997). The N-alkylPP results in HLCL in this study contrast with results previously obtained with AIA in BIICL in which 0.23 nmol of N-AIAPP were formed per nanomole of CYP2D6 (Lavigne et al., 2002).

When TTMS (0.5 mM), 4-ethylDDC (1.0 mM), or AIA (1.0 mM) were incubated in vitro with microsomes from HLCL possessing cDNA-expressed human CYP1A2, only 4-ethylDDC elicited significant N-alkylPP formation (2.8 times the minimal significant value) in a NADPH-dependent manner (Table 1). The average ratio of N-ethylPP formation to the amount of CYP1A2 present was 0.22. With TTMS and AIA, no significant amounts of N-alkylPPs were detected. In the previous studies of McNamee et al. (1997), 4-ethylDDC and TTMS but not AIA caused mechanism-based inactivation of CYP1A2 in microsomes from HLCL. Thus, the lack of N-alkylPP formation after AIA administration accorded with its inability to cause mechanism-based inactivation of CYP1A2. The detection of N-ethylPP after 4-ethylDDC administration shows that mechanism-based inactivation of CYP1A2 by 4-ethylDDC proceeds by a route that includes N-ethylPP formation. The fact that no N-ethylPP was detected shows that mechanism-based inactivation of CYP1A2 by TTMS proceeds by a route that does not include N-ethylPP formation. The formation of N-alkylPPs after administration of porphyrinogenic xenobiotics previously observed in microsomes from BIICL coincided with the present results using microsomes from HLCL (Table 1).

When TTMS (0.5 mM), 4-ethylDDC (1.0 mM), or AIA 1.0 mM) were incubated in vitro with microsomes from HLCL possessing cDNA-expressed human CYP3A4, TTMS elicited N-alkylPP formation in a NADPH-dependent manner. The average ratio of N-vinylPP formation to the amount of CYP3A4 present was 0.24 (Table 1). On the other hand, N-alkylPP formation was not significant with 4-ethylDDC and was borderline with AIA. In the previous results of McNamee et al. (1997), TTMS and 4-ethylDDC caused mechanism-based inactivation of CYP3A4 in microsomes from human lymphoblastoid cell lines. The detection of N-vinylPP after TTMS administration shows that mechanism-based inactivation of CYP3A4 proceeds by a route that includes N-vinylPP formation. The fact that no significant amount of N-ethylPP formation was detected shows that mechanism-based inactivation of CYP3A4 by 4-ethylDDC occurs either by direct alkylation of the apoprotein or destruction of the heme moiety. This result is consistent with a report by Correia et al. (1987) that mechanism-based inactivation of rat CYP3A2 by 4-ethylDDC involves fragmentation of the heme moiety to reactive metabolites that irreversibly bind to the P450 apoprotein. In the previous study of McNamee et al. (1997), AIA (1 mM) caused 19% mechanism-based inactivation of CYP3A4, which, however, did not reach statistical significance. In the present study the amount of N-AIAPP detected was at the borderline of significance. Our new N-alkylPP results contrast with those previously obtained with AIA in microsomes from BIICL in which 0.30 nmol of N-AIAPP were formed per nanomole of CYP3A4 (Lavigne et al., 2002).

When TTMS (0.5 mM), 4-ethylDDC (1.0 mM), or AIA (1.0 mM) were incubated in vitro with microsomes from HLCL possessing cDNA-expressed human CYP2C9, only AIA elicited N-alkylPP formation in a NADPH-dependent manner (Table 1). With TTMS and 4-ethylDDC, no significant amounts of N-alkylPP were detected. In previous studies of McNamee et al. (1997), 4-ethylDDC but neither TTMS nor AIA caused mechanism-based inactivation of CYP2C9 in microsomes derived from HLCL. Thus, the inability to detect N-vinylPP formation after TTMS administration accorded with its inability to cause mechanism-based inactivation of CYP2C9. The fact that no N-ethylPP was detected shows that mechanism-based inactivation of CYP2C9 by 4-ethylDDC occurs either by direct alkylation of the apoprotein or destruction of the heme moiety. The fact that the average ratio of N-AIAPP formation to the amount of P450 was 0.14 nmol/nmol was unexpected since no significant mechanism-based inactivation of CYP2C9 by AIA had been previously detected, and

<table>
<thead>
<tr>
<th>Xenobiotic</th>
<th>P450 Enzyme</th>
<th>Mechanism-based Inactivation in Microsomes from HLCL*</th>
<th>N-AlkylPP Formation in Microsomes from HLCL mmol N-AlkylPP/nmol P450</th>
<th>N-AlkylPP Formation in Microsomes from BIICL nmol N-AlkylPP/nmol P450 (Lavigne et al., 2002)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTMS</td>
<td>2D6</td>
<td>-</td>
<td>0.01 (0.01, 0.0)</td>
<td>0.08 (0.08)</td>
</tr>
<tr>
<td>4-EthylDDC</td>
<td>2D6</td>
<td>-</td>
<td>0.03 (0.04, 0.01)</td>
<td>0.08 (0.08)</td>
</tr>
<tr>
<td>AIA</td>
<td>2D6</td>
<td>+</td>
<td>0.02 (0.0, 0.04)</td>
<td>0.23 (0.24, 0.22)</td>
</tr>
<tr>
<td>TTMS</td>
<td>1A2</td>
<td>+</td>
<td>0.06 (0.07, 0.05)</td>
<td>N.D.</td>
</tr>
<tr>
<td>4-EthylDDC</td>
<td>1A2</td>
<td>+</td>
<td>0.22 (0.22, 0.22)</td>
<td>0.60 (0.24, 0.25)</td>
</tr>
<tr>
<td>AIA</td>
<td>1A2</td>
<td>+</td>
<td>0.05 (0.06, 0.04)</td>
<td>N.D.</td>
</tr>
<tr>
<td>TTMS</td>
<td>3A4</td>
<td>-</td>
<td>0.45 (0.45, 0.45)</td>
<td>0.87 (0.85, 0.89)</td>
</tr>
<tr>
<td>4-EthylDDC</td>
<td>3A4</td>
<td>+</td>
<td>0.04 (0.06, 0.03)</td>
<td>N.D.</td>
</tr>
<tr>
<td>AIA</td>
<td>3A4</td>
<td>-</td>
<td>0.08 (0.11, 0.06)</td>
<td>0.30 (0.28, 0.32)</td>
</tr>
<tr>
<td>TTMS</td>
<td>2C9</td>
<td>-</td>
<td>0.03 (0.01, 0.02)</td>
<td>N.D.</td>
</tr>
<tr>
<td>4-EthylDDC</td>
<td>2C9</td>
<td>-</td>
<td>0.24 (0.28, 0.20)</td>
<td>0.29 (0.39, 0.18)</td>
</tr>
<tr>
<td>AIA</td>
<td>2C9</td>
<td>-</td>
<td>0.14 (0.13, 0.15)</td>
<td>0.21 (0.26, 0.16)</td>
</tr>
</tbody>
</table>

N.D., Not Detected.
*-, denotes no mechanism-based inactivation; +, denotes mechanism-based inactivation.
N-alkylPP formation is believed to depend on mechanism-based inactivation of P450. A comparison of our results in HLCL with those previously obtained in microsomes from BIICL reveals similar results with TTMS and AIA (Table 1). However, in contrast to insignificant amounts of N-ethylPP detected after 4-ethylDDC interaction with microsomes of HLCL, the average ratio was found to be 0.29 nmol of N-ethylPP/nmol CYP2C9 in microsomes from BIICL.

In summary, N-alkylPP formation with TTMS and 4-ethylDDC in microsomes from HLCL was observed only with human P450 that had undergone mechanism-based inactivation. The converse was not the case and in some cases mechanism-based inactivation was not accompanied by N-alkylPP formation. This was to be expected since mechanism-based inactivation can occur by pathways that do not include N-alkylPP formation. In the case of AIA, despite the fact that mechanism-based inactivation was not observed with either CYP2D6, 1A2, 3A4, or 2C9, N-alkylPP formation was significant with CYP2C9 and at the borderline of significance with CYP3A4. A possible explanation for this result is that significant mechanism-based inactivation may not be detected due to relatively large standard deviations in the NADPH-treated and NADPH-untreated groups. On the other hand, detection of an N-alkylPP as by direct measurement and does not depend on group differences. In contrast to the results obtained with N-alkylPP formation in microsomes from HLCL, significant N-alkylPP formation was found with AIA in microsomes from BIICL for three of four P450 enzymes, 2D6, 3A4, and 2C9, in which mechanism-based inactivation had not been observed. We conclude that mechanism-based inactivation and N-alkylPP formation after porphyrinogenic xenobiotic administration should be compared in microsomes from the same cell system. The P450:NADPH-P450 reductase ratios for microsomes from HLCL were 1:0.11 (CYP1A2), 1:4.30 (CYP2C9), 1:0.60 (CYP2D6), and 1:0.77 (CYP3A4). These values differ in BIICL where the ratios were 1:10.4 (CYP1A2), 1:0.70 (CYP2C9), 1:13.28 (CYP2D6), and 1:0.31 (CYP3A4). However, there is no obvious correlation between differences in these ratios and N-alkylPP formation (Table 1). It is of interest that these ratios in general are considerably higher than the P450:NADPH-P450 reductase ratio in normal human liver microsomes (1:0.1) (Benet et al., 1996).

We had previously observed that when 4-ethylDDC and NADPH interacted with human liver microsomes containing 4.98 nmol of total P450 of which 25% was CYP1A2 and 29% was CYP2C9 (BD Gentest Donor HG56), N-alkylPP was not formed. This was a surprising finding since N-ethylylPP formation was observed in microsomes from BIICL containing either CYP1A2 or 2C9. This unexpected finding was explained as follows: human liver microsomal preparation HG56 also contains 12.5% of CYP3A4. Correia et al. (1987) have shown that 4-ethylDDC causes mechanism-based inactivation of rat CYP3A2, which is not accompanied by N-ethylPP formation. It was therefore suggested that a major portion of 4-ethylDDC may have undergone biotransformation by CYP3A4 thus diverting the xenobiotic from CYP1A2 and 2C9. The second objective of this study was to test this explanation by comparing N-ethylPP formation after administration of 4-ethylDDC to a mixture containing human cDNA-expressed CYP1A2 and 2C9 with formation of N-ethylPP in a mixture of human cDNA-expressed CYP1A2, 2C9, and 3A4. When a mixture of CYP1A2 (0.5 nmol) and 2C9 (0.5 mmol) was incubated with NADPH and 4-ethylDDC (1.0 mM), an average of 0.10 nmol of N-ethylPP/nmol P450 was formed (first experiment 0.11 nmol, second experiment 0.09 nmol). On the other hand, when a mixture of CYP1A2 (0.5 nmol), 2C9 (0.5 nmol), and 3A4 (0.25 nmol) was incubated with NADPH and 4-ethylDDC (1.0 mM) an average of 0.11 nmol of N-ethylPP/nmol P450 was formed (first experiment 0.09 nmol, second experiment 0.13 nmol). Thus, no significant difference was observed in the formation of N-ethylPP from 4-ethylDDC when CYP3A4 was added to a mixture of CYP1A2 and 2C9. Therefore, it is necessary to consider other possible explanations for the discrepancy between the ability of 4-ethylDDC to cause N-ethylPP formation when interacting with cDNA-expressed human CYP1A2 or 2C9, and the inability to cause N-ethylPP formation when interacting with human liver microsomes possessing elevated levels of CYP1A2 and 2C9. One possibility is that the human liver microsomes contained, in addition to CYP1A2, 2C9, and 3A4, 11.6% CYP2A6, 5% CYP2C19, 2.2% CYP2D6, and 13.2% CYP2E1, and results obtained with expression systems may not reflect the overall in vivo effects of these compounds, which is dependent on the specific activity and relative abundance of each P450 enzyme. In addition, the phospholipid environment, lipid to protein ratio and the P450:NADPH-P450 reductase ratio can differ between native microsomal systems and cDNA-expressed single P450 enzyme systems (Rodrigues, 1999). It is of interest that the P450: NADPH-P450 reductase ratio in the human microsomes (BD Gentest Donor HG56) was 1:0.34. This value contrasts with the P450: NADPH-P450 reductase ratios for microsomes from BIICL, which were 1:10.4 (CYP1A2), 1:0.70 (CYP2C9), and 1:0.31 (CYP3A4).

References


