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HUMAN LIVER MICROSOMAL CYTOCHROME P450 3A ISOZYMES MEDIATED VINDESINE BIOTRANSFORMATION

METABOLIC DRUG INTERACTIONS

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Abstract—Vindesine biotransformation was investigated using a bank of human liver microsomes. The drug was converted into one major metabolite (M) upon incubation with the microsomes. Large inter-individual variations were observed: vindesine biotransformation rates ranged from 1.2 to 12.9 pmol/min/mg protein. Vindesine metabolic processes followed Michaelis–Menten kinetics: K_m = 24.7 ± 9.4 μM, V_{max} = 1.5 ± 0.8 nmol/min/mg protein. The involvement of human cytochrome P450 3A isozymes in vindesine metabolism was demonstrated by: (1) competitive inhibition of vindesine biotransformation by compounds known to be specifically metabolized by human cytochrome P450 3A. Apparent K_i values were 3.6, 17.9 and 19.8 μM for quinidine, troleandomycin and erythromycin, respectively; (2) immunoinhibition of vindesine metabolism by polyclonal anti-P450 3A antibody; (3) significant correlation between immunoquantified P450 3A and vindesine biotransformation ($r = 0.800$, $P < 0.001$); and (4) significant correlation between erythromycin *N*-demethylase activity, which was supported by P450 3A in humans, and vindesine biotransformation ($r = 0.853$, $P < 0.001$). Other vinca alkaloids also exerted an inhibitory effect on vindesine biotransformation with apparent K_i values of 3.8, 10.6 and 19.2 μM for vinblastine, vincristine and navelbine, respectively, suggesting a possible involvement of the same cytochrome subfamily in their hepatic metabolism. Moreover, a number of anticancer drugs currently associated with the vinca alkaloids, such as teniposide, etoposide, doxorubicin, lomustine, folinic acid and mitoxantrone, significantly inhibited vindesine biotransformation.

Vinca alkaloids, including vinblastine, vincristine, vindesine and navelbine are anticancer drugs widely used both as single agents and in combination with other drugs in cancer chemotherapy [1, 2]. Although these molecules are structurally related, marked differences have been observed in their antitumor activity, toxicity and pharmacokinetic behavior [3]. The clinical pharmacokinetic parameters of vinca alkaloids present large inter- and intra-individual variations [4–10]. Some authors have suggested that intra- and inter-individual variations in vinca alkaloid pharmacokinetics may result from individual differences in hepatic drug disposition [6, 11] and from undefined time- and dose-dependent pharmacokinetics [10, 12, 13]. To date, however, there has been no complete elucidation of such phenomena. Clues might be found by studying the metabolism of these compounds and enzymes involved in such processes. Previous experiments performed by using *in situ* perfusion of rat liver demonstrated that the vinca alkaloids are largely excreted via the hepatobiliary system both as unchanged drug and metabolites [14, 15]. Upon incubation with freshly isolated rat hepatocytes in suspension, a number of metabolites of the vinca alkaloids were formed [14, 15]. However, whether these biotransformation products are formed in patients remains to be clarified. Deacetylated derivatives have been partially characterized as metabolites after i.v. injection of tritiated and non-radiolabeled vinblastine [16], vincristine [17] and navelbine [18]. Deacetylvinblastine has been shown to be four to five times more potent as an antitumor agent than vinblastine [19].

The cytochrome P450-dependent monooxygenase system is a multigene superfamily of hemoproteins, which is abundant in the endoplasmatic reticulum of hepatocytes. It is involved in the oxidative biotransformation of numerous endogenous substances (such as steroids and fatty acids) and xenobiotics (drugs, carcinogens). In humans, nine distinct cytochrome P450 gene families have so far been characterized in terms of molecular, spectral, enzymatic and immunologic properties [20]. These include the P450 1 family comprising only one subfamily (P450 1A1 and P450 1A2) inducible by polycyclic aromatic hydrocarbons; family P450 2 divided into six subfamilies: P450 2A (2A6 and 2A7), P450 2B (2B6 and 2B7P), P450 2C (2C8, 2C9, 2C10 and 2C19), P450 2D (2D6, 2D7P and 2D8P), ethanol inducible P450 2E (2E1) and P450 2F (2F1);

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family P450 3 comprising one subfamily (3A3, 3A4, 3A5 and 3A7) inducible by macrolide antibiotics and steroids; family P450 4 including two subfamilies: P450 4A (4A9) and P450 4B (4B1); family P450 11 divided into two subfamilies: P450 11A (11A1) and P450 11B (11B1 and 11B2); and family P450 21 comprising only one subfamily: P450 21A (21A1P and 21A2). For the three other families, i.e. P450 17, P450 19 and P450 27, no subfamilies have been identified yet.

It is now well known that liver microsomal cytochrome P450s are directly involved in a number of drug-induced hepatotoxicities and drug interactions. Some of these adverse effects are caused by genetic polymorphism; others come from drug interactions occurring between two or more co-administered drugs when they are metabolized by the same cytochrome P450, and when one of them is a specific inhibitor or a specific inducer of the cytochrome P450 in question.

To date, research on the identification of specific cytochrome P450 isozymes in the metabolism of cancer chemotherapeutic agents has been limited to a few compounds such as cyclophosphamide, procarbazine and 1-(2-chloroethyl)-3-(cyclohexyl)-1-nitrosourea and 1-(2-chloroethyl)-3-(*trans*-4-methylcyclohexyl)-1-nitrosourea, where rat hepatic microsomes were used [21]. Very little knowledge of the human enzymes involved in the metabolism of cancer chemotherapeutic agents is available. Most anticancer drugs produce a number of side effects. Thus, a better understanding of the enzyme systems that process these compounds would be of great use in designing therapy protocols that will predict adverse drug interactions and avoid unwanted toxicities. In that context, we evaluated the biotransformation of vindesine by using a bank of human liver microsomal fractions. The aim of the present report was to identify human liver microsomal cytochrome P450 isozymes involved in vindesine biotransformation and to evaluate metabolic drug interactions possibly existing between vindesine and other anticancer agents often associated with the vinca alkaloids.

MATERIALS AND METHODS

Drugs and chemicals. Vindesine, vinblastine and vincristine were generously supplied by Eli Lilly Research Laboratories (Indianapolis, IN, U.S.A.). Navelbine was obtained from Pierre Fabre Médicament (Boulogne, France). [^{3}H]Vindesine (1.0 Ci/mmol) was purchased from Moravek Biochemical Inc. (Brea, CA, U.S.A.) and was >98% pure as ascertained by HPLC described below. Benzo[*a*]pyrene, theophylline, hexobarbital, spartein, quinidine, troleandomycin, nifedipine, erythromycin, aniline, 1-β-D-arabinofuranosylcytosine (Ara-C), lomustine and NADPH were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Etoposide, teniposide and cyclosporin A were obtained from Sandoz Ltd (Basel, Switzerland). Doxorubicin, cisplatin and lomustine were obtained from Roger Bellon Laboratories (Neuilly, France). Debrisoquine and 5-fluorouracil were supplied by Hoffmann La Roche Laboratories (Basel, Switzerland). Methotrexate, mitoxantrone and folinic acid were gifts of Lederle Laboratories (Pearl River, NY, U.S.A.).

Human liver microsomes. A bank consisting of 29 human liver microsomal samples is available in our laboratory. Human livers were obtained under strict ethical conditions from multi-organ donors. The use of such samples was authorized by the French National Ethics Committee in the cases where the organs could not be used for transplantation. The microsomes were prepared by a differential centrifugation technique described previously [22]. After preparation, microsomal samples termed HL1 to HL39 were stored at −80° prior to use. Microsomal protein concentrations were measured by the method of Bradford [23] using bovine serum albumin as standard. Table 1 summarizes the characteristics of the human microsome bank.

Inter-individual variability of vindesine biotransformation. Vindesine (1 μM final concentration) was incubated with 29 microsomal samples (2 mg protein/mL final concentration) in phosphate buffer (0.1 M, pH 7.4) at 37°. Reactions were initiated by the addition of NADPH (1 mM final concentration). Final incubation volume was 250 μL. After a 30 min incubation, reactions were stopped by adding an equal volume of methanol. Proteins were removed by centrifugation at 15,000 *g* for 5 min, and 200 μL aliquots of the supernatant were analysed by HPLC as described below.

Enzyme kinetics. Initial experiments were conducted to determine optimal incubation conditions. They were selected so that vindesine biotransformation was linear with respect to (incubation) time and (microsomal) protein concentration. These experiments demonstrated that vindesine metabolism was linear during 20 min and at a protein concentration of 0.5 mg/mL. NADPH was used at a final concentration of 1 mM throughout all experiments. Final incubation volume was 250 μL. Under these conditions, eight vindesine concentrations (ranging from 1 to 50 μM) were assessed. The Michaelis–Menten parameters (apparent K_m and V_{max}) were determined for three microsomal samples (HL1, HL30 and HL31, HL for human liver) by using the Lineweaver–Burk plot.

Inhibition of vindesine metabolism by various compounds. A number of compounds known to be metabolized by specific human liver cytochromes P450 were assessed for their inhibitory effects on vindesine biotransformation. These compounds included benzo[*a*]pyrene and theophylline which are metabolized by cytochrome P450 1A; hexobarbital (P450 2C); spartein and debrisoquine (P450 2D); aniline (P450 2E); troleandomycin, erythromycin, nifedipine, quinidine and cyclosporin A (P450 3A). Vinblastine, vincristine and navelbine, which are structurally related to vindesine, were also tested. Assays were performed using microsomes HL31 in the above mentioned optimal conditions. Final vindesine concentration was set at 10 μM; inhibitor concentration was 5-fold the K_m value determined on the same microsomal sample. When inhibition was over 50%, full kinetic studies were performed to determine the apparent K_i values, and to characterize the inhibition mechanism by Dixon

Table 1. Characteristics of the human liver microsome bank

HL	Sex	Age (year)	Co-medication	P450 contents (nmol/min/mg protein)	Protein contents (mg/mL)
1	M	29	Corticoids	0.25	30.0
5	M	36	Corticoids Penthotal	0.27	38.8
7	M	30	Narcoprep	0.21	34.2
8	M	20	Corticoids	0.47	29.8
9	M	32	Corticoids Dopamine	0.37	22.5
10	M	46	Dopamine	0.25	29.5
11	F	38	Dopamine	0.34	32.9
13	M	23	Dopamine	0.46	34.9
14	M	56	Dopamine	0.40	26.1
15	M	19	Dopamine	0.44	31.8
17	M	41	Dopamine (alcoholic past)	0.44	24.6
18	F	40	Dopamine	0.44	32.8
20	F	38	Dopamine	0.32	21.5
21	F	24	Hydrocortisone Dopamine	0.46	28.1
22	M	26	Dopamine	0.37	17.6
23	M	26	Dopamine Kerlone Dihydroergotamine	0.58	10.0
24	F	27	Dopamine Corticoids	0.37	34.0
26	M	45	Dopamine	0.21	30.0
27	F	42	Dopamine	0.27	33.0
28	M	42	—	0.29	27.0
29	M	33	Dopamine	0.30	26.3
30	F	17	Dopamine	0.51	27.6
31	M	36	Dopamine	0.35	27.1
33	F	31	Dopamine	0.43	15.4
34	F	37	Dopamine Penthotal Phenobarbital	0.78	20.4
35	M	41	—	0.30	34.1
36	M	27	Dopamine	0.14	15.6
37	M	36	Dopamine (alcoholic past)	0.26	24.0
39	F	40	Dopamine	0.54	36.2

—, No co-medication.

plot. For these assays, three vindesine concentrations (5, 7 and 10 μM) and a series of inhibitor concentrations (ranging from 0.25 to 60 μM) were used.

Immunoinhibition of vindesine metabolism by anti-cytochrome P450 antibodies. Four polyclonal antibodies directed against cytochrome P450 1A, 2B, 2C and 3A were prepared and purified as described previously [24–26]. Microsomes (HL31) were used at a final concentration of 1 mg protein/mL and were pre-incubated for 3 min at 37° and 20 min at room temperature in the absence or in the presence of increasing amounts of antibodies (from 1 to 5 mg/mL final). Final vindesine concentration was 5 μM and assay was allowed to proceed as indicated above.

Correlation between immunoquantified microsomal P450 3A and vindesine metabolism. Western blot analysis of human microsomes was carried out as reported elsewhere [27]. Briefly, proteins from human liver microsomes (10 μg/mL) were subjected to electrophoresis on a 10% polyacrylamide–SDS gel according to the Laemmli procedure [28]. Separated proteins were then electrophoretically transferred to nitrocellulose filters (Bio-Rad Laboratories, Richmond, CA, U.S.A.) followed by incubation with anti-P450 3A antibody, and species-specific horseradish peroxidase-labeled polyclonal antibody. Finally, the blot was developed with diaminobenzidine and hydrogen peroxide. The relative amount of P450 3A in various human microsomes was estimated from densitometric analysis of the blot with a Shimadzu model CR-930 dual-wavelength scanner (Shimadzu Co., Kyoto, Japan). The immunoquantified P450 3A of the human microsome bank was correlated with vindesine biotransformation by linear regression.

Correlation between microsomal monooxygenase activity and vindesine metabolism. Erythromycin *N*-demethylase activity of the human microsome bank

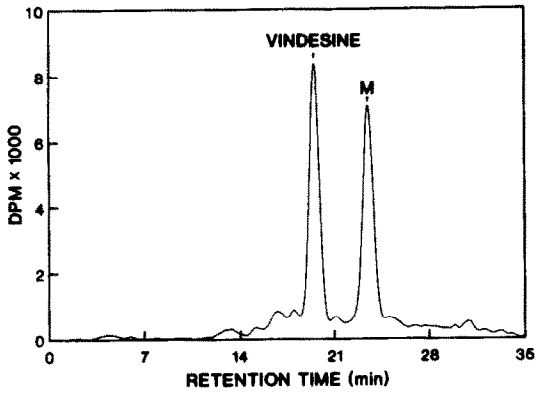

Fig. 1. High performance liquid chromatogram of vindesine and its major metabolite. Vindesine (1 μM) was incubated 20 min at 37° in a total volume of 250 μL of 0.1 M phosphate buffer (pH 7.4), with microsomes HL1 (2 mg protein/mL) in the presence of 1 mM NADPH. After quenching by addition of an equal volume of methanol, the reaction mixture was centrifuged at 15,000 *g* for 5 min and a 200 μL aliquot of the supernatant was analysed by HPLC.

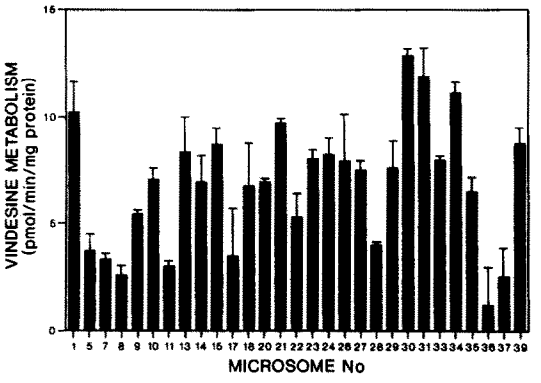

Fig. 2. Inter-individual variability of vindesine biotransformation. Vindesine (1 μM) was incubated, 30 min at 37° in a total volume of 250 μL of 0.1 M phosphate buffer (pH 7.4), with 29 microsomal samples (2 mg protein/mL). After incubation, reactions were stopped by adding methanol. Proteins were removed by centrifugation at 15,000 *g* for 5 min and 200 μL aliquots of the supernatant were analysed by HPLC. Numbers, 1, 5, . . ., 39, represent human liver microsomal samples from donors 1, 5, . . ., 39, respectively.

was determined by colorimetric measurement of the formaldehyde formed [29] and was correlated with vindesine biotransformation by linear regression.

Metabolic drug interactions. A number of anticancer drugs frequently associated with the vinca alkaloids in cancer chemotherapy were assessed for their inhibitory effects on vindesine biotransformation. These drugs included Ara-C, doxorubicin, etoposide, teniposide, cisplatin, 5-fluorouracil, folinic acid, lomustine, methotrexate and mitoxantrone. Assays were carried out using microsomes HL31 in the optimal conditions described above. Final vindesine concentration in incubation medium was 10 μM; the anticancer drugs were used at 100 μM. All assays in the present study were done in triplicate.

HPLC. Vindesine and its metabolites were separated using HPLC with a Hewlett–Packard model 1084 liquid chromatograph equipped with an automatic injection system. Detection of tritiated compounds was performed on a radioactive flow detector (Flow-one beta, Radiomatic Instruments & Chemical Co., Inc., Tampa, FL, U.S.A.). Reversed phase chromatography was carried out using a μBondapak phenyl column (10 μm, 3.9 × 300 mm, Waters Associates, Milford, MA, U.S.A.). The mobile phase consisted of methanol and perchlorate buffer (45 mM sodium perchlorate/5 mM perchloric acid). Elution was performed by a programmed linear methanol gradient (from 42 to 75%) for 35 min at a constant flow rate of 0.9 mL/min.

RESULTS

Metabolic pattern of vindesine by human liver microsomes. Figure 1 illustrates the metabolic pattern of vindesine obtained after a 20-min incubation with microsomes (HL1) in the presence of NADPH. Vindesine was converted into one major metabolite (M).

Inter-individual variability of vindesine biotransformation. Vindesine biotransformation rates were studied in 29 human liver microsomal samples. Results indicate a significant variability among individuals (Fig. 2): microsomes HL30 metabolized vindesine at a mean ± SD rate of 12.9 ± 0.4 pmol/min/mg protein, which was about 10-fold more rapid than did the HL36 (1.2 ± 1.7 pmol/min/mg protein).

Enzyme kinetics. Vindesine biotransformation by human liver microsomes followed Michaelis–Menten kinetics as demonstrated by Lineweaver–Burk plot. The monophasic kinetics suggested a single enzyme binding site for vindesine. Apparent K_m values were 18.4, 35.5 and 20.3 μM for microsomes HL1, 30 and 31, respectively; apparent V_{max} values were 0.9, 2.2 and 1.2 nmol/min/mg protein. Mean ± SD values of K_m and V_{max} were, respectively, 24.7 ± 9.4 μM and 1.5 ± 0.8 nmol/min/mg protein.

Inhibition of vindesine metabolism by various compounds. These assays were carried out using microsomes HL31. Final concentration of the tested compounds was set at 100 μM, which was approximately 5-fold the K_m value determined for microsomes HL31 (K_m = 20.3 μM). Results are presented in Table 2. Among the tested compounds, only the known substrates of cytochrome P450 3A significantly inhibited vindesine biotransformation. Other vinca alkaloids also exhibited marked inhibitory effects on vindesine metabolism. Vindesine biotransformation was reduced to (mean ± SD % of control) 0 ± 0, 6.0 ± 3.4, 33.9 ± 9.8, 74.5 ± 16.3, 80.7 ± 7.5, 10.9 ± 9.8, 24.8 ± 5.1 and 26.7 ± 9.3% by quinidine, troleandomycin, erythromycin, cyclosporin A, nifedipine, vinblastine, navelbine and vincristine, respectively. The other tested compounds such as benzo[*a*]pyrene, theopylline, hexobarbital, spartein and aniline had no inhibitory effects. The

Table 2. Effects of various compounds on vindesine biotransformation

Tested compounds (100 μM)	Related P450	Vindesine metabolism (% of control)
Benzo[a]pyrene	1A	96.3 ± 9.1
Theophylline	1A	95.4 ± 6.1
Hexobarbital	2C	95.7 ± 13.5
Debrisoquine	2D	97.8 ± 14.7
Spartein	2D	95.8 ± 11.9
Aniline	2E	95.3 ± 6.5
Quinidine	3A	0 ± 0
Troleandomycin	3A	6.0 ± 3.4
Erythromycin	3A	33.9 ± 9.8
Cyclosporin A	3A	74.5 ± 16.3
Nifedipine	3A	80.7 ± 7.5
Vinblastine	ND	10.9 ± 9.8
Navelbine	ND	24.8 ± 5.1
Vincristine	ND	6.7 ± 9.3

ND, not determined.

Vindesine (10 μM) was incubated 20 min at 37° in a total volume of 250 μL of 0.1 M phosphate buffer (pH 7.4), with microsomes HL 31 (0.5 mg protein/mL) in the presence of 1 mM NADPH and 100 μM of various compounds as potential inhibitors. After quenching by addition of an equal volume of methanol, the reaction mixture was centrifuged at 15,000 *g* for 5 min and 200 μL aliquots of the supernatant were analysed by HPLC.

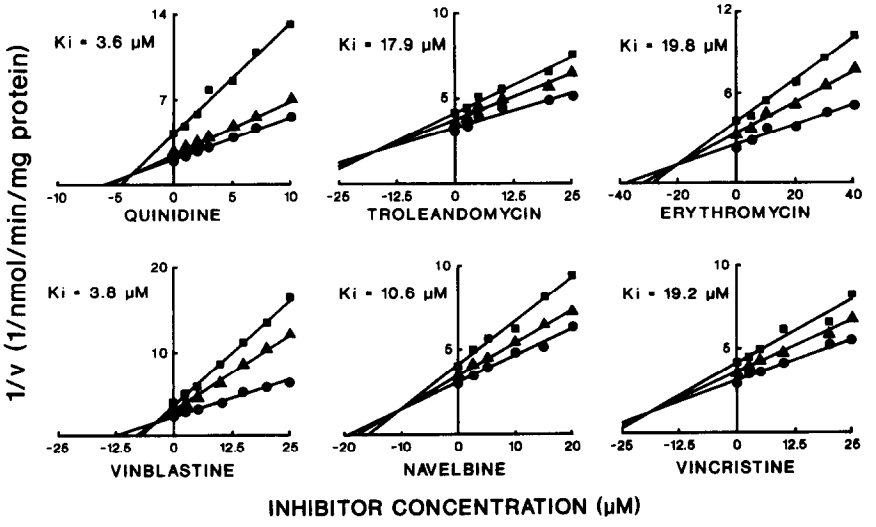

Fig. 3. Dixon plot for the determination of the inhibition of vindesine biotransformation. Vindesine (5, ■; 7, ▲; and 10, ● μM) was incubated 20 min at 37° in a total volume of 250 μL of 0.1 M phosphate buffer (pH 7.4), with microsomes HL31 (0.5 mg protein/mL) in the presence of 1 mM NADPH and various compounds including quinidine, troleandomycin, erythromycin, vinblastine, navelbine and vincristine (from 0.25 to 60 μM). After stopping the reactions by adding methanol, proteins were removed by centrifugation at 15,000 *g* for 5 min; 200 μL aliquots of the supernatant were analysed by HPLC.

apparent K_i values were determined by a Dixon plot for compounds inhibiting vindesine metabolism by more than 50%. They were 3.6, 17.9, 19.8, 3.8, 10.6 and 19.2 μM for quinidine, troleandomycin, erythromycin, vinblastine, navelbine and vincristine, respectively. Moreover, the inhibition mechanism was competitive, as demonstrated by the Dixon plot of Fig. 3.

Immunoinhibition of vindesine metabolism by anti-cytochrome P450 antibodies. In order to confirm P450 3A as the major enzymes involved in vindesine biotransformation by human liver microsomes, immunoinhibition experiments were carried out by using various anti-P450 antibodies. As shown in Fig. 4, only antibody anti-P450 3A inhibited, in a concentration-dependent manner, vindesine metab-

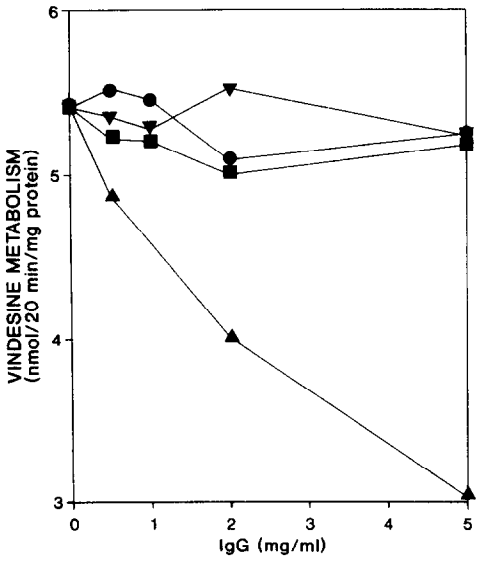

Fig. 4. Immunoinhibition of vindesine biotransformation by polyclonal anti-cytochrome P450 antibodies. Microsomes HL31 were diluted in 0.1 M phosphate buffer to a final concentration of 1 mg protein/mL and were pre-incubated 3 min at 37° and 20 min at room temperature in the absence and in the presence of increasing amounts of antibodies (from 1 to 5 mg/mL). After addition of 5 μM vindesine, reactions were initiated by adding 1 mM NADPH. At the end of incubation (20 min), the reactions were quenched by addition of methanol, proteins were removed by centrifugation at 15,000 *g* for 5 min and 200 μL aliquots of the supernatant were analysed by HPLC. Immunoglobulin G anti-cytochrome P450 1A (●), 2B (▼), 2C (■) and 3A (▲).

olism. Other antibodies including immunoglobulin G anti-P450 1A, 2B and 2C, exhibited no inhibitory effect on vindesine biotransformation.

Correlation between immunoquantified microsomal P450 3A and vindesine metabolism. Levels of cytochrome P450 3A of the human microsome bank were immunoquantified by western blot as shown in Fig. 5. Since vindesine appears to be predominantly metabolized by human liver P450 3A, the level of the latter should be correlated with vindesine biotransformation. Indeed, we found a significant correlation between P450 3A levels of the human microsome bank and vindesine biotransformation ($r = 0.800$, $P < 0.001$) (Fig. 6, line A).

Correlation between microsomal monooxygenase activity and vindesine metabolism. Another argument in favor of an implication of P450 3A in vindesine metabolism was that erythromycin *N*-demethylase activity of the human liver microsome bank, which is known to be supported by P450 3A, was found to be significantly correlated with vindesine biotransformation ($r = 0.853$, $P < 0.001$) (Fig. 6, line B).

Metabolic drug interactions. Metabolic drug interactions between vindesine and a number of anticancer drugs frequently associated with vinca alkaloids were evaluated *in vitro* using microsomes HL31. Final concentrations of vindesine and the anticancer drugs in incubation medium were set at 10 and 100 μM, respectively (see Materials and Methods). Results are reported in Table 3. Among the anticancer agents studied, teniposide, etoposide, doxorubicin, lomustine, folinic acid and mitoxantrone strongly inhibited vindesine biotransformation (vindesine metabolization was less than 85% of control). Other agents such as cisplatin, 5-

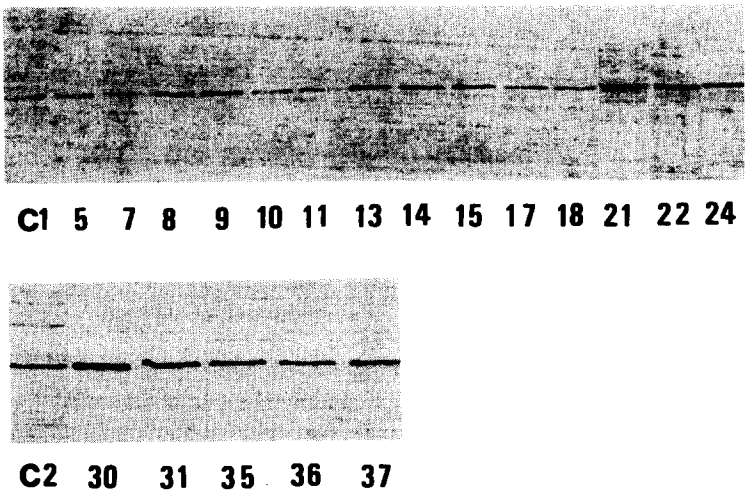

Fig. 5. Western blots of human liver microsomes developed with anti-P450 3A antibodies. Human liver microsomes (10 μg) were subjected to electrophoresis on 10% SDS–polyacrylamide gels and electrophoretically transferred onto nitrocellulose papers. The blots were developed with anti-P450 3A polyclonal antibodies. C1 and C2 were positive controls using purified P450 3A (cyclosporin A oxidase). Numbers, 5, 7, ..., 37, represent human liver microsomal samples from donors 5, 7, ..., 37, respectively.

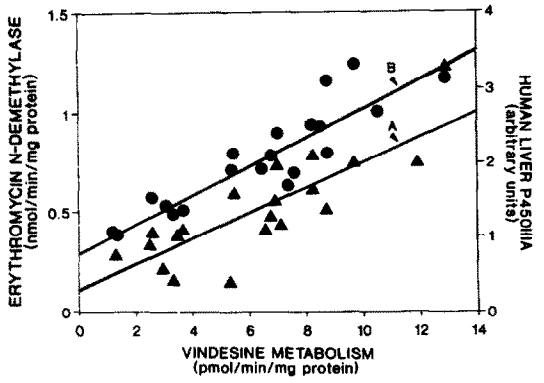

Fig. 6. Correlation of vindesine biotransformation rates with immunoquantitated P450 3A and erythromycin *N*-demethylase activity of the human liver microsome bank. Vindesine biotransformation rates were correlated, respectively, to immunoquantitated cytochrome P450 3A (▲, line A) and erythromycin *N*-demethylase activity (●, line B) determined for the human liver microsome bank. The lines through the points were drawn from a linear regression analysis of the data.

Table 3. Metabolic drug interactions

Anticancer drugs (100 μM)	Vindesine metabolism (% of control)
Ara-C	91.1 ± 4.9
Doxorubicin	56.7 ± 3.7
Etoposide	72.6 ± 6.5
Teniposide	25.0 ± 6.8
Cisplatin	94.1 ± 11.2
5-Fluorouracil	95.3 ± 9.7
Folinic acid	83.9 ± 6.4
Lomustine	48.8 ± 8.8
Methotrexate	95.2 ± 9.5
Mitoxantrone	84.3 ± 14.1

Vindesine (10 μM) was incubated 20 min at 37° in a total volume of 250 μL of 0.1 M phosphate buffer (pH 7.4), with microsomes HL 31 (0.5 mg protein/mL) in the presence of 1 mM NADPH and 100 μM of various anticancer agents frequently associated with the vinca alkaloids. After stopping the reactions by adding methanol, proteins were removed by centrifugation at 15,000 *g* for 5 min; 200 μL aliquots of the supernatant were analysed by HPLC.

fluorouracil and methotrexate exhibited only slight inhibitory effects (vindesine metabolization was about 5% of control).

DISCUSSION

The biotransformation of vindesine was investigated using a bank of human liver microsomes. Vindesine is biotransformed into one major metabolite (M), which, in our chromatographic conditions, eluted just after the unchanged drug. The formation of the metabolite followed Michaelis–Menten kinetics as demonstrated by Lineweaver–Burk plot. The monophasic linear kinetics suggest a single enzyme binding site for vindesine. The structure of the metabolite is unknown. Studies are in progress to prepare this metabolite in quantities allowing its structural identification. The vindesine metabolic pattern obtained upon incubation with human liver microsomes is markedly different from that after incubation with freshly isolated human hepatocytes in suspension. In the latter case, at least four metabolites were detected [30]. These observations suggest the involvement of both cytosolic and microsomal enzymes in vindesine metabolism in humans.

Vindesine biotransformation by human liver microsomes showed large inter-individual variations. We found a variability factor of 10 between the lowest and the highest metabolic rates. This variability could be partly responsible for the variations of the clinical pharmacokinetic parameters of the drug. The limited number of microsome samples did not allow us to evaluate the genetic polymorphism of the cytochrome P450 responsible for vindesine biotransformation. Recently, a genetic polymorphism of nifedipine oxidation, which is mediated by cytochrome P450 3A, has been suspected on the basis of the observation that bimodal variations in the metabolism of the drug occurred in humans [31].

The data presented in this paper demonstrate that human liver microsomal cytochrome P450 3A isozymes were predominantly involved in the biotransformation of vindesine. This conclusion was drawn from the following experiments: (1) among various tested compounds, only those known to be specifically metabolized by cytochrome P450 3A significantly inhibited vindesine biotransformation. Of these compounds, troleandomycin, erythromycin and quinidine exhibited competitive inhibition on vindesine metabolism. (2) Antibody directed against P450 3A strongly inhibited vindesine metabolism. (3) Immunoquantified P450 3A levels of the microsome bank significantly correlated with vindesine biotransformation rates. (4) Erythromycin *N*-demethylase activity, which is known to be supported by human cytochrome P450 3A, significantly correlated with the biotransformation of vindesine. Moreover, vinblastine, vincristine and navelbine, which are analogs of vindesine, competitively inhibited its metabolism. Our preliminary results indicated a possible involvement of cytochrome P450 3A in the biotransformation of vinblastine (data not shown). These data suggest that the antitumor vinca alkaloids may be metabolized by the same cytochrome P450 isozymes in humans.

Combination chemotherapy has become standard in the treatment of cancer because of a number of theoretical advantages and proven clinical efficacy. Protocols with as many as 10 drugs have been used in a variety of combinations, administered concurrently or sequentially. Yet, many unrecognized drug interactions undoubtedly occur. Therefore, investigations on interactions between anticancer drugs are of considerable interest with regard to the optimization of cancer chemotherapy. In the

present report, we found that the epipodophyllotoxins are potent inhibitors of vindesine biotransformation. Of the other anticancer drugs studied, doxorubicin, lomustine, folinic acid and mitoxantrone strongly inhibited vindesine biotransformation. These agents may clinically interfere with the vinca alkaloids and alter their metabolic processes.

Finally, vinca alkaloids are subject to multidrug resistance [32]. Calcium channel blockers and calmodulin inhibitors are known to reverse the resistance to vinca alkaloids. The mechanism of the reversal of multidrug resistance by these agents remains to be completely elucidated. Interestingly, some of these agents such as quinidine, nifedipine and cyclosporin A [33] are metabolized by cytochrome P450 3A. Moreover, they exhibit high inhibitory effects on vindesine biotransformation. These observations lead to the hypothesis that metabolic drug interactions could be involved in the appearance of multidrug resistance. Experiments with human malignant cell lines are in progress in our laboratory. These studies will allow us to better understand the coregulation between multidrug resistance and the metabolism of the anticancer drugs.

In summary, our data clearly demonstrate the involvement of human liver cytochrome P450 3A isozymes in the biotransformation of vindesine and significant metabolic drug interactions between this drug and a number of anticancer agents currently associated with the vinca alkaloids. These findings have both fundamental and clinical implications and should be taken into account in the evaluation and design of combination cancer chemotherapy regimens.

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