Cancer Chemother Pharmacol (1993) 32: 491–495

Cancer
Chemotherapy and
Pharmacology

Short communication

Inhibitory effects of anticancer drugs on dextromethorphan-*O*-demethylase activity in human liver microsomes

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Received: 24 September 1992/Accepted: 14 April 1993

Abstract. The dextromethorphan-*O*-demethylase activity determined in human liver microsomes was used to screen various anticancer drugs for their ability to inhibit this cytochrome CYP2D6-dependent activity. Competitive inhibition indicates that the drug binds the enzyme and is potentially subjected to a polymorphic metabolism. Among the 13 anticancer drugs tested, 4 compounds caused competitive inhibiton of dextromethorphan-*O*-demethylation: lomustine (Ki = 7.7 μ*M*), doxorubicin (Ki = 75 μ*M*), vinorelbine (Ki = 22 μ*M*), and vinblastine (Ki = 42 μ*M*). The results of these studies indicate that the metabolism of the drugs concerned is possibly altered in poor metabolizers of debrisoquine and requires further investigation to study their specific routes of biotransformation. The metabolism of these drugs probably involves various biotransformation pathways, among which the CYP2D6-dependent route would be of minor importance. A second hypothesis is that these drugs could be inhibitors of the isozyme without being a substrate.

Introduction

Drug biotransformation by hepatic cytochrome P450 monooxygenases is a major determinant of interindividual differences in drug responses. Among the factors that are responsible for this variability, genetic polymorphism of drug-metabolizing enzymes is of major concern. One of the best-studied examples of a genetic variation of drug oxidation is the debrisoquine-sparteine type of polymorphism [4–11], which occurs in approximately 5%–10% of Caucasian populations [16] and causes the impaired biotransformation of more than 20 drugs. The poor-metabolizer trait has been shown to be due to differences in the activity of the CYP2D6 isozyme in human liver [5]. Interactions between many drugs and CYP2D6 activity have been widely investigated in vivo and in vitro. In contrast, only a few studies have assessed the effects of anticancer drugs on CYP2D6 activity. However, the clinical relevance of polymorphic-dependent metabolism for these drugs exhibiting a narrow therapeutic range or the existence of drug interactions would be of major importance. Indeed, some of them exhibit highly variable pharmacokinetic parameters whose origins remain unclear.

To our knowledge, no anticancer drug is known to be metabolized by a genetically regulated polymorphic enzyme, although several compounds are metabolized by cytochrome P 450 enzymes. On the other hand, recent work [13] has demonstrated that some anticancer drugs are capable of inhibiting CYP2D6 activity. The authors have demonstrated that vinblastine inhibits bufuralol hydroxylation. Nevertheless, conflicting results have been observed, since it has been demonstrated that the CYP3A subfamily is responsible for the major route of vinca alkaloid biotransformation [17].

The aim of the present study was to determine the type and intensity of metabolic interactions of vinca alkaloids and other anticancer drugs with CYP2D6 to obtain a better understanding of these phenomena. For this purpose, dextromethorphan-*O*-demethylase activity in human liver microsomes was assessed in the presence of various anticancer drugs.

Materials and methods

Materials. Dextromethorphan (DMT) and dextrorphan were kindly provided by Hoffman-La Roche (Basel, Switzerland). Quinidine, debrisoquine, and reduced nicotinamide adenine dinucleotide phosphate (NADPH) were supplied by Sigma. Anticancer drugs were obtained from commercial sources. Other chemicals were of analytical or high-performance liquid chromatography (HPLC) grade.

Liver microsomes. Human liver samples were obtained from kidney transplant donors and microsomal fractions were prepared by differential ultracentrifugation as previously described [6]. They were resuspended in 0.1 *M* phosphate buffer (pH 7.4) containing 20% glycerol and 1 m*M*

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Table 1. Kinetics of the *O*-demethylation of DMT by 16 different microsomal fractions of human liver

Liver sample	Kinetic parameters	
	Km (μ*M*)	Vm (nmol mg^{-1} h^{-1})
HL01	9.14	25.1
HL07	3.18	7.89
HL09	4.51	11.4
HL11	11.1	6.45
HL13	2.50	10.7
HL14	13.2	1.82
HL15	5.19	29.7
HL20	2.61	6.44
HL22	3.42	10.3
HL23	5.05	17.4
HL27	6.10	1.36
HL29	8.45	7.30
HL30	15.2	5.23
HL31	3.78	10.3
HL32	7.43	6.11
HL33	3.27	12.4

Data represent mean values for three determinations

Table 2. Effects of various anticancer drugs on the formation of DMT metabolite in human liver microsomes

Anticancer drug	Inhibitory effects
Methotrexate	No inhibition
Cisplatin	No inhibition
Mitoxantrone	No inhibition
Doxorubicin	Competitive inhibition
Etoposide	Not defined
Teniposide	Not defined
Aracytine	No inhibition
Folinic acid	No inhibition
Lomustine	Competitive inhibition
Elliptinium	No inhibition
Vincristine	No inhibition
Vinblastine	Competitive inhibition
Vinorelbine	Competitive inhibition

ethylene diaminetetraacetic acid (EDTA) and were immediately frozen and stored at –80° C until use. Microsomal protein concentrations were measured by the method of Bradford [2]. Spectrophotometric measurement of cytochrome P450 and b5 concentrations were performed according to Omura and Sato [12].

Assay for microsomal dextromethorphan-O-demethylase activity. The incubation mixture contained dextromethorphan (0–50 μ*M*), microsomal proteins (50 μg), and phosphate buffer (0.1 *M*, pH 7.4) in a final incubation volume of 200 μl. After 5 min incubation at 37° C in a water bath, the reaction was started by the addition of 20 μl NADPH (10 m*M*). Incubations were conducted for 30 min and the reaction was stopped by the addition of 20 μl of 70% (w/v) perchloric acid. The mixture was centrifuged at 10,000 *g* for 5 min and 50 μl of supernatant was finally transferred for HPLC analysis. For the selection of an optimal microsomal sample, DMT-*O*-demethylase activity was measured in 16 human microsome samples. Inhibition studies were performed with an HL15 sample that exhibited an extensive metabolizer phenotype.

For a comparison of the effect of the tested compounds with those of classic inhibitors of CYP2D6, the inhibitors were incubated at six concentrations (debrisoquine: 0, 5, 10, 20, 50, and 100 μ*M*; quinidine: 0, 0.02, 0.05, 0.1, 0.2, 0.5, and 1 μ*M*) with DMT concentrations of 5, 10, and 20 μ*M*. For screening of the inhibition properties of anticancer drugs, two concentrations of test drug (2 and 25 μ*M*) were used with three concentrations of DMT (5, 10, and 20 μ*M*). When inhibition was observed during these screening experiments, full kinetic studies were performed to determine apparent Ki values. Each drug was studied at six different concentrations with three concentrations of DMT. The tested drugs and concentration ranges were: lomustine, 5–75 μ*M*; vinblastine, 20–200 μ*M*; vinorelbine, 10–100 μ*M*; and doxorubicin, 20–200 μ*M*.

Measurement of the dextrorphan formed was performed by HPLC using fluorescence detection as described by Kronbach et al. [8], with slight modifications: reverse-phase chromatography was carried out using a Nucleosil C18 5-μm column. The mobile phase was 0.1 *M* perchlorate buffer (pH 2.2): acetonitrile (55/45, v/v) delivered at a flow rate of 1 ml/min. Excitation and emission wavelengths were set at 270 and 312 nm, respectively. Under these conditions, the retention times of DMT and dextrorphan were 10.9 and 5.90 min, respectively.

Analysis of results. Apparent Vm and Km values were determined by linear regression of Lineweaver and Burk plots. The apparent Ki values were determined from Dixon plots of the data. All values represented the mean of three determinations.

Results

Kinetics of DMT-O-demethylation

Dextrorphan formation was linear at DMT concentrations ranging from 0.5 to 20 μ*M*. The Michaelis-Menten kinetic parameters of each sample of the liver bank are given in Table 1. The values of apparent Vm are distributed in two groups; the activities found in livers from extensive metabolizers (EM) varied from 6.10 to 29.7 nmol mg^{-1} h^{-1} (mean, 12.0 ± 0.64 nmol mg^{-1} h^{-1}), and those found in two livers from poor metabolizers were 1.80 and 1.35 nmol mg^{-1} h^{-1} (mean, 1.59 ± 0.20 nmol mg^{-1} h^{-1}). On the basis of these results, we chose the HL15 sample for inhibition studies.

Effects of classic inhibitors

As shown in Fig. 1, both quinidine and debrisoquine were confirmed as competitive inhibitors of DMT demethylation. The apparent Ki values were 0.04 and 32 μ*M* for quinidine and debrisoquine, respectively.

Inhibitory effects of anticancer drugs

The anticancer drugs listed in Table 2 were tested for their ability to inhibit DMT-*O*-demethylation by liver HL15. First, we determined which drugs inhibited DMT demethylase activity by at least 20% in relation to the control activity. When the anticancer drug concentration was 2 times lower (i.e., 2.5 μ*M*) than that of DMT (i.e., 5 μ*M*), none of the compounds caused more than 20% inhibition of DMT demethylation. At higher drug concentrations (25 μ*M*), four agents caused more than 20% inhibition of DMT demethylation. These drugs were studied for Ki determination and showed competitive inhibition, with Ki values ranging from 2 to 10 times the Km of the reaction (Fig. 1). Hence, the apparent Ki values obtained by analy-

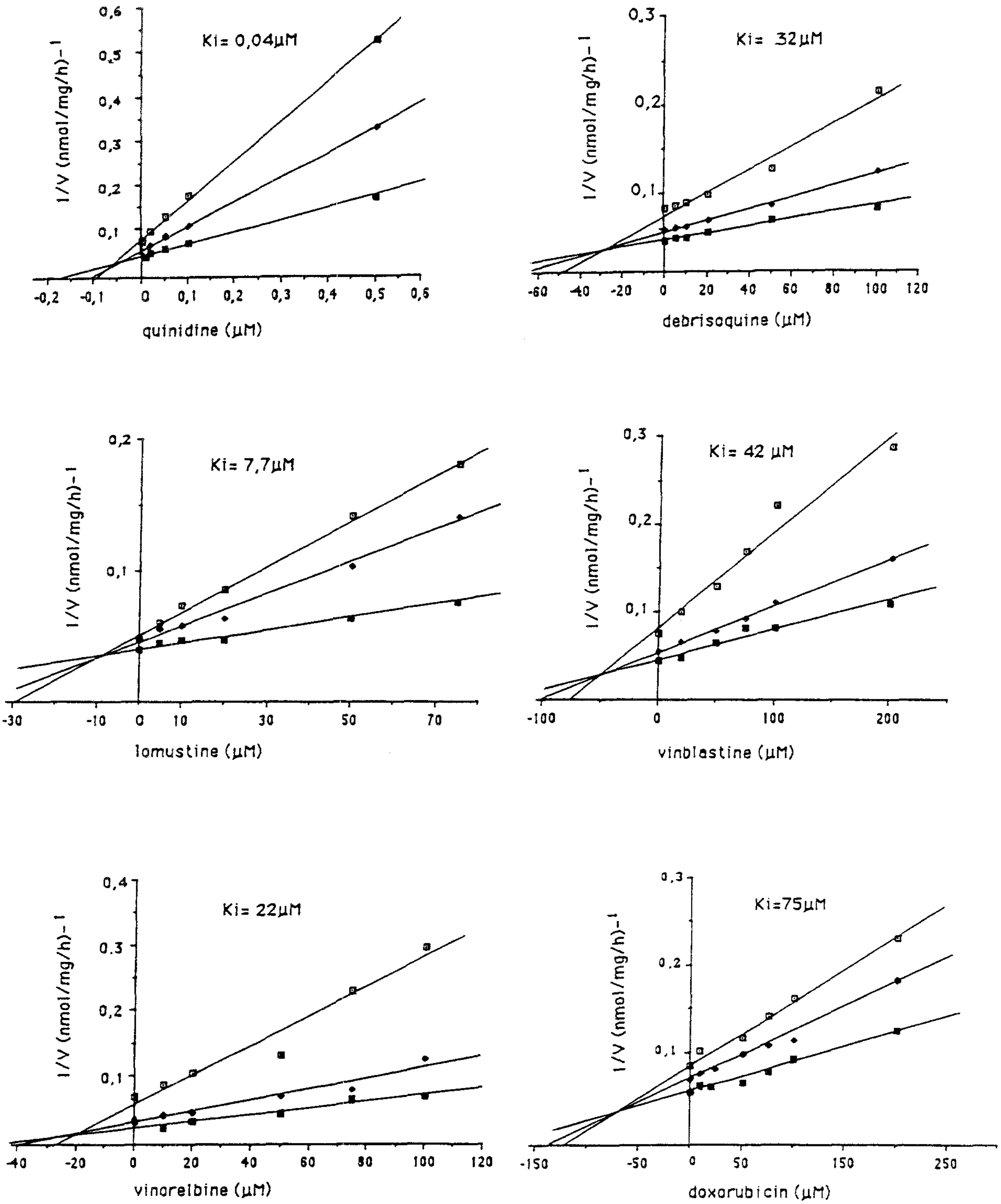

Fig. 1. Dixon plots illustrating the inhibition of DMT demethylation by known inhibitors and anticancer drugs at various concentrations. The DMT-*O*-demethylase activity in microsomal fractions of human liver was determined at concentrations of 5 (☐), 10 (◆), and 20 (■) µ*M* DMT

sis of the Dixon plots were: lomustine, 7.7 µ*M*; doxorubicin, 75 µ*M*; vinorelbine, 22 µ*M*; and vinblastine, 42 µ*M*. It should be noted that teniposide and etoposide showed analytical interference with the DMT assay (the peak of these substances co-eluted with that of dextrophan); hence, it was not possible to assess their inhibitory potency.

Discussion

CYP2D6 expression in 16 human liver samples was assessed by measuring DMT-*O*-demethylase activity. One liver microsomal sample was selected due to its great metabolizing capacity (EM phenotype) to evaluate the inhibitory effects of various anticancer drugs.

The high-affinity component of the isoenzyme involved in DMT demethylation has been shown to correlate with the debrisoquine-4-hydroxylation supported by CYP2D6. This correlation disappeared at the higher DMT concentrations [3]. Therefore, in the present study, all kinetic and inhibition studies were performed at concentrations below the 50 μ*M* substrate level.

The results obtained with known potent inhibitors of isoenzyme (i.e., quinidine and debrisoquine) confirmed that DMT-*O*-demethylation activity was an appropriate prototype reaction for studying the in vitro inhibitory effects of various drugs on CYP2D6 in human liver. Indeed, the Ki values obtained for the inhibition of DMT-*O*-demethylase by quinidine and debrisoquine were similar to those estimated using other prototype reactions of the debrisoquine-type polymorphism. The in vitro inhibition assay of DMT-*O*-demethylation permitted rapid screening of numerous drugs for their propensity to inhibit CYP2D6 activity.

Among the 14 anticancer drugs tested, 4 (lomustine, doxorubicin, vinorelbine, and vinblastine) were shown to inhibit competitively DMT metabolism in vitro. These findings confirm the results previously described by Relling et al. [13] concerning the inhibitory effects of vinblastine on bufuralol hydroxylation. These authors showed evidence for a competitive inhibitory action of this anticancer drug on CYP2D6. Nevertheless, studies performed recently on the vinca alkaloid family [17] have demonstrated that the CYP3A subfamily is involved in the metabolism of vinblastine. Such discrepancies may be explained by the very complex metabolism of these drugs involving various biotransformation pathways that can be under the control of several different isozymes. Thus, vinblastine could be a substrate of several cytochrome P450 isoenzymes, including CYP2D6, and then be subjected to the polymorphism of the latter. Therefore, this metabolic pathway involving CYP2D6 is probably of minor proportion and would not be of clinical consequence. Nevertheless, the role of CYP2D6 on vinblastine metabolism will have to be evaluated by specific studies of each of its metabolic pathways.

As for doxorubicin, vinorelbine, and lomustine, which are known to be extensively metabolized, one or more metabolic pathways could involve the CYP2D6 isoform. However, it must be emphasized that competitive inhibition may indicate merely that a drug is capable of binding to the active site of the enzyme catalyzing the inhibited reaction and is consistent with but does not prove that this drug is oxidized by the same enzyme [1]. Hence, anticancer drugs that inhibit DMT-*O*-demethylation could be, as is quinidine, inhibitors of the isozyme without being a substrate.

The specificity of oxidation of CYP2D6 is based on stereochemical requirements involving a good electrostatic fit between the substrate and the protein near the site of oxidation. The isozyme is known to metabolize exclusively substrates containing a protonated nitrogen atom that interact with an anion site of the protein. A molecular template was derived for several known CYP2D6 substrates that can be used as a screen to determine whether other related molecules are likely to be CYP2D6 substrates [7]. The four drugs suspected to interact with CYP2D6 exhibit a nitrogen atom. However, it would be interesting to determine the X-ray structure and the possible sites of attack and protonation of the four compounds for which we demonstrated a possible interaction with CYP2D6.

An important consideration for evaluation of the potential clinical relevance of these in vitro findings is the magnitude of the Ki value for the competitive inhibition and its relationship with the concentration of inhibitor that is clinically achievable. If the Ki value is much higher than the plasma concentrations achieved in vivo, such interactions may occur in vitro but not in vivo. Among our four anticancer drugs, vinblastine exhibited a Ki value much greater than the clinically achievable plasma concentrations (1.5–2 ng/ml) [14] and is therefore not likely to be a clinically important substrate or inhibitor. On the other hand, plasma concentrations achieved in vivo for doxorubicin (5–10 μg/ml) [15], vinorelbine (1 μg/ml) [10], and lomustine (10 μg/ml) [9] reached or exceeded the Ki values and, hence, these drugs may have clinical importance as inhibitors of DMT demethylation. Thus, one could predict that administration of these anticancer drugs could increase the prevalence of the poor-metabolizer phenotype. Moreover, future studies should investigate whether these drugs are substrates for the CYP2D6 isozyme and determine the consequences of this phenomenon on interpatient variability in response or toxicity.

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