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IN VITRO CHARACTERIZATION OF CYTOCHROME P450 CATALYSED METABOLISM OF THE ANTIEMETIC TROPISETRON

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Abstract—The new 5-hydroxytryptamine type 3 ($5HT_3$) receptor antagonist tropisetron is used in the treatment of chemotherapy-related nausea. The drug is extensively metabolized in man, with the enzymes involved in tropisetron biotransformation being unknown. Identification of these enzymes would make it possible to predict both interindividual variability in plasma concentrations and metabolic interaction potential. The present *in vitro* study was therefore aimed at identifying and characterizing the cytochrome P450 enzymes catalysing tropisetron metabolism. Enzyme kinetics for formation of 5-hydroxy (5-OH-ICS), 6-hydroxy (6-OH-ICS) and *N*-demethyl tropisetron (*N*-De-ICS) were studied in the microsomal fraction of eight human livers (seven livers from extensive metabolizer (EM), one liver from a poor metabolizer (PM) for CYP2D6). Formation of 5-OH-ICS and 6-OH-ICS was biphasic with a high (5-OH: K_m 3.9 ± 2.1 μM; V_{max} 1.88 ± 0.73 pmol/mg/min; 6-OH: K_m 4.66 ± 1.84 μM; V_{max} 4.00 ± 1.77 pmol/mg/min) and low (5-OH: K_m 172 ± 51 μM; V_{max} 17.0 ± 9.4 pmol/mg/min; 6-OH: K_m 266.0 ± 76.0 μM; V_{max} 81.4 ± 27.9 pmol/mg/min) affinity component. The high-affinity component was identified as CYP2D6 which exhibits a genetic polymorphism in man. This component was absent in the PM liver. The low-affinity component was present in EM and PM livers and was identified as CYP3A4. LKM1 antibodies directed against CYP2D6 completely inhibited the high affinity component. Quinidine (0.5 μM) inhibited 5- and 6-hydroxylation at 10–80 μM tropisetron concentrations competitively by 70% with K_i values of 10 and 18 nM, respectively. Stably-expressed CYP2D6 catalysed the formation of both 5-OH-ICS and 6-OH-ICS. Both inhibition experiments and use of stably-expressed enzymes revealed formation of *N*-De-ICS to be mediated by CYP3A4. Based on *in vitro* intrinsic clearances CYP2D6-catalysed 5-OH-ICS and 6-OH-ICS is the predominant route of tropisetron elimination. Large phenotype-related differences in total clearance are to be expected after administration of tropisetron. However, in view of the wide therapeutic index of tropisetrone and the rather high K_i for inhibition of the metabolism of other drugs by tropisetron, both the interindividual variability and the interaction potential appear to be of no clinical relevance.

Key words: tropisetron; drug metabolism; cytochrome P450; variability; interactions

Cancer patients consistently rank nausea and vomiting as the most feared side-effects of chemotherapy. Since the early 1980s, considerable progress has been made in controlling chemotherapy-induced nausea and vomiting by use of high-dose metoclopramide [1], corticosteroids [2] and combination therapy including benzodiazepines [3]. However, this therapy causes a number of side-effects, particularly extrapyramidal symptoms resulting from high doses of metoclopramide [4]. Following the demonstration that high doses of metoclopramide block 5 HT_3† receptors [5], several 5 HT_3 antagonists such as ondansetrone and tropisetron have been developed for clinical use as antiemetics.

ICS (Navoban®, Novaban®), a highly potent and selective 5 HT_3 receptor antagonist, has been shown to be a powerful antiemetic agent, superior to metoclopramide and comparable to the most effective combinations of antiemetic compounds used thus far [6–10].

Studies in healthy volunteers, however, revealed pronounced interindividual variation in its pharmacokinetics [11, 12]. ICS is extensively biotransformed by hydroxylation of the indol ring in the 5, 6, and 7 positions (for chemical structure see Fig. 1). The hydroxymetabolites are subsequently conjugated with glucuronic acid and sulphate. The major metabolic pathway is 6-hydroxylation, which accounts for 45% of the urinary excretion, while 12% of dose is excreted as 5-OH-ICS in urine. Oxidative N-demethylation and N-oxygenation at the tropinyl nitrogen also occur but to a minor extent. Only traces of *N*-De-ICS and *N*-oxide metabolites were found [12].

Although recent *in vitro* data indicated involvement of the polymorphic CYP2D6 in the hydroxylation of tropisetron [13], the relative contribution of the individual pathways and enzymes to the overall metabolism of the drug has not yet been elucidated. Characterization of the enzymes involved

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† Abbreviations: $5HT_3$, 5-hydroxytryptamine type 3; ICS, tropisetron; 5-OH-ICS, 5-hydroxy tropisetron; 6-OH-ICS, 6-hydroxy tropisetron; 7-OH-ICS, 7-hydroxy tropisetron; *N*-De-ICS, *N*-demethyl tropisetron; UV, ultraviolet; EM, extensive metabolizers; PM, poor metabolizers.

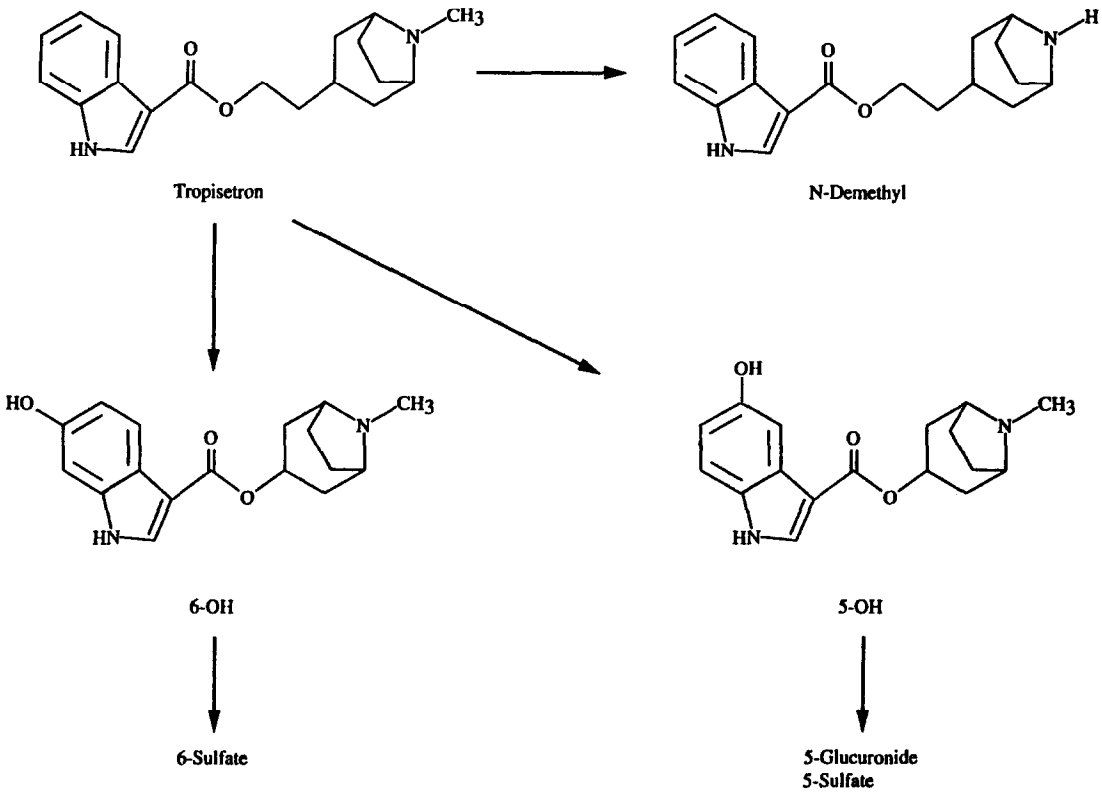

Fig. 1. Major metabolic pathways of ICS.

is pivotal for assessment of the interaction potential of ICS, of particular interest in the context of cancer chemotherapy with its administration of multiple drugs.

We therefore characterized the enzymes involved in biotransformation of ICS in great detail, employing the following *in vitro* approach: (i) development of an *in vitro* assay for ICS metabolism; (ii) investigation of the rate of formation of ICS metabolism in human liver microsomes in relation to the amounts of the various CYP enzymes expressed in the same liver samples; (iii) characterization of the enzymes by inhibition studies using both chemical inhibitors and antibodies; and (iv) use of stably-expressed human P450 enzymes.

These *in vitro* experiments allow one to predict the relative contribution of an individual pathway to the total clearance of ICS, interindividual variability in clearance and the potential for metabolic drug interactions.

MATERIALS AND METHODS

Chemicals. ICS, 5-OH-ICS, 6-OH-ICS, 7-OH-ICS and *N*-De-ICS were obtained from Sandoz Pharma Ltd (Basel, Switzerland). Vinblastine, quinidine chloride, and phenacetin were purchased from Sigma (Germany) and ketaconazole from Sigma (U.K.). Doxorubicin chloride and cisplatin were from Farmitalia Carlo Erba. Furaphylline was kindly provided by Dr. Fuhr (Frankfurt, Germany). All solvents and reagents used were of the highest purity grade commercially available.

Assay for 5-OH-ICS, 6-OH-ICS and N-De-ICS. Carbonate buffer pH 10.0 (7 mL) and a mixture of ethylacetate and 2-propanol (9:1, v/v, 2 mL) were added to the microsomal incubations. The samples were mixed for 30 sec on a Vortex type mixer. After centrifugation at 3250 *g* for 5 min, 1.6 mL of the organic phase were transferred into clean-tapered test tubes and the organic phase evaporated to dryness under a gentle stream of dry nitrogen in a water bath at 37°. The residue was dissolved in 110 μL of mobile phase and 100 μL aliquot injected onto the HPLC column.

The metabolites were separated on a 3 μm phenyl Hypersil® reverse-phase column (125 mm × 4.6 mm) operated at 50° and at a flow rate of 0.9 mL/min. The mobile phase consisted of acetonitrile, triethylamine and Millipore-Q-water (180:0.5:719.5, v/v/v). The pH was adjusted to 3.5 with 85% H_3PO_4. The eluate was monitored by UV detection at 220 nm. The retention times were 10.8 min for 5-OH-ICS, 11.8 min for 6-OH-ICS, 26.0 min for *N*-De-ICS and 29.3 min for ICS.

Preparation of human liver microsomes. Liver samples were obtained from patients who had undergone partial hepatectomy. All patients had

given written informed consent and the study protocol had been approved by the local Ethics Committee. The microsomal fractions were prepared from these liver samples as described previously [14]. Protein and cytochrome P450 contents were measured according to standard procedures [15, 16]. The contents of the microsomal preparations for CYP1A2, CYP2C, CYP2D6, CYP2E1 and CYP3A were determined by immunoblotting [17].

Incubation conditions. The samples were incubated at 37°, pH 7.4 for 40 min in the presence of 250 μg microsomal protein. The medium consisted of 6 mM $MgCl_2$ and 6.7 mM NADPH, Tris buffer (0.05 M) being added to a final volume of 200 μL. Under these conditions product formation was linear with respect to time (5–50 min) and protein amount (50–500 μg). For kinetic experiments tropisetron was added at concentrations ranging from 1.25 to 1280 μM. The reaction was stopped by adding 1 mL of 0.2 M carbonate buffer pH 10.0 and 2 mL of ethylacetate/2-propanol (9:1, v/v) and extracted thereafter as described above. Incubation conditions were identical for human liver microsomes and stably-expressed enzymes.

Inhibition studies with LKM1 antibodies. The serum from patients with chronic autoimmune hepatitis containing LKM1 antibodies directed against CYP2D6 [18] were kindly provided by M. Manns (Hannover, Germany). Microsomal protein (250 μg) was preincubated for 1 hr with 0.2, 1, 5 and 15 μL LKM1 serum at room temperature. Thereafter, tropisetron was added at concentrations of 10, 30 and 640 μM. Control experiments were performed with the same volumes of serum from healthy volunteers. Incubation conditions were identical to those described above.

Inhibition studies using inhibitors of CYP2D6, CYP3A4, CYP1A2 and cytostatic drugs. Quinidine was used as inhibitor of CYP2D6 (K_i = 0.07 μM) [19], ketaconazole as inhibitor of CYP3A4 (K_i < 1 μM) [20], and furaphylline (IC_{50} = 0.07 μM) [21] and phenacetin (K_i = 25 μM) as CYP1A2 [22] inhibitors. Quinidine at concentrations of 1, 5, 25, 100, and 500 nM was added simultaneously with tropisetron (5, 10, 20, 30, 80, 640 μM) to the incubation mixture. Further inhibition studies were performed with ketoconazole (10 μM) at 80 and 160 μM tropisetron, phenacetin (80, 160, 335 μM) at 30 and 640 μM ICS, and furaphylline (1 and 5 μM) at 80 and 160 μM ICS.

The concentrations of cytostatic drugs used in the inhibition experiments were of the same order of magnitude as the maximum plasma concentrations achieved in patients. The exception was vinblastine, which was used in the same concentration as described for the inhibition of bufuralol 1'-hydroxylation [23]. Vinblastine (220 μM), cyclophosphamide (358 μM) and cisplatinum (8.3 μM) were added to the incubation mixture at tropisetron concentrations of 30 and 160 μM. Doxorubicin (1, 2, 4 μM) was incubated together with 5, 10, 20, 30 μM ICS. The experiments were carried out under the same incubation conditions as described above.

Studies with yeast microsomes containing stably-expressed CYP2D6 and CYP3A4. The yeast microsomes prepared from AH22/pELT1 cells containing stably-expressed CYP2D6 (protein content 14.3 mg/mL; 46 pmol P450/mg protein) or CYP3A4 (protein content 7.3 mg/mL; 49 pmol P450/mg protein) and microsomes from control cells AH22/pMA91 (protein content 25.4 mg/mL; no P450 detectable) were a generous gift from Ellis *et al.* [24]. Yeast microsomal protein (250 μg) containing CYP2D6 was incubated together with 30, 160 and 640 μM tropisetron. Yeast microsomes containing CYP3A4 were incubated with 160 μM tropisetron. Incubation conditions were the same as described above for human liver microsomes.

Statistical evaluation. The kinetics of metabolite formation were analysed using ELSFIT, a non-linear extended least-squares algorithm [25]. The calculated parameters were the maximum rate of metabolite formation (V_{max}) and the Michaelis–Menten constant (K_m). Data were fitted by both one- and two-component models. The best fit for each model to the data was judged by the *F*-test. Intrinsic clearance Cl_{int} was calculated from V_{max}/K_m. The inhibition constants (K_i) were estimated graphically from Dixon plots.

RESULTS

Based on CYP2D6 content derived from immunoblotting, seven liver samples were characterized as originating from an EM subject and one from a PM subject. ICS was metabolized by microsomes yielding 5-OH-ICS, 6-OH-ICS and *N*-De-ICS. Recovery was 67.5 ± 12% (N = 8) for 5-OH-ICS, 70 ± 9% (N = 8) for 6-OH-ICS and 82 ± 6% (N = 4) for *N*-De-ICS. Due to incomplete separation of the *N*-De-ICS from ICS at substrate concentrations above 320 μM, the detailed kinetics could not be investigated. The coefficient of variation for the determination of *N*-De-ICS at 320 pmol was ± 10%.

Kinetics of formation of 5-OH-ICS and 6-OH-ICS

The formation of 5-OH-ICS and 6-OH-ICS was studied in seven EM and one PM liver. The formation of 5-OH-ICS and 6-OH-ICS was biphasic with a high and a low affinity component in all seven EM livers (Fig. 2). In the PM liver the high-affinity component was absent with only the low-affinity component being observed. Table 1 provides V_{max} and K_m values as well as the individual CYP2D6 content. V_{max} of the high-affinity component (V_{max1}) of the 5-OH-ICS and 6-OH-ICS was correlated with the amount of CYP2D6 (5-OH-ICS, r = 0.88, $P < 0.01$; 6-OH-ICS, r = 0.98, $P < 0.01$). In contrast no such correlation was observed between the low-affinity component and CYP2D6 content. There was no relationship between the V_{max} values of 5-OH-ICS and 6-OH-ICS formation and the contents for the other P450 enzymes.

Inhibition studies with LKM1 antibodies

The influence of LKM1 antibodies on metabolite formation was studied in the microsomes of one EM liver and one PM liver (livers 6 and 8 in Table 1). The inhibition pattern is shown in Fig. 3. In the EM liver the antibodies almost completely inhibited metabolite formation at both substrate concentrations. In the PM liver 5- and 6-OH-ICS formation

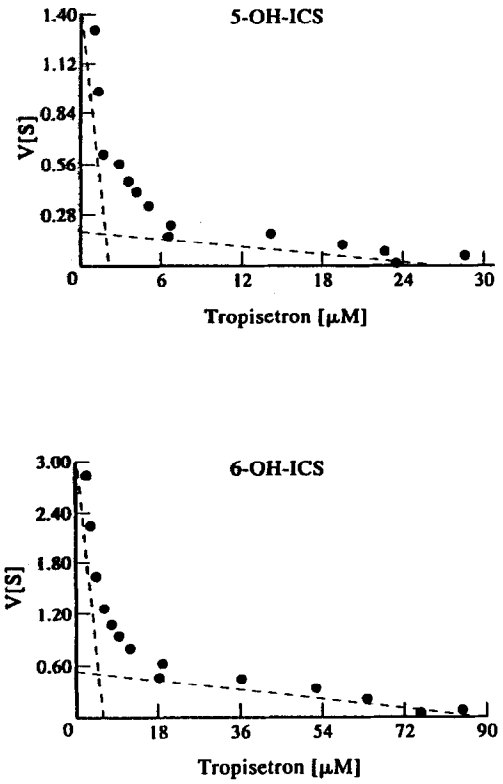

Fig. 2. Scatchard plots for 5-OH-ICS and 6-OH-ICS of ICS in presence of the microsomal fraction of liver 1 (250 μg of microsomal protein, 40 min at 37°; V, velocity; S, substrate; kinetic data are shown in Table 1). The symbols indicate the data as obtained from experiments, with the dotted lines being the best fit of the respective components.

was not affected by LKM1 at substrate concentrations of 640 μM. *N*-De-ICS formation was not inhibited by the antibodies in either EM or PM liver microsomes. The rate of formation of 5-OH-ICS and 6-OH-ICS in presence of LKM1 in three human livers (livers 1, 6 and 8 in Table 1) was correlated with the CYP3A4 content (5-OH-ICS, $r = 0.997$; 6-OH-ICS, $r = 0.844$).

Inhibition studies with quinidine

The effect of quinidine on ICS metabolite formation was studied in the microsomal fraction of 1 EM and 1 PM liver. In EM liver microsomes, a complete inhibition of 5- and 6-hydroxylation by quinidine was observed at substrate concentrations of 5 and 10 μM tropisetron. Dixon plots revealed competitive inhibition with a K_i value of 10 nM and 18 nM for 5-OH-ICS and 6-OH-ICS, respectively (Fig. 4). At substrate concentrations of 80 μM of tropisetron, quinidine inhibited 5-OH-ICS and 6-OH-ICS formation by 75% and 66%, respectively. N-Demethylation was only slightly inhibited (25%) by quinidine at a concentration of 500 nM. In contrast to EM microsomes, quinidine had no effect on the formation of 5-OH-ICS and 6-OH-ICS in the PM liver.

Table 1. Kinetic data of 5-OH-ICS and 6-OH-ICS formation in microsomal fractions of eight human livers (250 μg of microsomal protein, incubation time 40 min at 37°). Cl_{int} was calculated as V_{max}/K_m and is expressed in mL/min/g microsomal protein. CYP2D6 content is given in arbitrary units/mg protein determined by densitometry of the immunoblots

		5-OH-ICS						6-OH-ICS					
Liver	CYP 2D6	V_{max1} (pmol/mg/min)	K_{m1} (μM)	Cl_{int1} (mL/min/g)	V_{max2} (pmol/mg/min)	K_{m2} (μM)	Cl_{int2} (mL/min/g)	V_{max1} (pmol/mg/min)	K_{m1} (μM)	Cl_{int1} (mL/min/g)	V_{max2} (pmol/mg/min)	K_{m2} (μM)	Cl_{int2} (mL/min/g)
1	1.3	2.17	1.5	1.45	27.67	139	0.20	4.67	1.5	3.11	90.00	169	0.53
2	0.5	1.50	7.9	0.19	5.83	280	0.02	1.83	7.7	0.24	36.67	277	0.13
3	0.5	1.17	4.2	0.28	8.33	213	0.04	2.00	4.1	0.49	53.33	360	0.15
4	0.8	1.67	3.2	0.52	29.17	137	0.21	3.67	4.7	0.78	108.33	193	0.565
5	1.7	3.33	5.2	0.64	19.17	159	0.12	7.0	4.6	1.52	65.00	279	0.23
6	1.1	2.00	2.2	0.91	20.00	161	0.12	4.67	5.5	0.85	105.00	381	0.28
7	1.3	1.33	3.1	0.43	20.33	122	0.17	4.17	4.5	0.93	80.00	205	0.39
8	0.0	0	0	0	5.50	166	0.03	0	0	0	113.33	266	0.43
Mean		1.88	3.9	0.63				4.00	4.66	1.13			
± SD (for livers 1–7)		0.73	2.1	0.43				1.77	1.84	0.96			
Mean					17.00	172	0.11				81.42	266	0.34
± SD (for all livers)					9.4	51	0.08				27.93	76	0.17

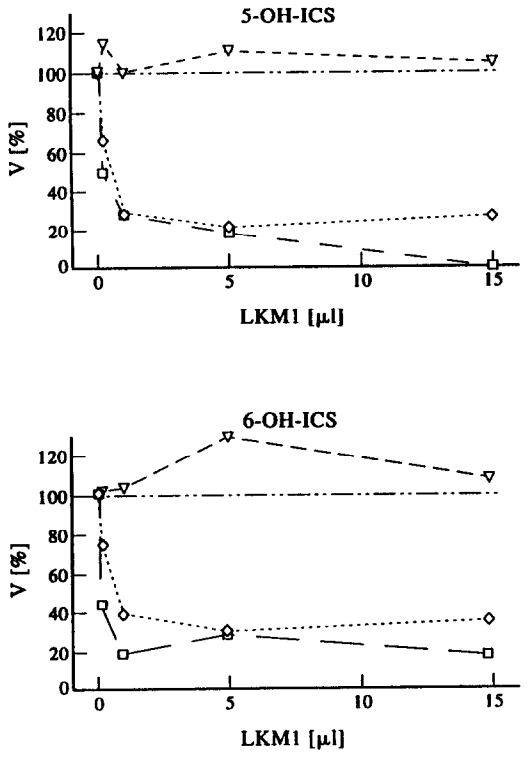

Fig. 3. Inhibition of 5-OH-ICS and 6-OH-ICS, respectively, formation by LKM1 antibodies in the microsomal fraction of human livers (250 µg of microsomal protein, 40 min at 37°; liver 6 at 10 µM (□) and 640 µM (◇), and in liver 8 at 640 µM (▽) tropisetron; kinetic data are shown in Table 1). Data are displayed as percentages of the initial velocity in presence of antibodies.

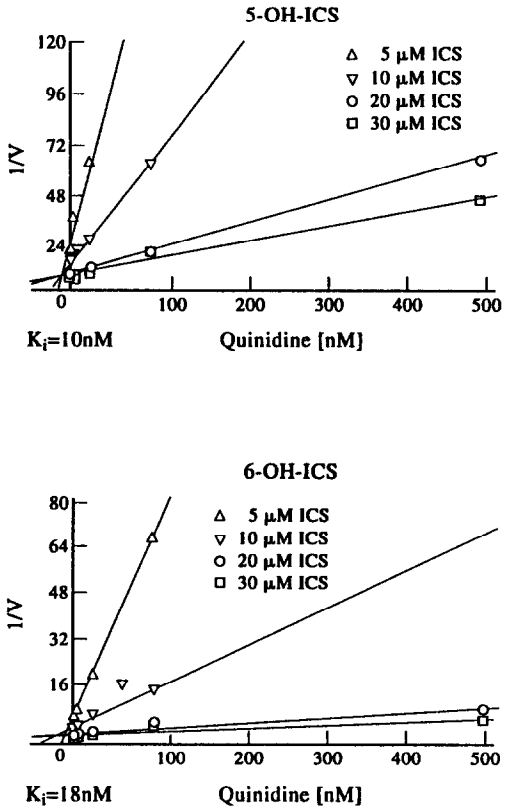

Fig. 4. Dixon plots for the inhibition of 5-OH-ICS and 6-OH-ICS formation (250 µg of microsomal protein, 40 min at 37°) by quinidine.

Inhibition studies with ketoconazole, furaphylline and phenacetin

Ketoconazole at a concentration of 10 µM inhibited the formation of 5-OH-ICS and 6-OH-ICS 17 ± 8% (N = 3). In contrast *N*-De-ICS formation was inhibited by 85 ± 5% (N = 3). Furaphylline and phenacetin had no effect on formation of any of the three metabolites.

Inhibition studies with cytostatic drugs

The only cytostatic drug which inhibited *in vitro* tropisetron metabolism to a significant extent was vinblastine. At a concentration of 220 µM vinblastine inhibited 5- and 6-hydroxylation by 72.5 ± 5% and 64 ± 13%, respectively. N-Demethylation was not affected. Doxorubicin (4 µM) and cisplatinum (8.3 µM) had no inhibitory effect on ICS biotransformation.

Incubations in presence of the microsomal fraction from yeast cells stably expressing human CYP2D6 and CYP3A4

Incubations with yeast microsomes containing CYP2D6 resulted in the formation of 5-OH-ICS and 6-OH-ICS. When ICS was incubated with CYP3A4 containing yeast microsomes the major metabolite formed was *N*-De-ICS while only small amounts of 5- and 6-hydroxy metabolites were generated. Data obtained with stably-expressed enzymes are displayed in Table 2.

DISCUSSION

The use of $5HT_3$ antagonists offers a new therapeutic strategy for the treatment of chemotherapy-induced nausea. As these drugs are almost exclusively used in combination with other agents in cancer chemotherapy, knowledge of the pharmacokinetic interaction potential is a critical issue. Besides altering the disposition of the HT_3 antagonist ICS itself, such interactions may change the metabolism of the concomitantly administered cytostatic drugs. For example, recent data [26] demonstrated bioactivation of iphosphamide to be mediated by CYP3A4. Simultaneous administration of other CYP3A4 substrates could reduce formation of the active moiety and hence reduce therapeutic efficacy of this drug. This scenario may occur with the new HT_3 antagonist ICS. We therefore identified and characterized the enzymes involved in metabolism of ICS and predicted both interindividual variability and interaction potential.

We observed biphasic enzyme kinetics in the 5-

Table 2. Formation of 5-OH-ICS, 6-OH-ICS and *N*-De-ICS in yeast cells expressing human CYP2D6 and CYP3A4. Activities are expressed as pmol/min/mg microsomal protein after subtraction of control yeast activities (values in parentheses are turnover numbers (min^{-1}))

ICS (μM)	Enzyme	5-OH-ICS (pmol/mg/min)	6-OH-ICS (pmol/mg/min)	*N*-De-ICS (pmol/mg/min)
30	CYP2D6	1.22 (0.026)	3.55 (0.077)	0.0
160	CYP2D6	3.15 (0.068)	10.10 (0.21)	0.0
640	CYP2D6	4.32 (0.093)	15.67 (0.34)	NS*
160	CYP3A4	0.25 (0.005)	0.28 (0.005)	22.17 (0.452)

* NS, not separated.

and 6-hydroxylation of ICS with a high- and low-affinity component. These data clearly indicate that at least two cytochrome P450 enzymes are involved in formation of 5-OH-ICS and 6-OH-ICS. The high affinity component was identified as CYP2D6, evidence for this assumption being based on the following observations. V_{max} of this component was significantly correlated with the expression of CYP2D6 in the liver samples. The high-affinity component was absent in the liver of a PM where no CYP2D6 is present. LKM1 antibodies directed against CYP2D6 completely inhibited formation of 5-OH-ICS and 6-OH-ICS at low substrate concentrations. Finally, quinidine, an inhibitor of CYP2D6, completely inhibited 5-OH-ICS and 6-OH-ICS formation at low substrate concentrations. Moreover, 5-OH-ICS and 6-OH-ICS were formed in yeast microsomes expressing CYP2D6. The latter findings are in agreement with recently published data [13].

In contrast, the low-affinity component involved in the formation of 5-OH-ICS and 6-OH-ICS tropisetron is not CYP2D6, since it could not be inhibited by antibodies directed against this enzyme and CYP2D6 inhibitors. Furthermore, this component was also present in the PM liver. This low-affinity component was identified as CYP3A4 which is again supported by several lines of evidence. The amounts of 5-OH-ICS and 6-OH-ICS formed in the presence of CYP2D6-blocking LKM1 antibodies correlated with the CYP3A4 content of the livers. The CYP3A4 inhibitor ketoconazole [20] reduced formation of 5-OH-ICS and 6-OH-ICS by 20%. The degree of inhibition in 5-OH-ICS and 6-OH-ICS formation by ketoconazole corresponds to the percentage of metabolites formed in the presence of LKM1. Finally, small amounts of the 5- and 6-hydroxy metabolites were formed in yeast microsomes containing CYP3A4.

The kinetics of the N-demethylation reaction could not be studied in detail since measurable amounts of this metabolite were formed only at tropisetron concentrations >40 μM. Metabolite formation at saturating substrate concentrations could not be investigated either because at concentrations above 320 μM the *N*-De-ICS peak could no longer be separated from the parent compound. Despite these experimental limitations several lines of evidence indicate formation of *N*-De-ICS to be catalysed by CYP3A4. Incubation of tropisetron in presence of the microsomal fraction of yeast cells expressing CYP3A4 resulted in a high rate of formation of the *N*-demethyl metabolite (Table 2). Moreover, the CYP3A inhibitor ketoconazole substantially reduced formation of this metabolite. In contrast, CYP1A2 is not involved in the N-demethylation of tropisetron since inhibitors of this enzyme such as phenacetin and furaphylline did not impair *N*-De-ICS formation. The modest inhibition in *N*-De-ICS formation by 0.5 μM quinidine is not at odds with an involvement of CYP3A4 since the 3-hydroxy and *N*-oxide metabolites of quinidine are formed by this enzyme [27]. Although the quinidine concentration of 0.5 μM is below the K_m values of 4 μM for 3-hydroxy and 33 μM for *N*-oxide metabolite formation, the quinidine concentration nevertheless could be sufficient to slightly inhibit CYP3A4-catalysed *N*-De-ICS formation.

If one evaluates the contribution of CYP2D6 and CYP3A4 to the *in vivo* elimination of ICS on the basis of *in vitro* intrinsic clearance, several predictions can be made. CYP2D6-catalysed 5- and 6-hydroxylation is the predominant route of elimination and hence large phenotype-related differences in the formation of these metabolites and total plasma clearance are to be expected. With intrinsic clearance for the low-affinity component being considerably smaller, formation of these two metabolites by CYP3A4 contributes only slightly to elimination and cannot compensate for the loss of CYP2D6 activity in PM. Furthermore, clearance to 6-OH-ICS should be two to three times higher than to 5-OH-ICS. Metabolism via CYP3A4-catalysed N-demethylation is a minor pathway, since ICS concentrations achieved *in vivo* are far below the concentrations at which measurable amounts of *N*-De-ICS are formed *in vitro*. As in the case of CYP3A4-catalysed hydroxylation this pathway cannot compensate for the loss of CYP2D6 in PM. These *in vitro* predictions are in good agreement with the *in vivo* situation. EM subjects excrete 50–60% of the dose as 5-OH-ICS and 6-OH-ICS whereas in PM only trace amounts of these two metabolites are formed (SANDOZ AG, data on file). The fraction of dose excreted in urine as 6-OH-ICS is on average three times higher than the corresponding values for the 5-hydroxy metabolite. The *N*-demethyl metabolite accounts for only a minor part of the dose.

Since CYP2D6 plays a major role in tropisetron metabolism these *in vitro* findings have several

implications for the *in vivo* disposition of the drug. PM will have considerably higher plasma concentrations than EM after administration of the same dose. However, side-effects due to high concentrations of the HT_3 receptor antagonist are unlikely in view of the rather wide therapeutic safety margin of this drug. It is rather the loss of therapeutic efficacy in so-called ultra-rapid metabolizers that may arise as a consequence of CYP2D6-catalysed biotransformation of ICS. Such loss of efficacy has been observed for the antidepressant nortriptyline where patients required doses three to four times above the highest recommended dose [28]. Recent data have identified the molecular base of the ultra-rapid metabolizers (e.g. patients with extremely high CYP2D6 activity [29]) to be gene amplification [29]. They reported three members of one family having 12 copies of the CYP2D6 gene, resulting in aberrantly high CYP2D6 activity. If ICS is given to a patient with this genetic disposition our *in vitro* data predict that regular doses will result in subtherapeutic plasma concentrations. Thus, this subset of patients will suffer from chemotherapy-induced nausea despite administration of a potent 5 HT_3 antagonist. As a consequence dosage of ICS should be adjusted according to the individual CYP2D6 activity in rapid metabolizers in particular.

Besides interindividual variability in drug metabolism the question of drug interactions may play a pivotal role in net drug effect resulting from multiple drug administration during chemotherapy. In general our *in vitro* data predict that drugs which are substrates for CYP2D6 and CYP3A4 can inhibit ICS metabolism and vice versa. Since CYP2D6-catalysed 5- and 6-hydroxylation is the main route of elimination, inhibition of this enzyme will have a major impact on ICS biotransformation. In view of their low K_i values drugs such as quinidine [30] and propafenone [31] are expected to inhibit CYP2D6 completely. Coadministration of drugs known to be inducers of cytochrome P450 enzymes such as rifampicin should have no major impact on 5- and 6-hydroxylation and hence on overall disposition since CYP2D6 cannot be induced [32]. However, a modest induction of ICS metabolism for the fraction of the dose metabolized via CYP3A4 can be expected. The same holds true for drugs which inhibit only CYP3A4. If drugs such as propafenone which inhibit both CYP2D6 and CYP3A4 are administered almost complete inhibition of ICS metabolism is to be expected in both EM and PM, with the effects being much greater in the EM subset. Again, in view of the wide therapeutic margin, increase in plasma concentrations arising from these interactions is not expected to cause side effects.

Of the cytostatic drugs studied, only vinblastine affected the 5- and 6-hydroxylation of ICS indicating that vinblastine is an inhibitor of CYP2D6. This is in agreement with data [23] which described vinblastine to be an inhibitor (K_i 90 μM) of the CYP2D6 catalysed 1-hydroxylation of bufuralol. However, it is very unlikely that vinblastine will inhibit ICS metabolism *in vivo*, because the high vinblastine concentrations required (K_i 220 μM) will not be achieved. Whether or not vinblastine is itself metabolized by CYP2D6 is presently unknown. If it is, ICS could inhibit vinblastine metabolism. Again the K_i value of 16 μM for inhibition of CYP2D6 with ICS will not be achieved *in vivo* at the doses employed.

The potential of ICS to cause interactions with drugs which are CYP2D6 and CYP3A4 substrates is rather small. ICS concentrations which inhibited sparteine and bufuralol oxidation *in vitro* are at least one order of magnitude greater than the plasma concentration of ICS which is achieved in patients.

In summary, we identified and characterized the cytochrome P450 enzymes involved in ICS metabolism. In view of the wide therapeutic index of ICS and the rather high K_i values observed for inhibition of the metabolism of other drugs by ICS, both interindividual variability and interaction potential appear to be of no clinical relevance.

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