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Preclinical paper

Pharmacokinetics and central nervous system toxicity of declopramide (3-chloroprocainamide) in rats and mice

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Declopramide (3-chloroprocainamide) has been identified in previous studies as a representative of a new class of chemosensitizers. In this study, the toxicity and pharmacokinetics of declopramide have been investigated and compared with a structural analog, metoclopramide (MCA). Declopramide has not induced central nervous system (CNS)-related side effects in rats at doses up to 200 mg/kg, whereas MCA does at 12.5 mg/kg. In addition, declopramide did not bind to dopamine D_2 receptors in subcellular preparations at doses up to 1000 μM, whereas MCA showed affinity at 1 μM. Declopramide bound with affinity to 5-hydroxytryptamine$_3$ receptors which are important in controlling vomiting. In contrast to MCA, declopramide has a rapid clearance from serum, a lower tissue concentration (about 15-fold lower than MCA) and a lower oral bioavailability (about 6-fold lower than MCA). However, declopramide was shown *in vitro* to possess a higher tumor cell absorption rate. One of the main metabolites of declopramide was identified as *N*-acetyl declopramide. Taken together, these data suggest that the clinical development of declopramide as a sensitizer of radio- and chemotherapies is an improvement over MCA, because it can be administered in a high dose and is devoid of CNS side effects. [© 1999 Lippincott Williams & Wilkins.]

Key words: Benzamides, declopramide (3-chloroprocainamide), metoclopramide, mice, pharmacokinetics, rats.

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Introduction

Declopramide (3-chloroprocainamide) is a new synthesized *N*-substituted benzamide of interest because of its structural similarity to metoclopramide (MCA; Figure 1). MCA is an effective antiemetic and has been used in the clinic for this purpose for more than 30 years.[1] Recently, MCA has been formulated into a highly concentrated injectable solution (Sensamide) and a neutralized injectable solution (Neu-Sensamide). Neu-Sensamide has a reduced CNS side effect profile.[2] Both Sensamide and Neu-Sensamide have been shown to potentiate the cytotoxicity of radiation and chemotherapies in several animal tumor models.[3–11] Moreover, Sensamide has been shown to enhance the effectiveness of radiation in patients with inoperable non-small lung cancer in a phase I/II clinical study.[12]

Declopramide has been shown to retard tumor growth and to enhance cytotoxicity induced by cisplatin as efficiently as Neu-Sensamide.[13] In addition, declopramide has also been shown to have comparable antitumor efficacy to Neu-Sensamide, but with reduced acute toxic symptoms.[13]

The main drug development issue for the MCA formulations (Sensamide/Neu-Sensamide) as sensitizers of radio- and chemotherapies has been the dose-limiting side effects on the central nervous system (CNS),[2] which have been well established over 30 years of clinical use.[1,14,15] The CNS side effects have been chemically related to the presence of the *ortho*-methoxy group in MCA, which planarizies the molecule and allows high affinity for dopamine D_2 receptors.[16] Declopramide was synthesized to avoid CNS side effects by eliminating the *ortho*-methoxy structure from the molecule. Here we report the pharmacokinetics and CNS side effects of declopramide and in comparison to MCA.

Materials and methods

Chemicals

Acidic metoclopramide (Sensamide) was purchased from Lundbeck (Copenhagen, Denmark). Neutral metoclopramide (Neu-Sensamide, nMCA), 3-chloroprocainamide (declopramide) and *N*-acetyl declopramide (*N*-acetyl-3-CPA) were provided by Oxigene Europe (Lund, Sweden). The formulations of Neu-Sensamide and declopramide have been described in previous studies.[13,17] Haloperidol was obtained from Sigma (St Louis, MO). Organic solvents were purchased from Labscan (Dublin, Ireland).

Animal experiments

Female Wistar-Furth rats (2 months old, about 200 g) and female scid mice (8–12 weeks old, about 23 g) were used in this study. The animals were fed a standard pellet diet and had free access to water during the experiments.

Evaluation of drug-induced CNS side effects

Female rats were randomly divided into several groups with five rats in each group and they were treated with Sensamide (25 or 12.5 mg/kg), declopramide (25 and 200 mg/kg) or physiological saline by intramuscular (i.m.) administration in a final injection volume of 180–210 μl. Two groups were orally administered declopramide by gavage in a volume of 250 μl. The CNS side effects were evaluated after 30 min, 2 h and 24 h. The rats were placed at the entrance of a $8 \times 10 \times 45$ cm tunnel located on top of a lab bench which was situated in an artificially lit room free from external stimuli such as in view of windows or other animals. Only the top of the tunnel was transparent for observation purposes and the end of the tunnel was left open as an escape stimulus. The time for each rat to travel the tunnel was recorded. If the time was longer than 120 s, it was recorded as 120 s. The rats used in this experiment were not previously trained to negotiate the tunnel.[2]

Measurement of binding affinities to D_2 and 5-hydroxytryptamine$_3$ (5-HT_3) receptors

The conventional procedures have been described in detail.[18,19] The binding affinities of Sensamide, Neu-Sensamide and declopramide were determined by competitive displacement of the high-affinity binding of radioligands [^{3}H]spipirone for D_{2A} and D_{2B} receptors and [^{3}H]GR63630 for 5-HT_3 receptors in crude 100 000 *g* membrane fractions. Soluble membrane fractions were prepared from Chinese hamster ovarian cells expressing the human D_{2A} and D_{2B} receptors, and from rabbit submucosal muscle layers of internal organs expressing 5-HT_3 receptors. The drugs were assayed in duplicate at six different concentrations and each experiment was repeated 3 or 6 times. Specific binding was plotted and subjected to Scatchard analysis. IC_{50} values were calculated by converting the binding data to logits and linear regression against the log of drug concentrations. The IC_{50} values were in turn used to compare the relative binding potencies of Sensamide, Neu-Sensamide and declopramide by calculating the K_i=IC_{50}/1+(ligand/K_D). The K_i values were analyzed statistically by the paired *t*-test.

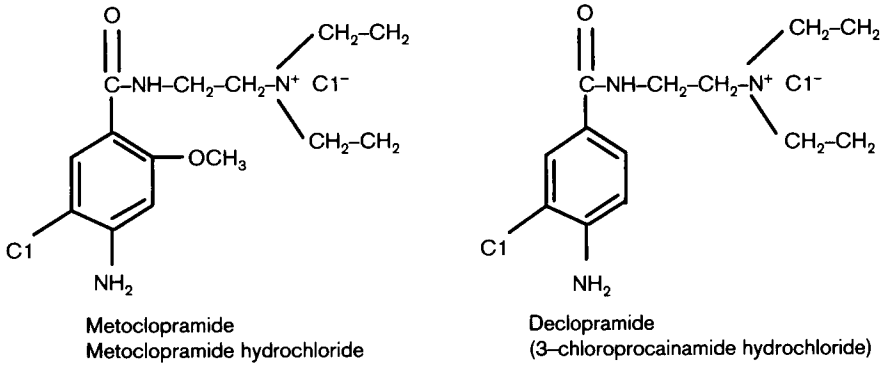

Figure 1. Molecular structures of metoclopramide and declopramide.

Pharmacokinetic studies

Female W/Fu rats were treated with declopramide by i.m. injection at doses of 25, 50, 100 and 200 mg/kg or by i.v. and oral administration at a dose of 25 mg/kg, or treated with Neu-Sensamide by i.v., i.m. and oral administration at a dose of 25 mg/kg. For i.v. administration, drugs were injected into the tail vein under ether anesthesia over a 2 min period in a volume of 180–200 μl. For i.m. administration, drugs were injected into the right leg of animals in a volume of 180–200 μl. For oral administration, drugs were given to animals by gavage in a volume of 250 μl. Scid mice xenografted with human lung adenocarcinoma (H2981, tumor volume 300 mm^3) were also treated with declopramide and Neu-Sensamide by i.m. and oral administration at 25 mg/kg in a volume of 100 or 250 μl. After 5, 15, 30, 45, 60 and 120 min of single administration, blood samples (three to five samples for each time point) were taken by periorbital puncture from rats and each rat was sampled three times. For mouse, the blood samples were collected by heart puncture. Serum samples were separated from the blood by centrifugation at 2000 *g* for 10 min. Rat urine samples were collected over 24 h following drug administration in a metabolic cage. The animals were sacrificed and tissue samples collected at the end of experiments for determination of drug uptake. All samples were stored at −20°C until being subjected to chemical analysis.

Drug extraction

The procedure of extraction for all *N*-substituted drug analogs was according to the method of Meyer *et al.*[20] with slight modifications. Briefly, 100–150 mg of tissue was homogenized and then internal standard (haloperidol) was added. After centrifugation, the supernatant was adjusted with saline to 2 ml before being extracted. Aliquots of 100 μl samples of serum or urine were directly mixed with internal standard and adjusted to 2 ml with saline. All samples were pH adjusted by addition of 100 μl of 5 M NaOH to convert the hydrochloride salt to free base, and then extracted with 6 ml of chloroform and isopropanol (96:4). The solvents were evaporated under nitrogen gas.

Thin layer chromatography (TLC) analysis

The chloroform:isopropanol extracts of urine or serum were spotted on precoated silica gel 60 F_{254} TLC plates and developed in chloroform:methanol:concentrated ammonium hydroxide (90:10:1). R_f values were calculated in a conventional manner and compared with authentic standards.

High-pressure liquid chromatography (HPLC) analysis

The dried chloroform:isopropanol extracts were taken up in 20 μl of methanol just before subjecting to HPLC analysis. The HPLC system consisted of a four-pump system (Perkin Elmer 410 LC), a variable UV detector (LC-95) set at 280 nm, an integrator, a 120-3C18 column and a 5 μl loop. Data were collected and analyzed by a Turbochrom 4 Perkin Elmer HPLC system. Declopramide has a shorter retention time than Sensamide or Neu-Sensamide (both have the same retention time) when eluted by a mobile phase gradually increasing from 5% acetonitrile to 50% in 100 mM sodium buffer (pH 5.0) and at a flow rate of 2 ml/min. The drug concentration was calculated as a function of the peak area divided by the peak area of the internal standard. The recovery of declopramide was 80–90% for serum and spleen samples, and for brain, liver and kidney it was 60–70%. The detection limit was 0.4 μg/ml when 100 mg tissue or 100 μl serum was used for the extraction. The quantification range was defined between 0.7 and 30 μg/ml (correlation coefficients=0.996 with CV less than 10%, N=5). The *N*-acetyl metabolite was detected and measured by using a longer column (8.3 cm C18) and 43% methanol with 0.03% triethylamine as constant mobile buffer.[21]

Pharmacokinetic analysis

The profiles of drug serum concentration versus time were evaluated with the program SAAM II (Seattle, WA). The curve produced by declopramide after i.v. injection was precisely fitted by a monoexponetial equation; curves produced by declopramide after i.m. and oral administration, or MCA after oral administration were fitted by biexponetial equations. All other analyses were best fitted by triexponetial equations.[22] The area under curve (AUC) of the serum concentration versus time was calculated by the tripezoidal rule, and significant parameters determined by fitted equations. The apparent distribution volume (V_d), the clearance (Cl) and bioavailablity were calculated as follows: $V_d = \text{dose}_{(i.v.)}/C_0$ and $Cl = V_d \times K_{et}$ for the monoexponetial equation; $V_d = \text{dose}_{(i.v.)}/AUC_{0\rightarrow\infty} \times \beta$ and $Cl = V_d \times \beta$ for the biexponetial equation; bioavailablity=$\text{dose}_{(i.v.)} \times AUC_{(oral)}/\text{dose}_{(oral)} \times AUC_{(i.v.)}$.[23] Differences between mean values were analyzed by Student's *t*-test.[24,25]

Pharmacokinetic study *in vitro*

HL-60 cells at the concentration of 2×10^6/ml were incubated with declopramide or Neu-Sensamide at a concentration of 100 μM for varying periods. The cells were washed twice with medium containing 10% fetal calf serum before biochemical analysis. Declopramide and Neu-Sensamide were extracted from cells with chloroform:isopropanol as outlined above. The conditions for quantification analysis by HPLC were the same as those used for analyzing rat serum samples but at a higher sensitivity. Drugs were analyzed in duplicate.

Statistics

Differences among groups were tested by one-way analysis of variance (ANOVA) at the significance level of 0.05.

Results

CNS side effects

The sedative effect of Sensamide is illustrated in Figure 2. Rats administered with Sensamide at 12.5 and 25 mg/kg (i.e. as a positive control) displayed a motionlessness and drowsiness immediately after treatment. The travel time through the tunnel was much longer than that for the placebo control and became more pronounced at 25 mg/kg, which has already been reported in previous studies.[2] However, rats administered with declopramide had no observ-able sedative side effects nor were there any other notable symptoms up to a dose of 200 mg/kg and over an observation period of 24 h. There were no significant differences between the control and the declopramide-treated group at any dosage or at any observation period. No difference between administration routes, demonstrating declopramide was very poor at inducing CNS side effects estimated by this animal model.

Affinity for dopamine D_2 and 5-HT_3 receptors

Since the sedative effect is believed to relate to dopamine release, the affinity of Sensamide, Neu-Sensamide and declopramide binding to dopamine D_2 receptors were examined. Both Sensamide and Neu-Sensamide strongly inhibited the high-affinity binding of siperone ligand in crude membrane fractions having K_i=1.0$\pm$0.1 μM for D_{2a} and K_i=3.3$\pm$0.5 μM for D_{2b},[2] whereas declopramide exhibited no dopamine D_2 affinity binding at 1000 μM for both the α and β subclasses of receptors. These data indicated declopramide binding to dopamine D_2 receptors is at least 300 times weaker than Sensamide and Neu-Sensamide. However, declopramide retained the capacity to bind 5-HT_3 receptors, although the K_i value is 3-fold lower than Sensamide and Neu-Sensamide (Table 1).

Pharmacokinetics in rats

A linear pharmacokinetics of declopramide was obtained at doses between 25 and 100 mg/kg. A less proportional increase in C_{max} and a longer serum half-life time was found at 200 mg/kg indicating a saturation of the tubular reabsorption process. Since the pharmacokinetics of Neu-Sensamide and Sensamide are identical,[2] here we have reported only the pharmacokinetics of declopramide compared to Neu-Sensamide. The profiles of serum concentration of declopramide and Neu-Sensamide versus time are presented on Figure 3(A and B). Other parameters are summarized in Table 2. The Neu-Sensamide curves obtained from i.v. and i.m. injection could be precisely fitted by biexponential equations which are in agreement with previous studies.[2,26,27] The corresponding other pharmacokinetic parameters were also similar to the results reported by other researchers.[28] The declopramide curve obtained from single i.v. injection could be fitted by a monoexponetial equation. The C_0 value has been calculated as 33.6$\pm$0.48 nmol/ml with no significant difference from that of Neu-Sensamide. However, the half-life of declopramide in serum (36.6$\pm$1.1 min) and the AUC value (1815$\pm$25 nmol min/ml) were much smaller than those of Neu-Sensamide ($p < 0.05$). The clearance value was much higher than that of Neu-Sensamide, indicating declopramide could be quickly removed from circulation.

The oral administration of declopramide gave about 6-fold lower bioavailability (14%) than Neu-Sensamide. However, the $T_{1/2abs}$ value of declopramide was 5.85$\pm$0.25 min and showed that drug could be absorbed through the gastrointestinal track as quickly as Neu-Sensamide (7.09$\pm$3.08 min), which in turn suggested that the lower oral bioavailability was due to the first-pass effect through the liver (Table 2).

Drug concentrations in tissues and cells

Generally, both declopramide and Neu-Sensamide could be detected in all main organs examined 30 and 120 min after administration, but the levels

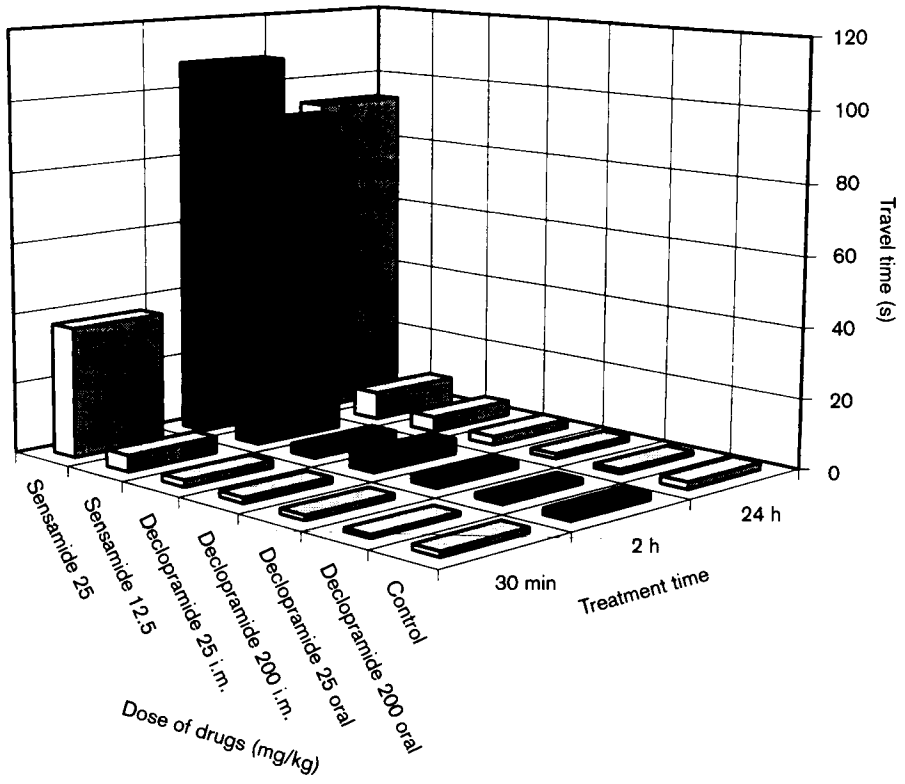

Figure 2. Comparison of sedative effects of Sensamide and declopramide. W/Fu rats were i.m. administered Sensamide at doses of 12.5 or 25 mg/kg and declopramide at doses of 25 or 200 mg/kg as well as orally administered declopramide at doses of 25 or 200 mg/kg. Data are mean of five rats. ANOVA comparison of declopramide with control was not significant. ANOVA comparison of Sensamide with control was significant ($p<0.05$).

Table 1. The K_i values for Sensamide, Neu-Sensamide and declopramide to inhibit the binding of the high-affinity ligands [^{3}H]spiperone to the dopamine D_{2a} and D_{2b} receptors and [^{3}H]GR65630 to the 5-HT_3 receptor

Drug	Dopmaine D_{2a} (K_i) (μM)	Dopmaine D_{2b} (K_i) (μM)	5-HT_3 (K_i) (nM)
Sensamide[a]	1.0±0.1	3.3±0.5	932±12
Neu-Sensamide[a]	1.3±0.1	4.6±0.4	733±41
Declopramide	>1000*	>1000*	3200±500*

The results are expressed as mean±SE (n=3), except for the dopamine D_{2b} data where n=6.
[a]The Sensamide and Neu-sensamide data have been published by Pero *et al.*
*P<0.05 when compared with Sensamide or Neu-Sensamide by Student's paired t-test.

differed from organ to organ (Tables 3 and 4). The highest concentrations were observed in the spleen and tumor. However, the tissue concentrations of declopramide were lower than those of Neu-Sensamide ($p<0.05$) and these differences were associated with the difference in rodent species. There was a 20-fold difference in rat tissues, whereas 2-fold differences were found in mouse tissues. The tissue concentrations of declopramide were also influenced by the administration routes where oral administration gave the lowest tissue concentration, which was consistent with the serum kinetics.

One main reason for a lower tissue concentration of declopramide could be explained by a poorer capacity of absorption through cell membranes. For evaluation of this possibility, a cell pharmacokinetic study has been performed in human leukemic cells (HL-60). The data clearly showed declopramide had a greater uptake into cells than Neu-Sensamide when evaluated by the AUC values (Figure 4), indicating lack of

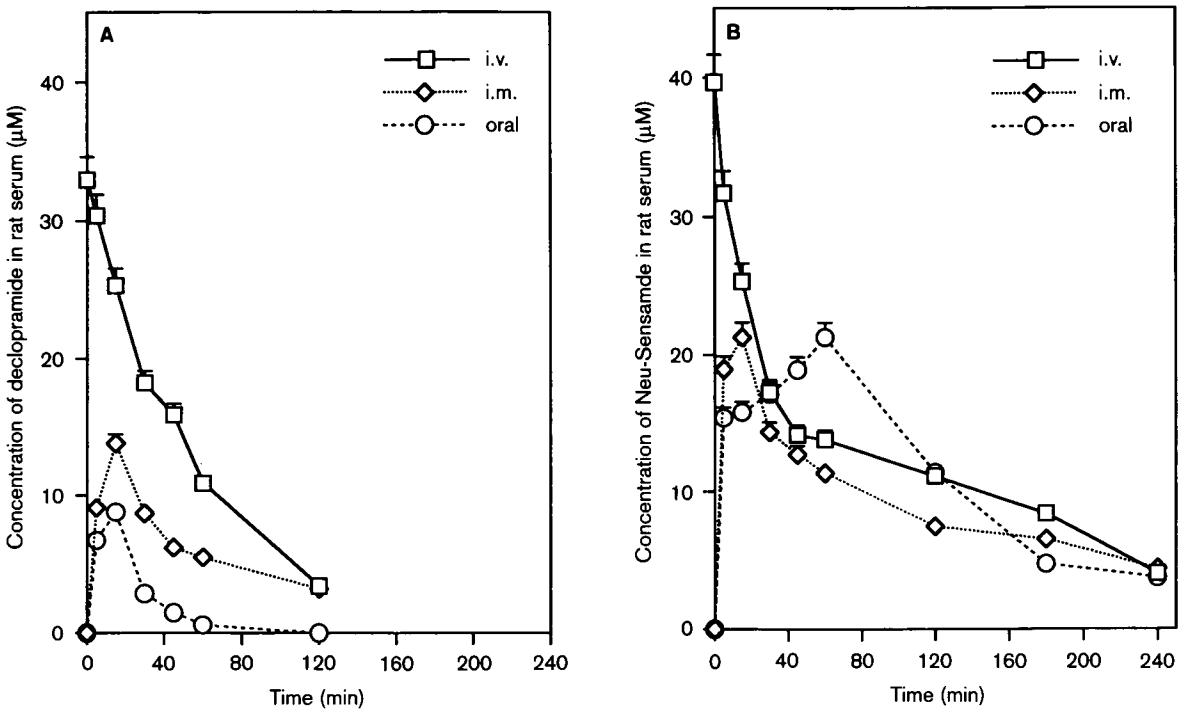

Figure 3. Pharmacokinetics of serum concentrations of declopramide (A) and Neu-Sensamide (B) in W/Fu rats after i.v., i.m. and oral administrations at a dose of 25 mg/kg. Data are expressed as means of four to five animals ± SEM.

Table 2. Parameters of pharmacokinetics of declopramide and Neu-Sensimide in W/Fu rats

Pharmacokinetic parameters	Declopramide			Neu-Sensamide		
	i.v.	i.m.	oral	i.v.	i.m.	oral
$T_{1/2abs}$ (min)	–	2.8±0.5	5.85±0.3	–	2.97±2.8	7.09±3.08
$T_{1/2\ (\alpha)}$ (min)	–	–	–	8.84±3.8	7.6±6.7	
$T_{1/2\ (\beta)}$ (min)	36.6±1.1	43.3±5.3*	8.8±0.3*	126±19.2	137±7.2	86.7±11.9
T_{max} (min)	–	11.8±1.3*	10.3±0.4*	–	6.5±1.6	27.9±7.3
C_{max} (nmol/ml)	33.6±0.48	11.3±0.5*	9.0±0.4*	39.5±4.7	20.6±2.0	17.9±1.9
V_d (l/kg)	2.4±0.0*	5.3±0.4	3.9±0.4	4.7±0.6	5.2±0.7	3.7±0.7
Cl (ml min/kg)	45±1.0*	85±0.1*	308±13*	24±1.0	26±2.4	29.6±1.9
AUC (nmol min/ml)	1815±25*	937±46*	258±11*	3544±153	3174±286	2830±180
Bioavailability (%)	–	52±2.5*	14.2±1.6*	–	90±8.1	80±5.1
24 h urine elimination (%)	–	6.2±2.7*	3.3±0.6*	–	23.6±5.5	20.9±6.8

Animals were treated with declopramide or Neu-Sensamide at 25 mg/kg. The profiles of serum concentration versus times were fitted by mono/polyexponential equations and the various parameters calculated as described in Materials and methods. Data are mean of three to five animals ± SD. A dash indicates no data under this specific curve fitting or no analysis.
*$p<0.05$ when compared with Neu-Sensamide administered in the same route.

absorption was not the explanation for lower serum levels of the drug.

Analysis of metabolites

The large differences in bioavailability between oral and i.v. administration of declopramide has suggested metabolism by the liver after oral administration. Hence, metabolite detection in serum and urine has been undertaken by TLC and HPLC. Metabolites were observed in both declopramide and Neu-Sensamide samples, but there was one metabolite found only in declopramide-treated samples with the same R_f value as *N*-acetyl declopramide (R_f=0.35) when the TLC plates were developed in a solvent system of

Table 3. Concentrations of declopramide and Neu-Sensamide in organs of W/Fu rats

	Declopramide (nmol/g)			Neu-Sensamide (nmol/g)		
	i.v.	i.m.	oral	i.v.	i.m.	oral
Serum	3.4±0.5*	2.5±0.8*	UD	11.1±1.8	7.5±1.0	11.4±2.1
Brain	–	3.4±0.3*	1.7±0.1*	–	17.8±2.3	12.1±0.9
Liver	8.2±1.5*	5.8±0.7*	5.2±1.4*	57±6.8	117±15	165±18
Kidney	29.2±3.4*	25.2±3.6*	5.0±0.4*	198±45	172±12	203±2.2
Spleen	33.1±3.9*	48.6±1.1*	18.2±1.8*	156±48	276±24	333±28

The animals were i.v., i.m. or orally administered declopramide and Neu-Sensamide at 25 mg/kg and sacrificed at 2 h. Data are mean of three to five animals ± SEM. UD, under detection limit. A dash indicates no analysis.
*$p<0.05$ when compared with Neu-Sensamide administered in the same route.

Table 4. Pharmacokinetics of declopramide and Neu-Sensamide in scid mice

Time (min)	Brain (nmol/g)		Spleen (nmol/g)			Tumor (nmol/g)		
	Declopramide	Neu-Sensamide	Declopramide		Neu-Sensamide	Declopramide		Neu-Sensamide
	i.m.	i.m.	oral	i.m.	i.m.	oral	i.m.	i.m.
30	18.0±2.7	19.4±1.5	25.4±6.3*	49.9±13.2**	80.5±14.6	11.3±4.3*	49.1±8.2**	73.4±3.8
60	8.5±1.0	9.6±1.0	3.6±0.6*	12.8±2.1**	33.8±5.4	8.2±1.6*	24.1±6.4**	31.0±3.7
120	1.2±0.3	1.7±0.1	UD	2.4±0.5**	9.7±1.5	3.4±1.1	4.1±0.4**	7.0±1.6
180	UD	UD	UD	2.1±0.4**	7.0±0.6	UD	2.7±0.6	5.5±1.5

Animals were i.m. or orally given declopramide or Neu-Sensamide at 25 mg/kg and sacrificed at different times to provide samples of sera. Data are mean of five animal ± SEM.
*$p<0.05$ when compared with i.m. injection of the same drug at same time point.
**$p<0.05$ when compared with Neu-Sensamide at the same condition.
UD, under detection limit.

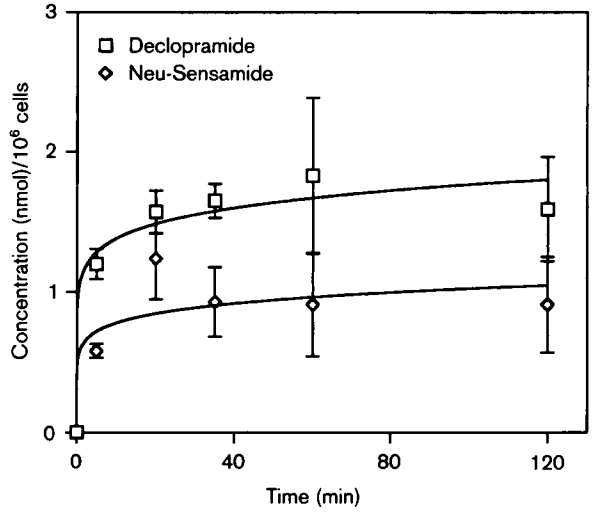

Figure 4. Cell pharmacokinetics of declopramide and Neu-Sensamide in HL-60 cells. Data are means of three to five samples ± SEM. The AUC value of declopramide was significantly different from that of Neu-Sensamide ($p<0.05$).

chloroform:methanol:concentrated ammonium. This metabolite was identified as *N*-acetyl declopramide by co-chromatography with authentic standard using TLC and HPLC. In addition, this metabolite was produced in urine *in vivo* and it has been eluded from TLC plates, extracted with chloroform, and also confirmed by HPLC as *N*-acetyl declopramide (Figure 5). The concentration of the *N*-acetyl metabolite has been quantified as about one-tenth the declopramide concentration in serum 30 min after oral administration and 2% after i.m. injection.

Discussion

It is known that declopramide and Sensamide/Neu-Sensamide have similar potency as antitumor drugs (see Introduction), but CNS side effects have not been compared for these two drugs. Our data have clearly shown that both declopramide and Sensamide/Neu-Sensamide have the capacity of passing through the

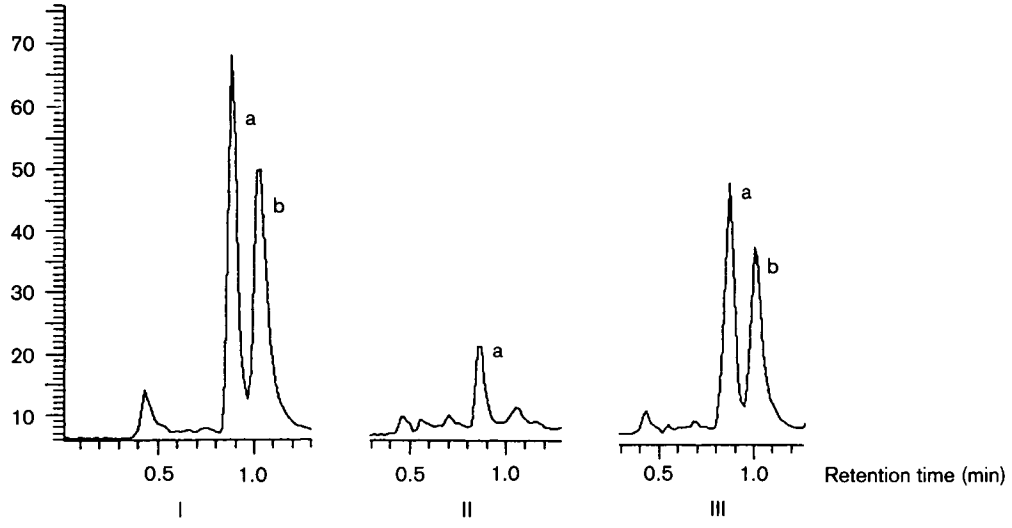

Figure 5. HPLC chromatograms of a standard solution of *N*-acetyl declopramide and declopramide. The analyzing conditions and procedures have been described in Materials and methods. (I) (a) Standard *N*-acetyl declopramide; (b) standard declopramide. (II) Extract of TLC spot which was isolated from urine extracts of declopramide-treated rats. (a) Peak having the same retention time as authentic standard of *N*-acetyl declopramide. (III) Urine extract spiked with standards of *N*-acetyl declopramide and declopramide. (a) *N*-acetyl declopramide + urine extract; (b) declopramide.

blood-brain barrier (Table 3),[17] but nevertheless declopramide did not induce CNS side effects at the dose up to 200 mg/kg. This is 16-fold higher than the dose causing CNS side effects by Sensamide. The tremendous difference in CNS side effects induced by these two drugs is explained by the fact that declopramide has no affinity to dopamine D_2 receptors (Table 1). The further explanation is the molecular structure of declopramide lacking an *ortho*-methoxy benzamide ring substitution compared to MCA (Figure 1). Previous chemical studies have established that the *ortho*-methoxy benzamide ring substitution is important in planarizing the conformational structure for binding to dopamine D_2 receptors.[16] Consequently, conformational chemistry supports the changes in binding affinities of these drugs.[2] Interestingly, declopramide still has affinity for binding to 5-HT_3 receptors, indicating that conformational changes did not influence other pharmacological properties of the drug and this in turn suggests it might have anti-emetic properties without inducing side effects.

The pharmacokinetics of declopramide are characterized by a rapid distribution, a fast elimination and a large clearance (Figure 3 and Table 2). Declopramide also readily crosses cell membranes and penetrates the blood-brain barrier (Tables 3 and 4). These features are common pharmacokinetic features for benzamide analogs, which are small lipid soluble molecules.[29,30] However, some pharmacokinetic features of declopramide were unexpected based on comparison to the model drug (MCA). Those features mainly manifested as (i) a quick decrease of serum concentration, (ii) a lower tissue accumulation and (iii) a lower oral bioavailability (Figure 3, and Tables 2 and 3). Reasons for such pharmacokinetic phenomena are usually related to poor absorption or quick elimination or both in combination. Our data has clearly shown declopramide has a higher absorption rate into cells (Figure 4) and the primary difference from Neu-Sensamide is thus suspected to be a rapid metabolic alteration of declopramide. First, a low oral bioavailability compared to i.v. or i.m. administration of declopramide suggests that there is an early metabolic transformation due to a first-pass effect through the liver. Second, a low portion of unchanged declopramide has been detected in 24 h urine samples (Table 2), indicating that most of declopramide has been metabolically altered. Third, differences in tissue concentrations of declopramide and Neu-Sensamide have been found between rats and mice implicating well-known different metabolic capacities of these species.[31,32] Finally, *N*-acetyl declopramide has been identified in urine and serum samples confirming a well-known characteristic of liver metabolism.

Our previous studies have shown a similar efficacy in inhibiting tumor growth by administration of declopramide and Neu-Sensamide when using a rodent tumor model,[13] but the data obtained in these studies

did not determine drug concentrations in tumors. This raises the question of whether metabolites such as *N*-acetyl declopramide have similar antitumor activity compared to unmetabolized declopramide. This point is currently under investigation in our laboratory.

Acetylation of declopramide has been considered because of the following reasons. First, declopramide is also an analog of procainamide. Procainamide has been well known for rapid acetylation, i.e. oral administration of procainamide is quickly acetylated into *N*-aceytl procainamide.[31,33-35] This metabolite of procainamide retains the procainamide property of antiarrhythmic activity but without the side effect of inducing lupus disease.[37-39] Second, none of the metabolites of MCA have been identified as acetylated products so far in metabolic studies.[40,41] Third, a higher acetylation rate has been found in rats than in mice, which is consistent with the difference observed in this study between rats and mice.[42] Fourth, a metabolite, *N*-acetyl declopramide, produced by metabolic acetylation of declopramide has been identified in this study (Figure 6). However, neither *N*-acetyl procainamide nor procainamide are shown to possess antitumor properties. Using the procainamide structure as a basis for metabolic explanation, *N*-acetyl declopramide could function as an antitumor agent like declopramide and if so then the lower oral bioavailability would not seriously influence the antitumor activity.

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