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Disposition of Quinidine in the Rabbit

THEODOR W. GUENTERT *, JIN-DING HUANG, and SVEIN ØIE ˣ

Received June 18, 1981, from the *Department of Pharmacy, School of Pharmacy, University of California, San Francisco, CA 94143.* Accepted for publication October 15, 1981. *Present address: Pharmazeutisches Institut, Universität Basel, Totengässlein 3, CH-4051 Basel, Switzerland.

Abstract □ Quinidine shows two-compartment characteristics in rabbits with a terminal half-life of 67 min for total drug and 58 min for unbound drug. Statistically, the values are not significantly different from each other ($p > 0.05$). The clearances for total and unbound drug are 52 and 464 ml/min/kg, respectively, and the total and unbound apparent volumes of distribution at steady state are 4.2 and 27.3 liters/kg, respectively. The unbound clearance and unbound apparent volume of distribution were inversely related to the unbound fraction of quinidine in plasma. The total clearance and apparent volume of distribution showed no relationship to the binding. Approximately 0.5% of the dose was excreted as unchanged quinidine. Six identifiable metabolites were found in the urine, accounting for ~14% of the dose. Two unknown metabolites were also observed in the urine. With the exception of 2′-quinidinone, these metabolites were formed in the rate-limiting step in the metabolite kinetics. The quinidine unbound fraction ranged from 0.06 to 0.23 in the eight rabbits studied. The binding of the metabolites was less pronounced, and only 3-hydroxyquinidine showed a significant correlation with quinidine binding.

Keyphrases □ Quinidine—disposition in rabbits, metabolites, binding □ Metabolites—binding, quinidine disposition, rabbits □ Binding—quinidine disposition, metabolites, rabbits □ Pharmacokinetics—disposition of quinidine, rabbits

Very little information exists regarding quinidine disposition in the rabbit, especially when unbound quinidine and metabolite concentrations are considered. The rabbit is, however, an attractive animal model for quinidine studies because it allows for sampling of sufficiently large blood volumes to determine both total and unbound concentrations; blood and urine sampling is simple; and if drug responses are desired, the response, measured as EKG changes, can be readily obtained (1).

As part of a long-term study of the disposition and response interrelationship between binding of quinidine to plasma proteins the pharmacokinetic picture of quinidine was evaluated in the rabbit.

EXPERIMENTAL

Quinidine Administration—Eight male New Zealand white rabbits (2.0–3.3 kg) were injected with 5.2 mg/kg of quinidine base as the gluconate salt[1] dissolved in 1 ml of saline into an ear vein over 2 min. Blood samples of 3-ml volume were obtained from the marginal vein in the other ear before the injections and again at 4, 8, 20, 40, 60, 90, 120, 150, 195, and 240 min after the injections. The blood was heparinized[2] to a final concentration of 5 U/ml. The blood was centrifuged and the plasma was stored at −20° until assayed. In addition, a 300-μl whole blood aliquot from the 4- and 150-min sampling time was also stored frozen until assayed. The red blood cells from the samples obtained at 20 min and at later time points were suspended in an equal volume of 6% dextran 75 in isotonic saline and reinfused within 10 min of sampling.

The urethra of each animal was cannulated with a catheter[3]. To ensure complete urine collection over the 4-hr study period, the bladder was rinsed twice with normal saline at the end of the study. The total urine collected was stored frozen until assayed.

Hepatic Blood Flow Determination—The hepatic blood flow was estimated in the individual animals 20–40 min after the end of the quinidine experiment by determining the indocyanine green blood clearance. Indocyanine green[4] (1 mg/kg) was infused over 30 sec into a marginal ear vein. Blood was collected from the marginal ear vein in the other ear by continuous withdrawal at a speed of 0.36 ml/min over a 12-min period, starting at the time of indocyanine green infusion.

The indocyanine green concentration in plasma of the withdrawn blood (C_{ICG}) was determined spectrophotometrically at 800 nm. Because the half-life of indocyanine green is 1 min and it does not enter the red blood

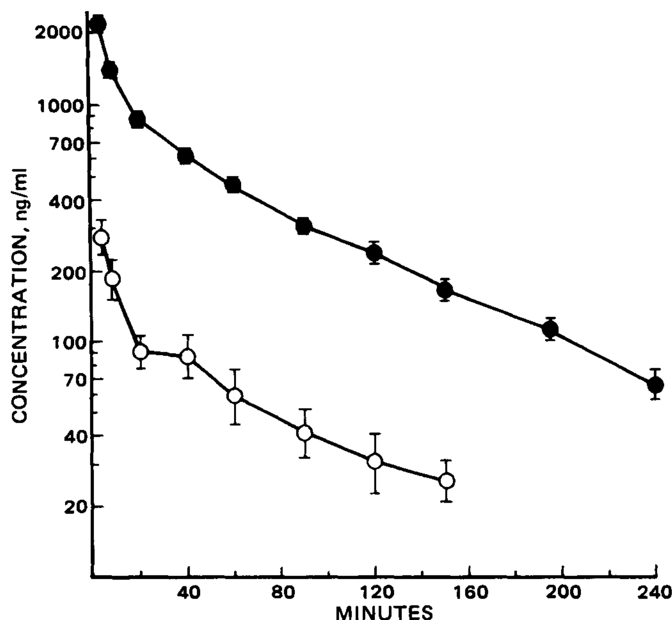

Figure 1—*Log-average plasma concentrations of total (●) and unbound (○) quinidine in eight rabbits after a 5.2-mg/kg intravenous injection.*

[1] Quinidine gluconate injection, USP, 80 mg/ml; Lilly, Indianapolis, Ind.
[2] Sodium heparin injection, USP, 1000 U/ml, Lilly, Indianapolis, Ind.
[3] French Foley Catheter #8, D. R. Bard, Ind., Murray Hill, N.J.
[4] Hynson, Westcott, & Dunning, Inc., Baltimore, Md.

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Table I—Pharmacokinetic Parameters of Quinidine in Rabbits

Rabbit Number	Unbound Fraction	Clearance, ml/min/kg		Renal Clearance, ml/min/kg		Volume of distribution steady state, liter/kg		$t_{1/2,\alpha}$[a], min		$t_{1/2,\beta}$[a], min	
		Total	Unbound	Total	Unbound	Total	Unbound	Total	Unbound	Total	Unbound
1	0.06	51	797	0.3	4.3	2.8	31.3	1.6	2.3	50	34
2	0.19	53	277	0.5	2.7	4.5	21.4	9.9	3.3	77	76
3	0.15	44	295	0.5	3.1	4.5	24.2	11.4	4.3	90	71
4	0.18	47	254	[b]	[b]	4.0	22.7	3.1	0.7	64	81
5	0.06	51	922	0.05	0.9	3.4	48.4	3.7	2.5	60	45
6	0.16	64	401	0.04	0.3	5.0	28.0	6.0	4.7	65	59
7	0.23	48	211	[b]	[b]	4.0	12.6	4.7	2.3	70	54
8	0.11	58	554	0.3	2.4	5.0	28.8	6.2	4.8	61	45
Mean	0.14	52	464	0.3	2.3	4.2	27.3	5.8	3.1	67	58
SD	0.06	6	268	0.2	1.5	0.8	10.3	3.4	1.4	12	17

[a] $t_{1/2,\alpha}$ = fast disposition half-life; $t_{1/2,\beta}$ = terminal half-life. [b] Incomplete urine collection.

cells, the blood clearance of indocyanine green (Cl_{ICG}) can be determined by:

$$Cl_{ICG} = \frac{\text{Amount infused}}{C_{ICG}\,\tau\,(1 - H)}$$

where τ is the time of withdrawal and H is the hematocrit value.

Protein Binding—Protein binding was determined in each plasma sample using an equilibrium dialysis technique (2): A 700-μl volume of freshly obtained plasma and of Krebs–Ringer bicarbonate buffer (pH 7.4) (3) was placed into the two half-cells of a 1-ml dialysis cell[5], separated by a dialysis membrane[6] (average pore radius 24 Å). The membrane was pretreated by soaking in water (10 min), ethanol (15 min), and buffer (120 min) before use. The cells were equilibrated in a shaker bath[7] at 37° for 6 hr ± 20 min. The plasma and buffer were then removed and assayed immediately, or frozen until assayed for quinidine and metabolite content.

Plasma Protein Concentration—The total plasma protein concentration was determined before the injection of quinidine and again 30 min after concluding the study. Plasma (100 μl) was mixed with 5 ml of biuret reagent[8], and the absorbance at 540 nm was determined within 1 hr. An aqueous solution containing 80 g/liter of total protein was used as a standard[9].

Quinidine Assays—Quinidine and quinidine metabolites in plasma, whole blood, or buffer from equilibrium dialysis were determined using a specific reversed-phase high-pressure liquid chromatographic method. A 100-μl sample to be analyzed was mixed with 200 μl of a methanolic solution containing 170 ng/ml of dihydroquinidine internal standard (quinidine-free). Plasma and blood samples were centrifuged in a microhematocrit centrifuge[10] at 12,000×g for 2 min. A 20-μl aliquot of the sample was then injected into a C-18 μBondapak column of 30-cm length and 3.9-mm bore[11], connected to an HPLC pump[12]. Methanol–water–phosphoric acid 85% (27:72.95:0.05, v/v/v) was used as the mobile phase at a flow rate of 1.0 ml/min. Detection was achieved using a fluorescence detector[13] with excitation at 245 nm and emission at 440 nm (cutoff filter). Correction of dihydroquinidine contamination in the injection contributing to the internal standard peak was made from injection of the samples prepared as described previously, without addition of the internal standard. All samples were analyzed within 1 week of collection. However, the frozen samples have been found to be stable for up to 12 months without change in analyzed concentration.

Quinidine and quinidine metabolites in urine were assayed by a method described by Guentert *et al.* (4): Urine (200 μl) or 500 μl of urine hydrolysate was mixed with 150 μl of aqueous solution of 34 μg/ml pronetalol (internal standard), and 1.5 ml of 1 *M* borate buffer (pH 9.4). The mixture was extracted into 10 ml of methylene chloride–isopropanol (4:1, v/v). The organic phase was removed and extracted with 1 ml of 0.1 *N* sulfuric acid. The aqueous phase was then alkalinized with 1.5 ml of 1 *M* borate buffer (pH 9.4) and extracted with 8 ml of methylene chloride–isopropanol (4:1, v/v). The organic layer was evaporated to dryness and the residue reconsitituted in 200 μl of eluent. A 50-μl aliquot was injected

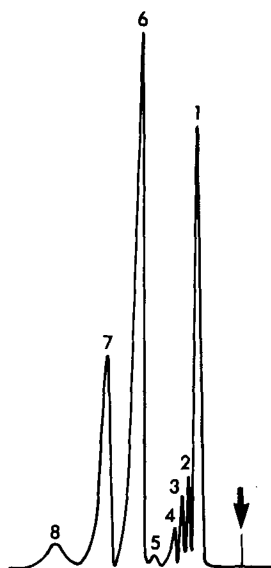

Figure 2—*Chromatogram obtained from plasma of rabbit no. 5 after a quinidine dose of 5.2 mg/kg. The arrow indicates time of injection. 1, quinidine-10,11-dihydrodiol; 2, 3-hydroxyquinidine; 3, 6-desmethylquinidine; 4, unknown metabolite; 5, quinidine-N-oxide; 6, quinidine; 7, dihydroquinidine (used as internal standard); 8, 2'quinidinone.*

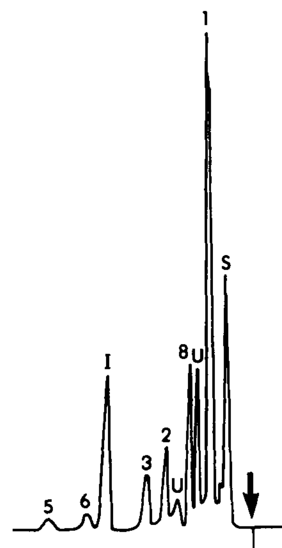

Figure 3—*Chromatogram obtained from the urine of rabbit No. 1 after a quinidine dose of 5.2 mg/kg. Identifications of peak numbers, (Fig. 2). 1, Pronetalol (internal standard); U, unknown metabolites.*

[5] Technilab Instruments, Inc., Pequannacock, N.J.
[6] VWR Scientific, Inc., San Francisco, Calif.
[7] Braun Melsungen AG, Germany.
[8] Sigma Chemical Co., St. Louis, Mo.
[9] Protein standard solution, Sigma Chemical Co.. St. Louis, Mo.
[10] IEC Model MB, Needham Heights, Md.
[11] Waters Associates, Inc., Milford, Mass.
[12] Altex Scientific model 110, Berkeley, Calif.
[13] Schoeffel Inst. Corp., model GM 970.

Table II—Rate Constants for the Various Metabolites, Min^{-1}

Rabbit Number	Peak 1 Quinidine-10,11-dihydrodiol		Peak 2 3-Hydroxyquinidine		Peak 3 6′-Desmethylquinidine		Peak 4 Unknown		Peak 5 Quinidine-*N*-oxide		Peak 8 2′-Quinidinone	
	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast	Slow
1	0.142	0.010	[a]	[a]	[a]	[a]	0.606	0.009	0.104	0.011	0.020	0.004
2	0.024	0.011	0.098	0.007	[b]	[b]	0.140	0.006	[a]	[a]	0.012	0.0012
3	0.048	0.006	0.130	0.005	[b]	[b]	0.162	0.004	[a]	[a]	[a]	[a]
4	0.019	0.014	[a]	[a]	[a]	[a]	0.053	0.009	[a]	[a]	[a]	[a]
5	0.036	0.014	0.026	0.026	0.061	0.012	0.075	0.010	0.198	0.011	0.013	0.0002
6	0.039	0.010	0.104	0.009	[b]	[b]	0.103	0.008	0.290	0.008	0.024	0.0001
7	0.017	0.014	0.059	0.011	[b]	[b]	0.111	0.009	[a]	[a]	[a]	[a]
8	0.052	0.008	0.119	0.009	[b]	[b]	0.081	0.007	[a]	[a]	[a]	[a]
Mean	0.047	0.011	0.089	0.013	0.061	0.012	0.166	0.008	0.197	0.010	0.017	0.0014
SD	0.040	0.003	0.039	0.009	—	—	0.181	0.002	0.093	0.002	0.006	0.0018
n	8	8	6	6	1	1	8	8	3	3	4	4

[a] Not detected. [b] Only trace concentration was detected.

onto a μBondapak-phenyl column[11]. The mobile phase was acetonitrile–tetrahydrofuran–0.05 *M* phosphate buffer (pH 4.75) (15:6:79, v/v/v). The eluent was monitored using a variable wavelength detector[14] set at 230 nm. Quantitation of the compounds was achieved by using standard curves of quinidine and metabolites (5–8). The unknown metabolites were assumed to have identical spectroscopic behavior to quinidine. All standards were made from spiked blank plasma or urine samples and treated as described previously.

Hydrolysis of Urine—Urine (200 μl) was adjusted to pH 5.0 by addition of acetic acid. One milliliter of β-glucuronidase[8] (5000 Fishman U/ml) was added to the urine and incubated at 37° for 12 hr. The hydrolysate was analyzed for quinidine and metabolites as described previously. This method was at several instances compared to results obtained by hydrolysis with 0.1 ml of sulfatase[8] (2970 U/ml), containing 92,000 Fishman U/ml β-glucuronidase activity, and with incubation of 1 ml of denaturated β-glucuronidase (denatured by heating over steam bath for 5 min). Furthermore, the results were also compared to results from urine that had been refluxed with 2 *N* HCl at 100° for 1 hr. As the other two modes of hydrolysis did not give different results from β-glucuronidase, only the β-glucuronidase hydrolysis results are reported here. Incubation with denatured β-glucuronidase gave no significant alteration of the original urine concentration of quinidine and its metabolites. Standards made from spiked urine were treated identically to that described previously.

Pharmacokinetic Analysis—An open two-compartment model with a short-term, zero-order input was found suitable to describe the observed total and unbound quindine plasma concentration with time in all animals studied.

The metabolite concentrations could adequately be described by an apparent one-compartment model with a first-order rate of formation. The values of the rate constants were obtained using a fitting procedure (9) available through a computer system (10). Plasma concentrations were weighted by their inverse value squared. The clearance was determined using the dose and area under the plasma concentration–time curve, and the apparent volume of distribution at steady state was obtained by the area under the moment curve as described previously (11).

RESULTS

The log-averaged plasma concentrations of total and unbound quinidine in the eight animals as a function of time are given in Fig. 1. The various pharmacokinetic parameters for total and unchanged drug are given in Table I.

From three to six metabolites could be detected in plasma of the different rabbits. Only in rabbit No. 5 could six different metabolites be distinguished (Fig. 2). Five of these metabolites represent known metabolites [quinidine-10,11-dihydrodiol (8), 3-hydroxyquinidine (5, 12), 6′-desmethylquinidine (7), quinidine-*N*-oxide (6), 2′-quinidinone (13)]. Metabolite number 4 is unknown. Rate constants for the various metabolites are given in Table II.

Urine was found to contain at least eight metabolites of quinidine, of which five could be associated with known metabolic products (5–8, 12, 13). In Fig. 3 a chromatogram from urine of rabbit No. 1 is presented. Metabolite number 1, quinidine-10,11-dihydrodiol, appears to have a shoulder on many of the chromatograms, possibly indicative of the two diastereoisomers of this compound. In addition to the metabolites shown, the 6′-desmethylquinidine peak substantially increased upon hydrolysis of urine with β-glucuronidase, indicative of a 6′-desmethylquinidine conjugate (probably a glucuronide).

The fraction of the dose recovered as unchanged quinidine in urine ranged between 0.001 and 0.010. The fraction of the dose recovered as known quinidine metabolites, including the 6′-desmethylquinidine conjugate, ranged between 0.110 and 0.202 for the six rabbits where the urine collection was complete (Table III).

The blood to plasma ratio of quinidine in the rabbits was 1.05 ± 0.08, which is not statistically significantly different from 1.

The indocyanine green clearance, indicative of liver blood flow, was found to be 58 ± 12 ml/mg/kg.

The unbound fraction of quinidine in plasma varied between 0.06 and 0.23 in the eight rabbits (Table I). Of the known quinidine metabolites, only quinidine-10,11-dihydrodiol and 3-hydroxyquinidine could be evaluated for their binding to plasma protein. The unbound fraction in the eight rabbits varied between 0.26 and 0.46, with an average value of 0.34 and a *SD* of 0.06 for quinidine-10,11-dihydrodiol. In six of the rabbits, where 3-hydroxyquinidine was detected, the unbound fraction for this metabolite ranged between 0.16 and 0.32, with an average value of 0.25 and a *SD* of 0.06. The unknown metabolite (4), showed negligible binding, with an average ±*SD* of 0.74 ± 0.09. Of these metabolites, only 3-hydroxyquinidine showed a statistically significant correlation with the binding of quinidine ($r^2 = 0.84$, $p < 0.01$).

DISCUSSION

The disposition of both total and unbound quinidine in the rabbit can be described by a two-compartment model. Statistically, the fast disposition half-lives for total and unbound drug were not significantly different from each other and were found to be 5.8 and 3.1 min, respectively, indicating fast distribution. The slow disposition half-life for total drug at 67 min was not significantly different from that reported for unbound drug, 58 min ($0.10 > p > 0.05$). However, in two of the animals, No. 1 and 8, the terminal slope and half-life for total drug was significantly different from that of the unbound drug. This suggests that the rate constants of total and unbound drug for individual animals cannot be assumed to be interchangeable, even though the average population values show no differences. The observed half-life is much longer than the average half-life of 29 min previously reported in rabbits (1). However, these studies were carried out for only 50 min after an intravenous injection and for 60 min after the termination of an infusion over 90 min. This short collection time and the use of Cramer and Isaksson's assay method for quinidine (14), which has been shown to be nonspecific for quinidine (15), could be contributing factors to this discrepancy. Ueda and Nichols (16) found a slow disposition half-life for total drug that was much longer than that of this study. In a 3-hr study they observed a terminal half-life of 132 min, almost twice the value in this study. Except for using much larger and presumably older animals than those in this study, no significant differences in the experimental protocol can be found to explain this difference. Similarly, Ueda and Nichols also reported a much smaller clearance of quinidine than that reported in this study (16.8 ml/min/kg *versus* 52 ml/min/kg).

The fraction of quinidine excreted unchanged in the urine is very low (Table III). Provided the extrarenal clearance is predominantly hepatic, quinidine shows a high extraction ratio in the rabbit liver. The metabolic clearance of quinidine (52 ml/min/kg) approaches the value of hepatic blood flow (58 ml/min/kg). According to theoretical considerations, the total drug clearance of quinidine in this case should be independent of

[14] Hitachi Model 110-30, Altex Scientific, Berkeley, Calif.

Table III—Fraction of Administered Dose of Quinidine Recovered in the Urine of Rabbits after 4 Hr

Rabbit Number	Quinidine	Quinidine 10,11-dihydrodiol	2′-Quinidinone	3-Hydroxyquinidine	6-Desmethylquinidine	6-Desmethylquinidine conjugate	Quinidine-*N*-oxide	Unknown[a] metabolites
1	0.005	0.064	0.005	0.002	0.003	0.030	0.001	0.007
2	0.009	0.077	0.012	0.005	0.005	0.039	0.002	0.010
3	0.010	0.114	0.014	0.006	0.007	0.050	0.001	0.009
4	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]
5	0.001	0.084	0.009	0.003	0.004	0.015	[c]	0.012
6	0.001	0.076	0.008	0.003	0.005	0.022	0.002	0.007
7	[b]	[b]	[b]	[b]	[b]	[b]	[b]	[b]
8	0.004	0.082	0.008	0.008	0.005	0.028	0.001	0.011
Mean	0.005	0.083	0.009	0.005	0.005	0.031	0.001	0.009
SD	0.004	0.017	0.003	0.002	0.001	0.012	0.001	0.002

[a] The sum of the two unknown metabolites (Fig. 3), assuming the metabolites have the same extractability and spectral characteristics as quinidine. [b] Incomplete urine collection. [c] Not detected.

the unbound fraction, and the unbound clearance inversely related to the unbound fraction (17). In accordance with this principle, the total clearance showed no statistically significant correlation with the unbound fraction ($p > 0.70$). The unbound clearance was strongly correlated with the inverse of the unbound fraction of quinidine in plasma ($p < 0.001$). These data confirm and support the previous observation in rabbits where the binding was altered by a bleeding procedure (18).

The apparent steady-state volume of distribution for total drug in this study, 4.2 liters/kg, was similar to the value calculated from the study of Ueda and Nichols (16). It showed no correlation with the unbound fraction in plasma ($p > 0.20$). The unbound apparent steady-state volume of distribution, however, showed a negative correlation with the unbound fraction ($p < 0.005$). This is the opposite to what could theoretically be expected for a compound with a large apparent volume of distribution (19) and is indicative of a correlation between tissue and plasma protein binding in individual animals.

Using the assay method described, the total recovery of the six identifiable metabolites and quinidine in the urine accounts for only 14% of the administered dose. Other primary or secondary metabolites must, therefore, account for the elimination of quinidine. However, only one conjugate of quinidine metabolites could be identified *via* enzymatic or acid hydrolysis of the urine.

Based on the fast elimination of unchanged quinidine in the rabbit, urine was collected for only 4 hr in this study. The terminal half-life of five of the metabolites in plasma was ~1 hr, suggesting that most of these compounds were eliminated in the collection period. The terminal half-lives of these metabolites are similar to that of quinidine, indicating that their formation is the rate-limiting step in their pharmacokinetic profile.

Accumulation of these metabolites after multiple doses will, therefore, only occur to a small degree. The sixth metabolite, 2′-quinidinone, exhibited a much slower elimination, with an apparent terminal half-life of 8 hr. The urinary recovery of this metabolite, with a study period of only 4 hr, therefore, was seriously underestimated and could possibly contribute to the overall low recovery of quinidine and metabolites in the urine. Because the observed half-life is greater than the study period, there is also little confidence in the estimated value of the half-life.

The protein binding of quinidine is variable; an unbound fraction varying from 0.06 to 0.23 was observed. Although two of the eight animals showed a statistically significant positive correlation between concentration and unbound fraction of quinidine in plasma, there was no difference in the terminal half-life between the total and unbound drug, suggesting that concentration-dependent binding is not a general phenomenon.

Because quinidine metabolites reportedly contribute to the pharmacologic activity (20), it may be of importance to note that all the metabolites for which protein binding was measured exhibited a lower binding than the parent compound. Of these metabolites for which binding could be evaluated, 3-hydroxyquinidine was the only one to show a statistically significant correlation with quinidine binding. This might suggest a common binding site and possible mutual displacement at high concentration.

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