The Liposome Partitioning System for Correlating Biological Activities of Imidazolidine Derivatives

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The partitioning of 10 imidazolidines in various liposome/buffer systems ($\log K'_m$) has been determined and compared to partitioning in the *n*-octanol/buffer system ($\log P'$). The $\log K'_m$, which was generally greater than the $\log P'$, increased or decreased upon the addition of dicetylphosphate (DCP) or stearylamine (STA), respectively, to dimyristoylphosphatidylcholine (DMPC) liposomes. Quantitative correlations of α_2-adrenergic potencies of imidazolidines have been made by regression analyses with $\log P'$, $\log K'_m$, binding affinity, and intrinsic activity. Both central and peripheral potencies correlated with $\log K'_m$ but not with $\log P'$. Multiple regressions yielded improved predictable quantification of these potencies. Thus, the liposomal membrane system shows certain advantages over the *n*-octanol/buffer system for the prediction of biological activities of the imidazolidines.

KEY WORDS: partitioning; liposomes; *n*-octanol/buffer system; imidazolidines; correlation analysis; quantitative structure–activity relationship (QSAR).

INTRODUCTION

The biological activities of weak electrolytes have been successfully quantified using the extrathermodynamic property of partitioning in organic solvent or lipid phases. Among these the *n*-octanol–water system has been predominantly advocated as more closely representing the distribution process in biological membranes. However, attention has recently been turned to the liposomal membrane as a possible better model since it possesses an ordered molecular arrangement, structural characteristics varying from a liquid crystalline state to a rigid, gel state, and having the option of being able to exert electrostatic influences, in contrast to the bulk oil/water system (1–9). Liposomes of various compositions have been shown to provide better correlations of membrane transport of drugs, pharmacokinetic behavior, and biological activities of certain classes of drugs than the *n*-octanol/buffer system (10–15). These data serve to support arguments that the partitioning of solutes into phospholipid membranes is fundamentally different, because of their ordered structures, from their partitioning into a bulk oil phase.

The α_2-adrenergic potency of imidazolidine derivatives has been described quantitatively in terms of receptor affinity and intrinsic activity as well as *n*-octanol/buffer partition coefficients (16). Hence, these results have been extended in the present study to compare the suitability of the *n*-octanol/buffer system versus the liposome system as a model membrane in quantitative structure–activity relationship (QSAR) studies of imidazolidine derivatives. Accordingly, improved quantitative assessments of their biological activities have been proposed.

MATERIALS AND METHODS

Materials

The chemical identities of the imidazolidine derivatives used in this study are shown in Table I. All compounds were obtained from Boehringer Ingelheim (Canada) Ltd. and used as received and included ST 476 hydrobromide, ST 475 hydrochloride, ST 2100 hydrobromide, ST 603 hydrochloride, ST 608 hydrochloride, STH 2224 hydrobromide, ST 600 hydrochloride, ST 585 hydrochloride, ST 606 hydrochloride, and ST 590 hydrochloride. L-α-Dimyristoylphosphatidylcholine (DMPC), cholesterol (CHOL), dicetylphosphate (DCP), and stearylamine (STA) were used as received from Sigma Chemical Co. (St. Louis, MO). All other solvents and chemicals were reagent grade.

Determination of Partition Coefficients

Partition coefficients of the imidazolidines in *n*-octanol/buffer solution (P'), pH 7.4 and 37°C, have been published (16). Apparent equilibrium partition coefficients in liposomes (K'_m) were determined by a method described previously (6,15). Briefly, rotary-evaporated films of phospholipids in round-bottom flasks were hydrated and hand-dispersed in aqueous, pH 7.4, phosphate buffer solution containing 1 mmol of drug then vortex-mixed for 5 min to form multilamellar liposomes (MLVs) at a concentration of 10 mg/mL. The MLVs were equilibrated at 37°C for 5 hr in a shaking water bath (Dubnoff metabolic shaking incubator, Precision Scientific), then centrifuged (Beckman Model L8-55 ultracentrifuge; 143,000*g*, 30 min, 37°C). The supernatants were analyzed by UV spectroscopy (Beckman Model 25 spectrophotometer) and calculations of K'_m were made as before (6). Averages of duplicate determinations are reported and repeated analyses of stock solutions confirmed the stability of the drugs under the experimental conditions.

RESULTS AND DISCUSSION

Correlations Between *n*-Octanol/Buffer and Liposome Partitioning

Experimentally determined $\log K'_m$ values using various liposome compositions and $\log P'$ values obtained from the literature are shown for comparison in Table I. As can be seen, $\log K'_m$ values are greater than $\log P'$ values in all cases, which is consistent with previous observations (6,9,15). The $\log K'_m$ yielded a poor correlation with the $\log P'$, but reasonable correlations were obtained for $\log K'_m$ results in different liposome compositions (Table II). Others (9,17,18) have reported that partitioning of solutes, particularly ionizable solutes, can be markedly influenced when a

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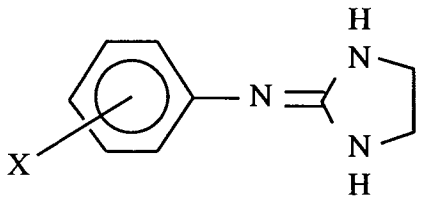
Table I. Apparent Partition Coefficients of Imidazolidine Derivatives in *n*-Octanol/Buffer System (P') and Liposome Systems (K'_m)



Compound No.	X	logP'[a]	logK'_m[b] (1)	(2)	(3)
ST 476	2, 3-di-Cl	0.85	1.56 ± 0.02	2.28 ± 0.01	1.35 ± 0.01
ST 475	2, 5-di-Cl	1.00	1.30 ± 0.01	1.86 ± 0.01	1.09 ± 0.03
STH 2100	2-Cl, 3-Br	1.04	1.74 ± 0.01	2.40 ± 0.01	1.48 ± 0.03
ST 603	2-Cl, 5-Br	1.21	1.28 ± 0.01	1.92 ± 0.01	0.98 ± 0.01
ST 608	2-Cl, 3-CH_3	−0.13	1.37 ± 0.03	2.08 ± 0.01	1.06 ± 0.01
STH 2224	2, 3-di-Br	1.14	1.79 ± 0.01	2.48 ± 0.02	1.45 ± 0.02
ST 600	2-CH_3, 5-F	−0.77	1.20 ± 0.03	1.83 ± 0.03	0.93 ± 0.01
ST 585	2-CH_3, 5-Cl	−0.27	1.39 ± 0.02	2.10 ± 0.02	1.07 ± 0.01
ST 606	2-CH_3, 3-Br	−0.02	1.64 ± 0.01	2.38 ± 0.01	1.41 ± 0.02
ST 590	2-CH_3, 5-Br	−0.25	1.38 ± 0.01	2.01 ± 0.03	0.99 ± 0.01

[a] From Ref. 16.

[b] Means ±SE (n = 3); numbers in parentheses represent the following liposome compositions: (1) DMPC; (2) DMPC/CHOL/DCP, 7:1:2 mol ratio; (3) DMPC/STA, 3:1 mol ratio.

net charge is located on the liposome surfaces. The addition of DCP to DMPC liposomes increased, whereas the addition of STA decreased the partitioning of the imidazolidines, because being weak bases, they are positively charged at the experimental pH. The rank order of logK'_m in liposomes is negatively charged > neutral > positively charged.

Correlation of logK'_m and logP' with Imidazolidine Intrinsic Activity and Receptor Affinity

The intrinsic activities and binding affinities of the α_2-adrenoceptor imidazolidines used in this study are listed in Table III (16). The results of regression analyses of these properties and logP' or logK'_m are presented in Table IV. The intrinsic activities of the imidazolidines and partition coefficients in the *n*-octanol/buffer or liposome systems did not yield acceptable correlations. On the other hand, the *in vitro* binding affinities correlated with logK'_m ($P < 0.05$) but not logP'. In this regard, the interaction between the imidazolidines and the α_2-adrenoceptors *in vitro* has been reported to be highly dependent on hydrophobic factors (16,19). Therefore, these results indicate that the liposome partitioning system might be a better predictor for the receptor–drug interactions than the *n*-octanol/buffer system, especially when taking lipophilicity into account.

Correlation of Central Hypotensive Activities of Imidazolidines with Their Partition Coefficients

Regression analyses of central hypotensive activities (pC_{20}) with apparent partition coefficients, intrinsic activities, and binding affinities are described in Table V. In the first instance, logK'_m in all liposome compositions correlated quite well with pC_{20} using univariant regression analysis. Among these, however, negatively charged liposomes yielded the highest correlation coefficient [Eq. (17)]. In comparison, the lowest correlation coefficients were generated using logP' applying either simple regression [Eq. (15a)] or second-order regression [Eq. (15b)] analysis, a finding that is

Table II. Correlations Between Apparent Partition Coefficients of Imidazolidines

	Equation	R/df/F/P[a]
(1)	logK'_m(1) = 1.50 + 0.26logP' − 0.22(logP')^2	0.561/7/1.61/0.266
(2)	logK'_m(2) = 2.21 + 0.30logP' − 0.30(logP')^2	0.533/7/1.39/0.311
(3)	logK'_m(3) = 1.22 + 0.29logP' − 0.23(logP')^2	0.603/7/2.00/0.206
(4)	logK'_m(1) = −0.34 + 0.84logK'_m(2)	0.980/8/192./0.0001
(5)	logK'_m(1) = 0.39 + 0.91logK'_m(3)	0.962/8/99.4/0.0001
(6)	logK'_m(2) = 0.91 + 1.03logK'_m(3)	0.940/8/60.8/0.0001

[a] Statistics generated by MacIntosh Statview 512. R, correlation coefficient; df, residual degrees of freedom; F, F-test value; P, probability.

Table III. Intrinsic Activity (pA_i), Binding Affinity (pK_i), Central Hypotensive Activity (pC_{20}), and Peripheral Bradycardic Activity (pD_2) of Imidazolidines[a]

Drug	pA_i	pK_i	pC_{20}	pD_2
ST 476	0.05	8.21	7.39	8.20
ST 475	0.43	7.79	5.95	7.61
STH 2100	0.09	8.71	7.64	8.42
ST 603	0.96	7.78	5.59	7.23
ST 608	0.05	8.42	6.70	8.07
STH 2224	0.16	8.70	7.78	8.14
ST 600	0.23	7.83	5.77	7.64
ST 585	0.23	7.92	6.34	7.65
ST 606	0.17	8.51	7.35	8.39
ST 590	0.19	8.27	6.14	7.47

[a] Detailed descriptions are given in Ref. 16. pA_i = −log(ia), where ia represents the intrinsic activity (maximal percentage inhibition) of the stimulation-induced increase in heart rate in pithed rats. K_i, $IC_{50}/(1 + 0.4/K_d)$, where IC_{50} was determined *in vitro* to inhibit the specific ^{3}H-clonidine binding to rat brain membrane by 50% and K_d was 3.6 n*M*. C_{20} represents the dose (mol/kg) to cause a 20% reduction in mean arterial pressure following i.v. administration to anesthetized normotensive rats. D_2 represents the dose (mol/kg) to elicit 50% of the maximal inhibition of the stimulation-induced increase in heart rate in pithed rats.

in accordance with previous partitioning analysis and α-adrenoceptor agonist activities (15). Table V also shows that the binding affinities of the imidazolidines were better predictors of hypotensive activities than their intrinsic activities [Eqs. (19) and (20)]. Again, this difference between these parameters might have a lipophilicity dependence as mentioned above.

Improved correlation equations were obtained by multiple regression analyses. Correlation coefficients were improved by the incorporation of pA_i to the regressions as a second variable to either log*P*′ or logK'_m [Eqs. (21)–(24)]. However, incorporation of pK_i as a third variable did not improve the correlations in liposome systems [Eqs. (27)–(29)] since aspects of pK_i are implicit in logK'_m. In comparison, the correlation coefficient for the *n*-octanol/buffer system was increased after incorporation of pK_i [Eq. (26)] since log*P*′ correlated poorly with pK_i [Eq. (11)]. Moreover, the multiple regression which excluded partition coefficients yielded a lower correlation coefficient [Eq. (25)] than that obtained by equations including either log*P*′ or logK'_m. Therefore, it follows that lipophilicity is an important factor in central α_2-adrenoceptor action. Also, all liposome systems were superior to the *n*-octanol/buffer system in correlations of the central hypotensive activities.

Correlation of Peripheral Bradycardic Activity of Imidazolidines with Their Partition Coefficients

Developing the regression analyses as above, again liposome systems provided better correlations with bradycardic activities (pD_2) than the *n*-octanol/buffer system. However, the correlation coefficients were lower in magnitude than those obtained using pC_{20}. On the other hand, positively charged liposomes yielded a slightly better correlation coefficient than either neutral or negatively charged liposomes [Eqs. (31)–(33); Table VI], a result which was not seen in the correlation of pC_{20}. Similar patterns of dependencies of liposome behavior on electrical surface charge have also been observed in earlier work (15). There was less of a difference between pA_i and pK_i for the correlation of pD_2, compared to the correlation of pC_{20}, although pK_i gave a somewhat higher value of the correlation coefficient than pA_i [Eqs. (34) and (35)].

Multiple regressions also yielded better correlations of pD_2. Thus, incorporation of pA_i in the regressions increased the correlation coefficients [Eqs. (36)–(39)], whereas the additional incorporation of pK_i did not have a significant influence [Eqs. (41)–(44)]. Also, a regression which did not include the partition coefficients yielded a lower correlation coefficient [Eq. (40)] than those which included either log*P*′ or logK'_m. Again, positively charged liposomes produced a better correlation of pD_2 than other liposome compositions or the *n*-octanol/buffer system. Therefore, a tentative conclusion is that the peripheral α_2-adrenoceptor action requires not only a lipophilic factor, but also electrostatic interaction at receptor sites.

Plots of values of pC_{20} and pD_2 calculated from Eqs. (23) and (39), respectively, against reported values have indicated the reliability of the correlations since most of the data points fell within the 95% confidence bands of the true values (Figs. 1 and 2). Although the activity of the agonist could be estimated with reasonable certainty only if both intrinsic activity and binding affinity were known (16), the best estimations were obtained when logK'_m were used instead of the binding affinity. Using univariant regressions of both pC_{20} and pD_2, the best correlations were found with logK'_m rather than with log*P*′, pA_i, or pK_i. It may be concluded, therefore, that the liposome system provides a rea-

Table IV. Correlations of Apparent Partition Coefficients with Intrinsic Activity and Receptor Affinity of Imidazolidines

Equation	*R*/df/*F*/*P*
(7) $pA_i = 0.07 - 0.09\log P' + 0.34(\log P')^2$	0.542/7/1.46/0.296
(8) $pA_i = 5.46 - 6.41\log K'_m(1) + 1.92\,[\log K'_m(1)]^2$	0.523/7/1.32/0.327
(9) $pA_i = 7.76 - 6.46\log K'_m(2) + 1.36\,[\log K'_m(2)]^2$	0.554/7/1.55/0.278
(10) $pA_i = 2.90 - 3.90\log K'_m(3) + 1.37\,[\log K'_m(3)]^2$	0.472/7/1.00/0.414
(11) $pK_i = 8.36 + 0.38\log P' - 0.45(\log P')^2$	0.436/7/0.819/0.479
(12) $pK_i = 5.88 + 1.59\log K'_m(1)$	0.882/8/28.0/0.0007
(13) $pK_i = 5.30 + 1.37\log K'_m(2)$	0.880/8/27.5/0.0008
(14) $pK_i = 6.62 + 1.35\log K'_m(3)$	0.793/8/13.5/0.006

Table V. Correlation Testing of the Central Hypotensive Activity (pC_{20}) of Imidazolidines as a Function of Various Parameters

	Equation	R/df/F/P
Univariant regression: pC_{20} =		
(15a)	$6.53 + 0.37\log P'$	0.327/8/0.961/0.356
(15b)	$6.94 + 1.04\log P' - 1.05\log(\log P')^2$	0.527/7/1.35/0.320
(16)	$1.04 + 3.84\log K'_m(1)$	0.957/8/86.8/0.0001
(17)	$-0.48 + 3.35\log K'_m(2)$	0.969/8/122./0.0001
(18)	$2.40 + 3.61\log K'_m(3)$	0.950/8/73.6/0.0001
(19)	$7.17 - 1.97\mathrm{p}A_i$	0.650/8/5.87/0.042
(20)	$-9.61 + 1.98\mathrm{p}K_i$	0.890/8/30.3/0.0006
Multiple regression: pC_{20} =		
(21)	$7.07 + 0.72\log P' - 2.66\mathrm{p}A_i$	0.884/7/12.6/0.005
(22)	$1.94 + 3.36\log K'_m(1) - 0.76\mathrm{p}A_i$	0.982/7/96.8/0.0001
(23)	$0.45 + 2.99\log K'_m(2) - 0.62\mathrm{p}A_i$	0.984/7/109./0.0001
(24)	$3.17 + 3.14\log K'_m(3) - 0.83\mathrm{p}A_i$	0.980/7/86.2/0.0001
(25)	$-7.73 + 1.77\mathrm{p}K_i - 0.47\mathrm{p}A_i$	0.898/7/14.5/0.003
(26)	$-2.83 + 0.44\log P' - 1.39\mathrm{p}A_i + 1.18\mathrm{p}K_i$	0.948/6/17.9/0.002
(27)	$2.50 + 3.48\log K'_m(1) - 0.80\mathrm{p}A_i - 0.09\mathrm{p}K_i$	0.983/6/55.7/0.0001
(28)	$0.29 + 2.95\log K'_m(2) - 0.61\mathrm{p}A_i + 0.03\mathrm{p}K_i$	0.984/6/62.3/0.0001
(29)	$-0.41 + 2.58\log K'_m(3) - 0.60\mathrm{p}A_i + 0.51\mathrm{p}K_i$	0.988/6/80.6/0.0001

Table VI. Correlations of the Peripheral Bradycardic Activity (pD_2) of Imidazolidines as a Function of Various Parameters

	Equation	R/df/F/P
Univariant regression: pD_2 =		
(30a)	$7.85 + 0.08\log P'$	0.139/8/0.158/0.701
(30b)	$8.06 + 0.42\log P' - 0.54\log(\log P')^2$	0.446/7/0.867/0.461
(31)	$5.48 + 1.64\log K'_m(1)$	0.812/8/15.5/0.004
(32)	$4.75 + 1.47\log K'_m(2)$	0.842/8/19.4/0.002
(33)	$5.91 + 1.67\log K'_m(3)$	0.873/8/25.7/0.001
(34)	$8.16 - 1.10\mathrm{p}A_i$	0.725/8/8.84/0.018
(35)	$0.35 + 0.92\mathrm{p}K_i$	0.818/8/16.1/0.004
Multiple regression: pD_2 =		
(36)	$8.13 + 0.26\log P' - 1.35\mathrm{p}A_i$	0.841/7/8.43/0.014
(37)	$6.26 + 1.22\log K'_m(1) - 0.67\mathrm{p}A_i$	0.899/7/14.7/0.003
(38)	$5.67 + 1.11\log K'_m(2) - 0.60\mathrm{p}A_i$	0.907/7/16.2/0.002
(39)	$6.49 + 1.31\log K'_m(3) - 0.63\mathrm{p}A_i$	0.947/7/30.2/0.0004
(40)	$2.50 + 0.67\mathrm{p}K_i - 0.53\mathrm{p}A_i$	0.862/7/10.1/0.009
(41)	$4.11 + 0.14\log P' - 0.84\mathrm{p}A_i + 0.48\mathrm{p}K_i$	0.885/6/7.20/0.021
(42)	$5.81 + 1.12\log K'_m(1) - 0.64\mathrm{p}A_i + 0.07\mathrm{p}K_i$	0.899/6/8.44/0.014
(43)	$5.34 + 1.04\log K'_m(2) - 0.58\mathrm{p}A_i + 0.06\mathrm{p}K_i$	0.907/6/9.29/0.011
(44)	$6.02 + 1.24\log K'_m(3) - 0.60\mathrm{p}A_i + 0.07\mathrm{p}K_i$	0.947/6/17.4/0.002

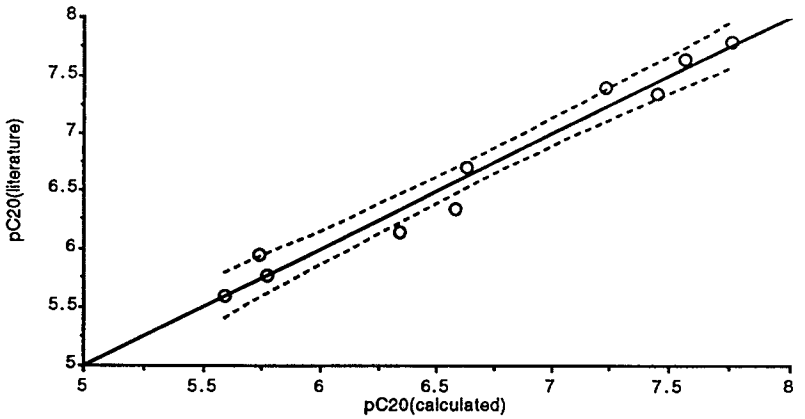


Fig. 1. Plot of central hypotensive activity (pC_{20}) versus calculated pC_{20} from Eq. (23) (r^2 = 0.969). The dashed lines represent 95% confidence bands for the pC_{20} (literature) values.

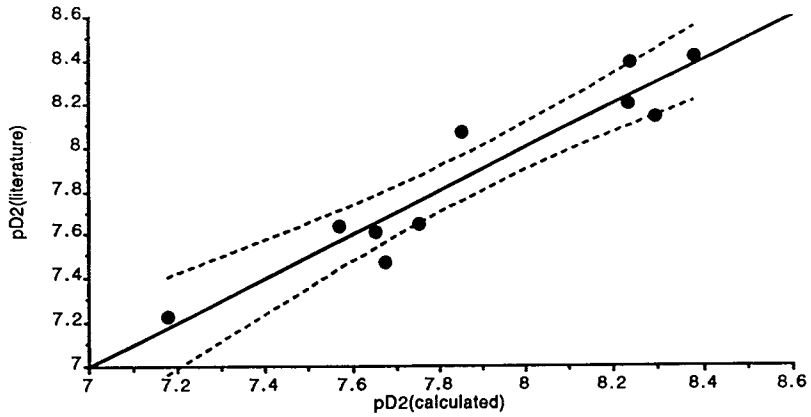


Fig. 2. Plot of peripheral bradycardic activity (pD_2) versus calculated pD_2 from Eq. (39) (r^2 = 0.896). The dashed lines represent 95% confidence bands for the pD_2 (calculated) values.

sonably good model membrane, especially for the prediction of α_2-adrenoceptor potency of the imidazolidine derivatives.

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