Department of Pharmaceutics[1], Hokkaido College of Pharmacy, Hokkaido, Japan; Department of Pharmaceutics[2], Shenyang Pharmaceutical University, Shenyang, China

Evaluating interactions of amphoteric molecules with phospholipid membrane using immobilized artificial membrane chromatography

J. Sun[1, 2], Y. Deguchi[1], J. Chen[2], R. Zhang[2] and K. Morimoto[1]

It has been demonstrated that bases exhibit a surprisingly higher membrane partitioning than acids and neutrals expected from their hydrophobicity and possess an attractive polar extra-interaction with ordered phospholipid membrane [1, 2]. Furthermore, pharmacokinetic results have shown that bases have higher volumes of distribution than acids and neutrals with comparable hydrophobicity [3]. However, amphoteric molecules have been neglected in the previous studies and their partitioning behavior into membrane has not been characterized in detail. Therefore, in the present study, we selected a test set consisting of 9 amphoteric, 5 basic and 13 acidic/neutral solutes, and investigated their interactions with membrane using immobilized artificial membrane (IAM) chromatography, expressed as membrane affinity, $\log k_{IAM}$. An *n*-octanol/buffer system was also employed as the reference hydrophobicity, defined as $\log D_{O/B,\,7.4}$.

The results were shown in the Table. We explored the relationship between $\log k_{IAM}$ and $\log D_{O/B,\,7.4}$ and observed a reasonable correlation between $\log D_{O/B,\,7.4}$ and $\log k_{IAM}$ values for 27 structurally diverse solutes (Fig.):

$$\log k_{IAM} = 0.44\ (\pm 0.07)\ \log D_{O/B,\,7.4} + 0.61\ (\pm 0.11) \qquad (1)$$

$$n = 27 \qquad r^2 = 0.62 \qquad s = 0.51$$

In this and following equations, n denotes the number of solutes contained in the regression equation; r^2 and s represent squared correlation coefficient and standard error of the estimate, respectively. Numbers in parentheses account for the standard error of regression coefficients.

Based on this correlation, the binding to IAM surface was to the larger extent contributable to hydrophobicity. However, the low r^2 and high s values indicating a much scattered $\log k_{IAM}$ population (Fig.), implied that not only hydrophobicity but also other driving forces ascribed to partitioning of solutes into membrane, and that the lipophilicity measurement scale of IAM chromatography was distinct from that of the *n*-octanol/buffer system.

As shown in the Fig., most of the bases and ampholytes were above the regression line of the total, while acids and neutrals were below the line. It was suggested that the set was composed of two separated subgroups, which behaved dissimilarly during partitioning into IAM membrane. Depending on their chemical structure, we accordingly classified all solutes into two categories, namely type I consisting of acidic and neutral solutes, and type II consisting of basic and amphoteric solutes. The more significant relationship between $\log D_{O/B,\,7.4}$ and $\log k_{IAM}$ for each subset was established, respectively (Fig.).

I type:

$$\log k_{IAM} = 0.57\ (\pm 0.06)\ \log D_{O/B,\,7.4} + 0.06\ (\pm 0.10) \qquad (2)$$

$$n = 13 \qquad r^2 = 0.89 \qquad s = 0.28$$

II type:

$$\log k_{IAM} = 0.51\ (\pm 0.05)\ \log D_{O/B,\,7.4} + 1.00\ (\pm 0.07) \qquad (3)$$

$$n = 14 \qquad r^2 = 0.89 \qquad s = 0.24$$

In the case of r^2 and s values, the above equations for separated subsets were notably improved in regression quality, showing no scattered $\log k_{IAM}$ population. Log $D_{O/B,\,7.4}$ accounted for 62% variation of $\log k_{IAM}$ for all solutes by virtue of eq. (1), while $\log D_{O/B,\,7.4}$ could explain 89% variation of $\log k_{IAM}$ for type I and type II solutes according to eq. (2) and (3), respectively. These conclusions seemed to be contradictive. In fact, if the driving forces excluding hydrophobic force, such as either attractive or repulsive polar extra-interactions with phospholipid, occurred in a constant extent for each class, these potential interactions could not be reflected by the above regression equations. Consequently, although the correlations of $\log D_{O/B,\,7.4}$ and $\log k_{IAM}$ were quite significant for separated subgroups, it was not allowed to conclude that membrane interactions with the solutes were uniquely hydrophobicity-based.

The comparison of eqs. (2) and (3) indicated that both had a comparable slope but an intercept of type I was 0.94 logarithm unit lower than that of type II. This illustrated that type II molecules generally displayed the higher membrane affinity than type I with comparable $\log D_{O/B,\,7.4}$. In order to further elucidate the polar extra-interaction with phospholipid for molecules of differing types, the relationship between $\log k_{IAM}$ and $\log D_{O/B,\,7.4}$ of the type I subset of a mainly hydrophobicity-based interaction mechanism, was used to calculate the values of $\log k_{IAM}$ expected from $\log D_{O/B,\,7.4}$. These values were subtracted from $\log k_{IAM}$ experimentally determined and differences

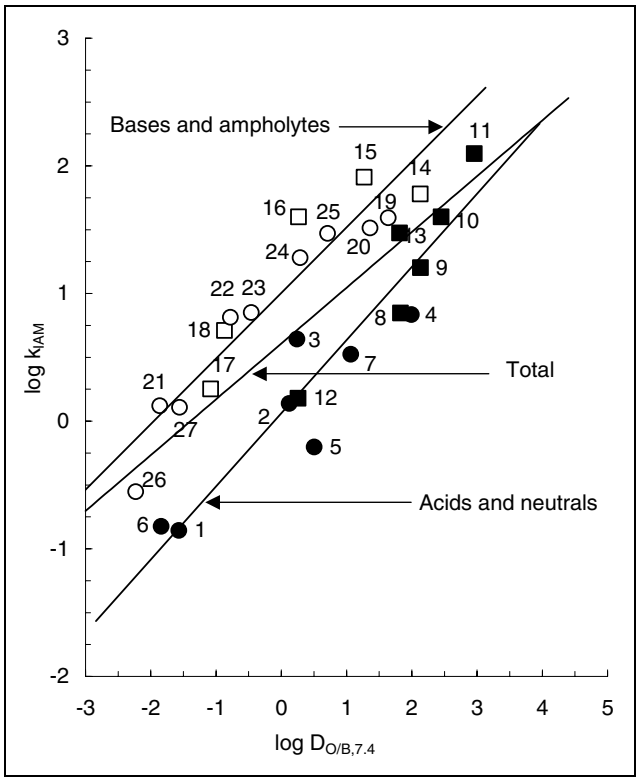

Fig.: Correlations between IAM chromatographic indexes ($\log k_{IAM}$) and hydrophobicity ($\log D_{O/B,\,7.4}$) of *n*-octanol/ buffer system for 27 structurally diverse solutes. Solid lines represent the regression lines of selected groups.
● (acid), ○ (ampholyte), ■ (neutral) and □ (base).

Table: Physicochemical parameters for 27 solutes

Drugs	log k_{IAM} [a]	log $D_{O/B,7.4}$ [b]	Δ log k_{IAM} [c]	Nature of drug	No.
p-Hydroxybenzoic acid	−0.86	−1.57	−0.02	Acidic	1
Nalidixic acid	0.14	0.12	0.01	Acidic	2
Oxolinic acid	0.64	0.24	0.44	Acidic	3
Pentobarbital	0.83	2.00	−0.37	Acidic	4
Barbital	−0.20	0.50	−0.55	Acidic	5
p-Aminosalicylic acid	−0.82	−1.84	0.17	Acidic	6
p-Nitrophenol	0.52	1.06	−0.15	Acidic	7
p-Hydroxybenzoic methyl ester	0.85	1.83	−0.26	Neutral	8
p-Hydroxybenzoic ethyl ester	1.20	2.13	−0.08	Neutral	9
p-Hydroxybenzoic propyl ester	1.60	2.45	0.14	Neutral	10
p-Hydroxybenzoic butyl ester	2.10	2.96	0.34	Neutral	11
Ampyrone	0.18	0.26	−0.03	Neutral	12
Dexamethason	1.48	1.81	0.37	Neutral	13
Quinidine	1.78	2.13	0.50	Basic	14
Propranolol	1.91	1.27	1.12	Basic	15
Erythromycin	1.60	0.26	1.39	Basic	16
Streptomycin	0.25	−1.08	0.81	Basic	17
Procainamide	0.71	−0.87	1.15	Basic	18
OPC−17203 [d]	1.59	1.64	0.59	Amphoteric	19
NR−762	1.51	1.36	0.67	Amphoteric	20
Pipemidic acid	0.12	−1.86	1.12	Amphoteric	21
Ciprofloxacin	0.81	−0.78	1.20	Amphoteric	22
Levofloxacin	0.85	−0.46	1.05	Amphoteric	23
HSR−903	1.28	0.29	1.05	Amphoteric	24
Grepafloxacin	1.47	0.71	1.00	Amphoteric	25
Amoxicillin	−0.55	−2.23	0.66	Amphoteric	26
Penicillin G	0.11	−1.56	0.94	Amphoteric	27

[a] log k_{IAM} represents IAM chromatographic index determined at pH = 7.4 phosphate buffered saline.
[b] log $D_{O/B,7.4}$ represents the apparent distribution coefficient in an *n*-octanol/buffer system at pH = 7.4 phosphate buffered saline.
[c] Δ log k_{IAM} quantifies the magnitude of polar extra-interaction with phospholipid.
[d] chemical structure is shown in [7].

(Δ log k_{IAM}) were assumed to quantify the magnitude of additive polar extra-interaction (hydrogen bond and electrostatic interaction). Δ log k_{IAM} values were by far greater for bases and ampholytes of type II ranging from 0.50 to 1.39, than those for acids and neutrals of type I with the scope from −0.55 to 0.44 (Table). These results again stressed that ampholytes showed much higher than the expected membrane affinity, behaving as alike as bases. Barbato et al. [2] also observed that piroxicam, an amphoteric molecule, showed attractive polar extra-interaction with phospholipid that was observed for bases, and proposed that the presence of basic function on analytes appeared as an essential prerequisite for the occurrence of additive polar extra-interaction.

It was clear that acidic and neutral molecules showed the less attractive membrane extra-affinity than did basic and amphoteric molecules. This highlighted the important perspective in rational drug design by introducing certain structural motifs (amino or zwitterionic functional groups) in ionized solutes to enhance membrane affinity (log k_{IAM}) but maintain low bulk hydrophobicity (log $D_{O/B,7.4}$). The higher log k_{IAM} is beneficial to good tissue distribution and pharmacological activity, and concurrently lower log $D_{O/B,7.4}$ leads to convenient physicochemical properties and suitable aqueous solubility. Grepafloxacin is an example of such an amphoteric compound, whose log $D_{O/B,7.4}$ was 0.71, but its higher log k_{IAM} conduced to better penetration into bacteria, concentrative uptake by alveolar macrophage and volume of distribution as large as 7000 ml/kg in humans [4–6].

Experimental

1. Determination of capacity factor for IAM chromatography

The eluents were mixtures of acetonitrile and 0.01 mol/l phosphate buffered saline (PBS, pH = 7.4) in different acetonitrile percentages (0 ~ 30% (v/v)) at a flow rate of 1 ml/min. The drugs were dissolved in PBS (200 μmol/l) and 10 μl samples were subjected into HPLC at 35 °C, monitored at 215 nm with UV detector. Chromatographic retention was expressed by the logarithm of capacity factor, defined as $\log k'_{IAM} = \log((t_r-t_0)/t_0)$, where t_r and t_0 are the retention times of drug and a non-retained solute (water), respectively. The logarithm of capacity factor extrapolated to (or measured at) 100% aqueous phase (log k_{IAM}) was used to express the membrane affinity of solutes.

2. Determination of solutes' hydrophobicity

The solutes' hydrophobicity (log $D_{O/B,7.4}$) was measured by shake-flask technique at *n*-octanol and PBS (pH = 7.4). All reported values were the average of at least three parallel measurements.

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Dr. J. Sun
Department of Pharmaceutics
School of Pharmacy
Shenyang Pharmaceutical University
No. 103 of Wenhua Road
Shenyang 110016
People's Republic of China
sunjin66@hotmail.com