Structure–Toxicity Relationships of Substituted Phenothiazines

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Abstract □ The goldfish test system is capable of discerning structure–toxicity relationships of substituted phenothiazine derivatives. A rank order correlation between dodecane partition coefficients, surface activity, and toxicity in goldfish was observed for various 2-substituted chlorpromazine derivatives (trifluprομazine > chlorpromazine > promazine > chlorpromazine sulfoxide). Dodecane partition coefficients were also found to correlate with the death of goldfish induced by the 1-chloro, 2-chloro, and 3-chloro analogs of chlorpromazine. The data imply that differences in the absorption of the phenothiazines could account, in part, for the observed differences in toxicity.

Keyphrases □ Phenothiazine derivatives, substituted—structure–toxicity relationships, goldfish test system data correlated with dodecane partition coefficients □ Structure–toxicity relationships—substituted phenothiazine derivatives, goldfish test system data correlated with dodecane partition coefficients □ Partition coefficients, dodecane—correlated with goldfish test system data, structure–toxicity relationships of substituted phenothiazine derivatives

An interesting relationship between the hydrophobic properties of phenothiazines and pharmacologic response has been suggested (1, 2). Apparently phenothiazine hydrophobicity, as characterized by surface activity and high oil–water partition coefficients, is related to pharmacological response, *i.e.*, the greater the hydrophobicity the greater the activity (3–6). In all of these studies, relatively complex pharmacological effects were monitored but no attempt was made to elucidate the role of the absorptive process in modifying pharmacological response.

The purpose of this report is to demonstrate that a relatively simple and inexpensive biological test system (death measurements in goldfish) can be utilized to correlate pharmacological effect with the physical–chemical properties of phenothiazines as well as to study the effect of the absorptive process in modifying such response.

EXPERIMENTAL

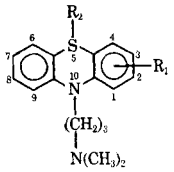
In all experiments the phenothiazines (chlorpromazine hydrochloride[1], chlorpromazine sulfoxide[1], 1-chloro and 3-chloro analogs of chlorpromazine hydrochloride[1], promazine hydrochloride[2], and trifluprομazine hydrochloride[3]) were dissolved in 0.06 *M* Sorensen's phosphate buffer, pH 5.3. Chemical structures and pKa values are given in Table I. In each case the drug was essentially protonated. All solutions (5×10^{-3} *M*) were freshly prepared and placed in light-resistant containers.

Surface tension measurements of the phenothiazines in a well-lighted laboratory demonstrated a gradual loss of surface activity, as indicated by a decrease in surface pressure. Accordingly, all experiments were conducted in a darkened laboratory where it was observed that surface activities were constant during the time course of the experiments (30 min.). The surface pressure of chlorpromazine sulfoxide was found to be constant for at least 6 hr., regardless of light conditions.

Goldfish, *Carassius auratus*, common variety, weighing about 3–4 g., were obtained locally and used as the test animal. Preliminary experiments with chlorpromazine indicated that bathing solution concentrations as low as 0.06–1.2 mcg./ml. were needed to obtain reasonable overturn times. Under these conditions, use of the overturn technique (7) yielded fluctuant results. It was reported (8) that goldfish exposed to chlorpromazine solutions of 4.0 mcg./ml. exhibited graded toxicity responses depending upon the volume of the bathing solution. This indicates that an appreciable adsorption of drug on the fish and/or glassware occurs at concentrations of 4.0 mcg./ml. and below. Use of higher chlorpromazine concentrations negates the adsorptive effect in that the bathing concentration probably remains constant but overturn is so rapid that it cannot be measured. Accordingly, the time of death induced by various 2-substituted derivatives of phenothiazine was determined by immersing each fish in 250 ml. of the drug solution at 26–28°, as described by Levy and Gucinski (9). Eight fish were used for each phenothiazine studied. Similarly, a separate lot of fish was used to determine the effect of changing the ring position of the chlorine atom on absorption of the 1-, 2-, and 3-chloro analogs.

Surface tension measurements were made at 25° using a surface tensiometer[4] having a ring circumference of 6.00 cm. Ten determinations, each involving fresh samples, were made of the various solutions. Surface pressure (π) was calculated as the surface tension of the buffer minus the surface tension of the phenothiazine solution.

Table I—Chemical Structures of Phenothiazines



Phenothiazine	R_1	R_2	pKa[a]
Trifluprομazine	2-CF_3	—	9.2
3-Chlorpromazine	3-Cl	—	9.2
2-Chlorpromazine	2-Cl	—	9.3
1-Chlorpromazine	1-Cl	—	9.4
Promazine	2-H	—	9.4
Chlorpromazine sulfoxide	2-Cl	O	9.0

[a] Data from *Reference 14*.

RESULTS AND DISCUSSION

The surface pressures and corresponding times of death for the phenothiazine derivatives and analogs are shown in Tables II and III. It is obvious from the π values reported in Table II that a phenothiazine containing a —CF_3 group at position 2 of the ring is more surface active than one containing a —Cl group, which in turn is more surface active than a phenothiazine containing a —H at the 2-position. Addition of oxygen to the sulfur produces a compound with virtually no surface activity. These findings are in agreement with the data of Zografi and Munshi (10). Although a quantitative comparison between the two studies is not possible due to differences in experimental conditions such as pH, buffer species, and phenothiazine concentration, a qualitative or rank order cor-

[1] Smith Kline & French Laboratories, Philadelphia, Pa.
[2] Wyeth Laboratories, Philadelphia, Pa.
[3] Squibb Institute for Medical Research, New Brunswick, N. J.
[4] Fisher.

Table II—Time of Death (T_d), Surface Pressure (π), and Apparent Partition Coefficient (P_a) of Phenothiazine Derivatives

Phenothiazine	T_d, min. ± SD[a]	π, dynes/cm.	P_a[b]
Triflupromazine	10.9 ± 0.8	28.8	172
2-Chlorpromazine	14.4 ± 1.0	18.3	73
Promazine	17.7 ± 1.7	12.9	8
Chlorpromazine sulfoxide	205 ± 38.2	1.1	0.0015

[a] All values significantly different from each other by Student's t test ($p < 0.05$). [b] Data calculated at pH 5.3 from *Reference 14*.

relation exists. As stated by Zografi and Munshi (10), the phenothiazine molecule appears to be oriented at the interface with the ring toward the nonpolar phase and the alkylamino group directed toward the bulk aqueous phase. Changes in hydrophobicity of the ring structure will, therefore, change surface activity significantly. The contribution of the substituent at position 2 of the ring to the hydrophobic character of the molecule is further illustrated in Table II.

The apparent partition coefficient at pH 5.3 for the various phenothiazine derivatives between dodecane and water shows the same rank order relationship as does surface pressure, *i.e.*, trifluprazine > chlorpromazine > promazine > chlorpromazine sulfoxide. Time of death determinations indicate that the greater the surface activity or partition coefficient, the greater the toxicity. Again the same rank order is seen: trifluprazine > chlorpromazine > promazine > chlorpromazine sulfoxide. This correlates well with experiments in other species using complex pharmacological responses (3–6, 11–13), thus illustrating that the simple goldfish experimental system is capable of differentiating pharmacological activity as a function of structural modification.

The effect of substituent position is seen in Table III. The 1-, 2-, and 3-chloro analogs of chlorpromazine caused death in goldfish with equal effectiveness and correlate with the apparent partition coefficients in dodecane at pH 5.3. Interestingly, the π values for these analogs exhibit the same rank order (3-chloro > 2-chloro > 1-chloro) as those reported by Zografi and Munshi (10). While these π values did not correlate with the apparent partition coefficient in dodecane or with times of death, they did exhibit a rank order correlation (61.3, 32.4, and 17.0 for 3-, 2-, and 1-chloro analogs, respectively) with *n*-octanol partition coefficients (14). Only the free base form of chlorpromazine partitions into dodecane, while in *n*-octanol both the free base and the ionic species partition, the latter as an ion pair (14). Since dodecane partition coefficients correlate with times of death in goldfish, it appears that it is the free base that is being absorbed. Thus, dodecane partition coefficients are a better parameter than π values or *n*-octanol partition coefficients for predicting phenothiazine absorption efficiency.

In spite of the fact that the phenothiazines are completely ionized at pH 5.3, absorption of the free base occurs due to the extremely high intrinsic partition coefficients (46,300–73,100) of these compounds.

The data presented in this report demonstrate that a relationship exists between phenothiazine absorption and hydrophobicity as indicated by partitioning into dodecane. Although a rank order correlation was also found between surface pressure and time of death for the 2-substituted derivatives, a similar relationship could not be demonstrated for the 1-, 2-, and 3-chloro analogs.

Table III—Time of Death (T_d), Surface Pressure (π), and Apparent Partition Coefficient (P_a) of Chlorpromazine Analogs

Phenothiazine	T_d, min. ± SD[a]	π, dynes/cm.	P_a[b]
3-Chlorpromazine	12.7 ± 0.9	21.8	58
2-Chlorpromazine	13.0 ± 1.4	18.3	73
1-Chlorpromazine	12.7 ± 1.2	13.2	49

[a] No statistical difference between values. [b] Data calculated at pH 5.3 from *Reference 14*.

It appears, therefore, that the goldfish test system is capable of discerning structure–toxicity relationships of substituted phenothiazines. The data reported in this study imply that differences in the appearance of phenothiazine-induced toxicity depend upon the ability of the free drug to partition into the fish and this process can be correlated with dodecane partition coefficients. One must, however, use caution in extrapolating these results to tranquilizing activity in higher animals since inherent activity differences between the various phenothiazines were reported (5).

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