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Report

Physicochemical Factors Associated with Binding and Retention of Compounds in Ocular Melanin of Rats: Correlations Using Data from Whole-Body Autoradiography and Molecular Modeling for Multiple Linear Regression Analyses

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The relationship between the physicochemical characteristics of 27 new drug candidates and their distribution into the melanin-containing structure of the rat eye, the uveal tract, was examined. Tissue distribution data were obtained from whole-body autoradiograms of pigmented Long–Evans rats sacrificed at 5 min and 96 hr after dosing. The physicochemical parameters considered include molecular weight, pK_a, degree of ionization, octanol/water partition coefficient (log $P_{o/w}$), drug–melanin binding energy, and acid/base status of the functional groups within the molecule. Multiple linear regression analysis was used to describe the best model correlating physicochemical and/or biological characteristics of these compounds to their initial distribution at 5 min and to the retention of residual radioactivity in ocular melanin at 96 hr post-injection. The early distribution was a function primarily of acid/base status, pK_a, binding energy, and log $P_{(o/w)}$, whereas uveal tract retention in rats was a function of volume of distribution (V_1), log $P_{(o/w)}$, pK_a, and binding energy. Further, there was a relationship between the initial distribution of a compound into the uveal tract and its retention 96 hr later. More specifically, the structures most likely to be distributed and ultimately retained at high concentrations were those containing strongly basic functionalities, such as piperidine or piperazine moieties and other amines. Further, the more lipophilic and, hence, widely distributed the basic compound, the greater the likelihood that it interacts with ocular melanin. In summary, the use of multiple linear regression analysis was useful in distinguishing which physicochemical characteristics of a compound or group of compounds contributed to melanin binding in pigmented rats *in vivo*.

KEY WORDS: melanin binding; physicochemical properties; tissue distribution; multiple linear regression approach; molecular modeling; quantitative whole-body autoradiography.

INTRODUCTION

The observation that chronic administration of phenothiazines (1) or long-term, high-dose chloroquine therapy (2) produced chorioretinopathy led to the awareness of an association between the toxic effects of some drugs and their high affinity for the pigment melanin. Since these early observations, melanin binding of drugs has been implicated not only in ocular toxicity, but also in ototoxicity and pigment disturbances of the skin and hair (3). In addition, studies on the molecular mechanisms of MPTP-induced neurotoxicity have shown that the active metabolite, MPP^+, binds with a high affinity to the melanin in the neurons of the substantia nigra (4). These authors suggest that MPP^+ bound intracellularly may be released gradually, resulting in damage to the neurons of the substantia nigra and producing the clinical symptoms of Parkinson's disease.

"Melanin" is a purely descriptive term, which conveys no chemical information and merely denotes a black pigment of biological origin. Melanins occurring naturally in the animal kingdom are known as "eumelanins," which are usually black, contain nitrogen, and are derived from tyrosine, dopa, and dopamine. Typical examples are the pigments of human skin, black hair, and brown eyes (5). In higher mammals, such as man, it is also present in the substantia nigra of the central nervous system. Although the exact structure remains elusive, these eumelanins appear to be irregularly structured polymers of 5,6-dioxoindole, frequently with conjugation to proteins. They are insoluble in virtually all solvents and lack well-defined spectral and physical characteristics.

The ability of drugs to interact with melanin has been a

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source of considerable concern within both the pharmaceutical industry and various drug regulatory agencies since much toxicological testing is still done using albino animals. To date, however, the nature of the interaction between melanin and drugs still has not been fully characterized. Electrostatic forces play an important role in the binding of drugs to melanin (6); however, nonelectrostatic contributions may also contribute to the binding for such drugs as chlorpromazine and chloroquine.

Any predictions of the tissue distribution of a drug from its physicochemical parameters must be grounded on a thorough knowledge of their mutual relationships. Studies to date have tended to focus primarily on relatively limited series of chemical homologues. Many of the diverse binding, transport, and metabolic phenomena which affect disposition are similar for closely related structures, and the investigator can isolate the variables which are relevant in a series. Although this approach has been highly successful in defining chemical parameters for a given series of drugs, it frequently says little about the propensity of a given organ or tissue to bind or exclude xenobiotics in general.

An alternate approach would be to examine the tissue affinities of a moderate to large number of diverse chemical structures and to attempt to assess the general physicochemical parameters that are important in determining the affinity of individual organs for the chemicals. This process is best carried out *in vivo,* and, while correlations cannot be expected to be as good as those found within a homologous series, statistical techniques such as multiple linear regression can be used to separate relative contributions of the measured physicochemical variables.

The present study focused on the disposition of 27 new drug candidates into the melanin-containing structure of the rat eye, the uveal tract (UT). Multiple linear regression analysis was used to identify the physicochemical and/or biological characteristics of these compounds which are important determinants of the initial distribution of drugs into the uveal tract, i.e., 5 min after intravenous injection, and of the retention of residual radioactivity associated with these same drugs 96 hr later.

MATERIALS AND METHODS

Animals. The primary animals used were male Long Evans Hooded (pigmented) rats received from Charles Rivers Laboratories, Wilmington, Massachusetts. The overall mean weight for the animals was 239.9 ± 38.4 g. For six of the compounds (denoted by a superscript asterisk in the tables), male Sprague–Dawley (albino) rats were used for the 5-min time points. These animals were also obtained from Charles River Laboratories, Wilmington, Massachusetts.

Determination of pK_a. The ionization constants (pK_a's) for 24 of the 27 compounds were either determined by an aqueous potentiometric titration method or determined using ultraviolet spectrophotometry (7). For three compounds (8515, 15337, and 19213 in Table I), the ionization constants were calculated using Hammett and Taft equations for organic acids and bases (8).

Determination of Partition Coefficient [log $P_{(o/w)}$*].* A version of the HPLC method of Garst and Wilson (9,10) was employed to determine the log $P_{(o/w)}$ for all 27 compounds. The method relates the logarithm of the capacity factor, k'_o, for a given HPLC column, solvent system, and temperature to log $P_{(o/w)}$. Linear correlation analysis gives the equation for the best line describing the relationship between the percentage solvent and the logarithm of a compound's retention time using that solvent mix as the eluent. The intercept of that line is defined as log k'_o.

According to Garst and Wilson (9), this intercept value must be corrected for the column and temperature used. Therefore, log k'_{oi} + (column/temperature correction factor) = log k'_o. Therefore, a standard curve using six chemically different compounds of known log $P_{(o/w)}$ (11) was developed to establish the relationship between log k'_o and log $P_{(o/w)}$. From this curve, the log $P_{(o/w)}$'s for the new drug candidates were determined. Implicit in this procedure is the assumption that the drug species which is retained on the HPLC column is in the nonionized form. Therefore, acidic compounds were examined in methanol which was acidified to pH 3.0 with 0.004 *M* trifluoroacetic acid, neutral compounds were examined in methanol alone, and basic compounds were examined in methanol alkalinized with 0.015 *M* triethylamine (pH 8.0).

The HPLC measurements were made using a Waters 840 system equipped with a 4-mm-i.d. × 25-cm Partisil C_8 column, maintained at 37°C. The mobile phase consisted of HPLC-grade methanol/water run isocratically beginning at a methanol concentration of 40% (v/v) and increasing, in successive runs, in 5–10% increments, to 70% methanol/water.

Calculation of Binding Energy. Binding energy was calculated using Macromodel, a molecular modeling program developed by Professor C. Still of Columbia University. A minimized structure of a tetramer of 5,6-dioxoindole (melanin monomer) was obtained using the multiconform mode and the MM2 Forcefield. Each of the 27 CGS compound structures was allowed to interact with the tetramer (which was kept fixed) at a close distance (3–5 Å); the energy was then minimized using the MM2 Forcefield. Each CGS compound was then moved to a distance of 15 Å, where the interaction with the tetramer would be negligible. The difference between the two energies was defined as the binding energy (14).

Treatment of Animals for Whole-Body Autoradiography. Each rat received a single intravenous injection of ^{14}C-labeled drug via either tail vein or jugular vein. The doses (ranging from 1.0 to 25 mg/kg) were specific to the individual compounds and corresponded to a pharmacologically active dose.

At 5 min or 96 hr postdose, animals were first anesthetized and then sacrificed by freezing in a −70°C dry ice/hexane (technical grade) bath. Each frozen animal was embedded at freezer temperatures in an aqueous solution of 3% carboxymethylcellulose. Whole-body sagittal sections were taken with a sledge microtome at various depths in a cryostat at freezer temperatures. The sections were allowed to dry in a freezer. Each dried section was placed on X-ray film and stored for an appropriate time in a lighttight container at freezer temperature. At the end of the exposure period, which was determined based on the specific activity and dosage of drug given, the film was separated from the radioactive tissue section and developed. The resulting autoradiograms were examined and levels were quantified using an

Table I. Physicochemical Parameters for 27 CGS Compounds

CGS No.[a]	Molecular weight	pK_a[b]	Log k'_o[c]	Octanol/water partition coefficient (log P)	Structural description and acid/base status[d]
10787B	267.3	2.60	3.52	3.57	Enolic acid
16617	363.4	2.87	−0.13	0.28	Amino acid—polar
15529	271.2	3.23	3.44	3.43	Enolic acid
14824A	465	3.28	3.48	3.53	Amino acid—polar
13080	232.3	4.51	1.20	0.94	Amphoteric: weak base, stronger acid
17348	489.4	6.00	5.07	5.26	Weak enolic acid
8515	307.3	7.00	2.86	2.76	*o*-Quinone: weakly acidic
9895	291.3	7.00	3.11	2.78	Planar, tricyclic—weak base, stronger acid
20625	309.4	10.00	3.46	3.06	Planar, tricyclic—weak base, stronger acid
17867A	299.8	10.00	3.55	3.45	Planar, tricyclic—weak base, stronger acid
1896	325.8	4.14	2.57	3.55	Weak base: benzodiazepine
10078B	373.4	7.00	3.12	2.45	Aromatic strong base
5218A	346.9	7.20	3.48	3.07	Aromatic base
8896	327.4	9.04	1.71	3.77	Aromatic strong base
10324	223.3	1.00	4.56	4.49	Enamide—essentially neutral
9343B	428.5	6.88	4.16	4.69	Large multicyclic base
19480A	292.8	6.95	2.73	4.24	Base: dialkyphenethylamine derivative
15337	299.3	7.00	2.84	2.63	Large multicyclic weak base
11760	281.4	7.00	3.37	3.35	Strongly basic, tricyclic
15943	285.7	7.00	3.46	3.45	Tricyclic, planar, weakly base
15040A	361.4	7.18	4.84	5.00	Pentacyclic, strong base
15855A	246.6	7.5	3.03	2.97	Fused phenolic dialkyphenethylamine: basic
15873A	246.6	7.5	3.12	3.07	Fused phenolic dialkyphenethylamine: basic
19213	269.3	7.5	3.19	3.15	Basic *o*-quinone
18102A	261.3	7.8	3.57	3.58	Base fused dialkyphenethylamine
16380A	255.4	8.13	4.42	4.90	Base: dialkyphenethylamine derivative
13135A	389.5	8.4	3.31	3.57	Aromatic strong base

[a] Compounds were administered as either salts, designated by a letter, or as free acids or bases.
[b] pK_a of acidic or basic functionality presented under structural description.
[c] Temperature-corrected intercept from partition coefficient determination.
[d] For multiple regression analysis, acids were given a value of 1 and bases assigned a value of 2.

Amersham RAS-1000 imaging system. The amounts of radioactivity in the uveal tract at 5 min, and for five compounds at 96 hr, were estimated using nonmatched standards exposed for the same length of time as the autoradiogram. The remaining 96-hr autoradiograms were placed on X-ray film with standards, and radioactivity was quantified.

Calculation of Volume of Distribution (V_1). The concentration of radioactivity (nCi/g) in each tissue on the 5-min autoradiograms was estimated. The tissues that were included in the calculations of V_1 for each animal were as follows: skeletal muscle, liver, myocardium, blood, lung, bone, kidney, brain, skin, and spleen. The total amount of radioactivity in each organ was calculated by multiplying the tissue concentration by the volume of that tissue. The tissue volumes were published values (12), based on a 250-g rat.

The sum of the total radioactivity (nCi) in the organs at 5 min was assumed to represent the total dose given to each animal. The volume of distribution, V_1, was then calculated using the following equation:

$$V_1 = \frac{\text{total dose (nCi)}}{\text{blood concentration (nCi/ml)}} \quad (1)$$

Statistical Analysis. All statistics for partition coefficient determinations were calculated using simple linear regression techniques. The multiple linear regression analyses were run using StatView II, a software package from Abacus Concepts, which runs on a Macintosh II computer. The multiple linear regression analyses were validated using programing developed on SAS (VMS SAS Production Release 5.16, SAS Institute, Inc., 1986). These programs were implemented on a VAX8650. A significance level of $P \leq 0.05$ was set for the multiple regression equation, while $P \leq 0.35$ was considered significant for addition of each regressor into the equation for the model. The latter P value was derived from a two-tailed t test to determine whether or not the associated regression coefficient is significantly different from zero. Significance was judged by the square of the t value for each regressor, called the partial F value and has (1, residual) degrees of freedom (from ANOVA table). In contrast to simple linear regression, which shows the relationship of individual variables to a single response [$y_{(i)}$], the addition of subsequent significant independent variables to the multiple regression model must be considered concurrently with the prior regressors. Hence, the model is dependent on the order in which each variable enters the equation. Finally, this type of analysis requires that the independent (x) variables are independent of each other, i.e., there is not significant statistical correlation among the x variables. Therefore, prior to the multiple linear regression analyses, the independent variables were examined for correlations

among themselves. In both analyses, the variables in the equations are independent of each other.

RESULTS

Determination of Octanol/Water Partition Coefficients [log *P*(o/w)]

Figure 1A illustrates the relationship of percentage methanol to log k' and Fig. 1B shows the regression line and equation for the standard curve relating log k'_o and log $P_{(o/w)}$ for the six compounds with known log $P_{(o/w)}$. The slope (1.15) of that line is used subsequently in determining log $P_{(o/w)}$ for the 27 compounds being studied. There was an excellent correlation for these six, structurally unrelated compounds as evidenced by the correlation coefficient, r = 0.973.

Figure 2 shows the regression lines relating percentage methanol to log k' for a subset of the 27 new drug candidates tested, representing five very different chemical structures. The correlation coefficients for the regression equations of these compounds were excellent, ranging from −0.985 to −1.000. Hence, this method provides a means for determining the log $P_{(o/w)}$ which is not limited to members of homologous series.

Table I shows the temperature-corrected intercept (log k'_o) and log $P_{(o/w)}$. The log k'_o and log $P_{(o/w)}$ are very similar, being related by the slope of the regression line derived from

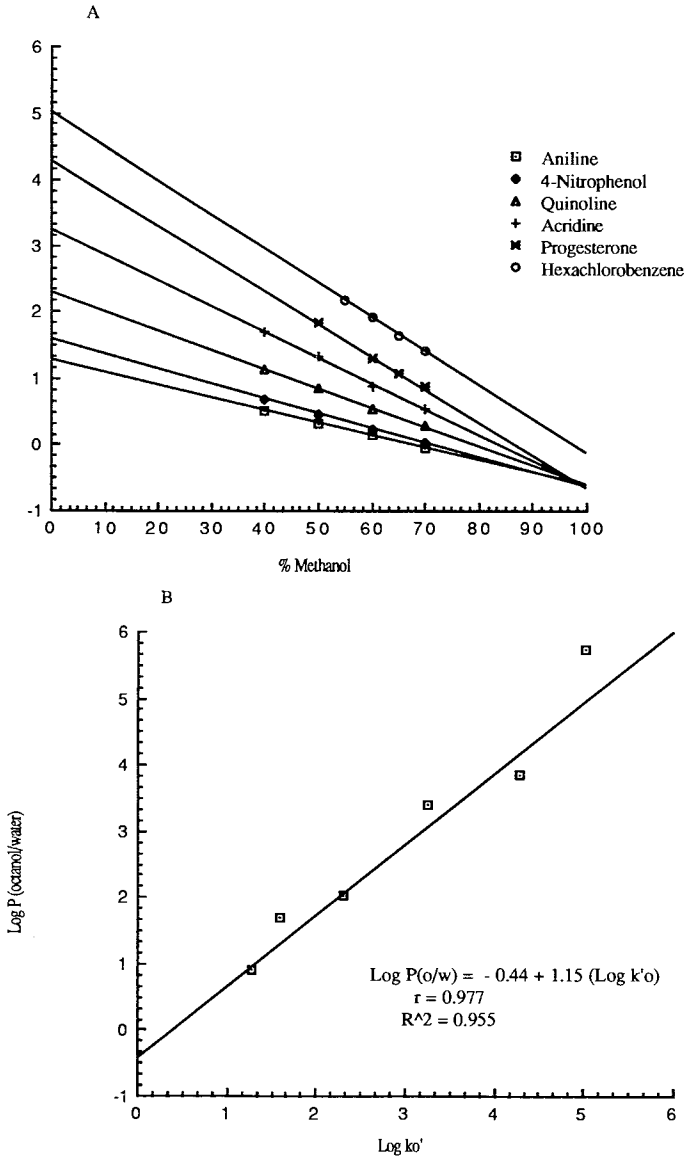

Fig. 1. (A) The relationship between the percentage methanol in the mobile phase of reversed-phase HPLC and the logarithm of the capacity factor (log k') for compounds with known octanol/water partition coefficients. (B) The relationship between the temperature-corrected intercept from reversed-phase HPLC and the log $P_{(octanol/water)}$ for compounds with known octanol/water partition coefficient—standard curve.

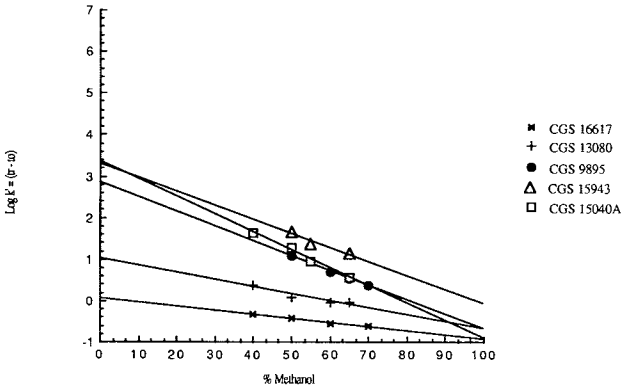

Fig. 2. The relationship between the percentage methanol in the mobile phase of reversed-phase HPLC and the logarithm of the capacity factor (log k') for five CGS compounds with unknown octanol/water partition coefficients.

the standard compounds. This similarity reflects the approximately 1:1 relationship between the capacity factor of the HPLC column and the octanol/water partition coefficient. Table I also presents the values of the other physicochemical parameters, molecular weight and pK_a, which were included in the regression analysis. Furthermore, Table I gives a brief description of major acid/base characteristics of the functionalities within each molecule. Based on the Bronsted-Løwry definition of acids and bases, those compounds containing acidic groups were considered as acids in the multiple linear regression model and compounds which contained basic functional groups were given the status of bases. Amphoteric compounds were classified as either acids or bases depending upon which functionality made the greatest contribution to the fraction nonionized at pH 7.4. The multiple linear regression analysis procedure treated acid/base status as a class variable, with recognition of two distinct groups or classes of molecules within the overall model. With inclusion of these two classes in the analysis, a model was developed which examined all the data simultaneously, while recognizing that the "microenvironment" of the functionalities may be very different for each class of chemical or drug.

Physicochemical Modeling Using Multiple Linear Regression Analysis

In order to examine the influence of physicochemical parameters on the early disposition (5 min postinjection) of drugs into the UT, multiple linear regression analysis was used. Table III shows the results of a stepwise regression analysis for three physicochemical properties listed in Table I versus log [UT/BL] (Table II). The investigation of the important determinants of early distribution into the uveal tract shows that the most highly correlated parameter was acid/base status, $r = 0.911$. When pK_a and binding energy were considered simultaneously with acid/base status, there was an increase in the correlation coefficient to 0.971. Si-

Table II. Biological Properties of 27 CGS New Drug Candidates

CGS No.	Volume of distribution (V_1)(L/kg)	Uveal tract-to-blood ratio, 5 min[a]	Uveal tract concentration, 96 hr (nCi/g)[b]	Binding energy (kJ)
10787B	0.25	*[c]	0.00	−16.0
16617	0.42	0.64	0.00	−70.0
15529	0.18	0.29	34.67[b]	−32.0
14824A	0.81	0.72	0.00	−60.0
13080	0.42	0.34	0.00	−26.0
17348	0.46	0.22	0.00	−6.0
8515	0.68	7.46	21.50	−9.0
9895	1.79	5.23	0.00	.[d]
20625	4.93	7.73	0.00	.
17867A	4.22	*	28.67	.
1896	10.38	*	31.00	.
10078B	6.54	*	651.9	−14.0
5218A	3.90	*	257.00	−11.0
8896	0.75	1.70	0.00	−8.0
10324	2.64	15.49	139.80[b]	−18.0
9343B	5.94	24.23	76.90[b]	−40.0
19480A	1.07	2.84	200.40[b]	−32.0
15337	0.38	1.62	0.00	.
11760	2.09	24.15	63.00[b]	−25.0
15943	1.24	12.16	0.00	−8.0
15040A	0.00	28.61	161.3[b]	−54.0
15855A	1.73	27.80	8.00[b]	−21.0
15873A	0.00	45.16	0.00	−20.0
19213	1.81	14.92	0.00	−24.0
18102A	0.00	41.20	3.00[b]	−36.0
16380A	3.14	20.81	106.67	−30.0
13135A	0.81	31.58	4.63[b]	−21.0

[a] Quantified using exposure-time matched standards; standards not on film.
[b] Quantified with standards on film.
[c] * denotes albino (Sprague–Dawley) rat used for 5-min time point.
[d] . Binding energy not calculated.

Table III. Stepwise Regression of log [Uveal Tract-to-Blood Ratio] at 5 min Postinjection Versus Physicochemical Characteristics: Final Equation, $\log [UT/BL]_{5\text{-min}} = 1.45$ (A/B status) + 0.127 (pK_a) − 0.007 (binding energy) − 0.051 [$\log P_{(o/w)}$] − 2.42

Step No.	Variable entered	r, correlation coefficient	R^2, coefficient of determination	df[a]	F test	P[b]
1	Acid/base status	0.939	0.881	1,16	118.32	≤0.0001
2	pK_a	0.965	0.931	2,15	101.86	≤0.0001
3	Binding energy	0.977	0.955	3,14	99.01	≤0.0001
4	$\log P_{(o/w)}$	0.981	0.961	4,13	78.29	≤0.0001

[a] Degrees of freedom in numerator, denominator.
[b] Significance level set at $P \leq 0.05$.

multaneous inclusion of log $P_{(o/w)}$ in combination with the three former parameters yielded a regression line that was significant ($P < 0.0001$) with $r = 0.983$. Further addition of physicochemical or biological parameters did not improve the correlation. For example, inclusion of molecular weight in the model still yielded a regression line with $r = 0.983$. This result suggested that molecular weight had no statistically significant effect in this weight range (223.27 to 489.40) and, hence, had little effect on the movement of the compounds into the uveal tract 5 min postinjection. Hence, the primary factors on UT association at 5 min (in decreasing order of effect) were acid/base status, pK_a, binding energy, and log $P_{(o/w)}$.

Figure 3 shows the observed and fitted values for the UT/BL ratios for the compounds used to generate the model. The fitted values were calculated using the final equation presented in Table III. With the exception of two compounds, there is good agreement between observed and fitted values. This is an illustration of the accuracy of this model to calculate the UT/BL ratios for the 17 compounds. When given the appropriate physicochemical parameters, one can use this model as a predictor of the uveal tract to blood level values for new drug candidate. The predicative capabilities of the model has been examined for two drugs with known affinity for melanin, chlorpromazine, and chloroquine. The model predicts substantial UT/BL 5 min postdosing (57.8 and 39.4) for these two compounds.

A similar approach was taken to dissect out the factors responsible for retention of drugs in the UT 96 hr postinjection. Table IV displays the results of the stepwise regression analysis for physicochemical and biological parameters versus log [1 + (concentration$_{drug}$ UT)]. The most highly correlated characteristic was V_1 ($r = 0.70$). There was improvement in the correlation coefficient when log $P_{(o/w)}$ was simultaneously considered ($r = 0.73$). Finally, when binding energy was also considered with the two former parameters, the multiple correlation coefficient r was 0.746.

The fact that the most statistically significant correlation with uveal tract retention was with the volume of distribution suggests that the more deeply a chemical is distributed within body fluids and tissues, the greater the likelihood that it will interact with ocular melanin. This result indicates that a relationship exists between the initial distribution of a compound into melanin-containing structures and its retention 96 hr later. In fact, log $P_{(o/w)}$ and binding energy were found in the regression models for both 5 min and 96 hr, while further additions of other physicochemical parameters did not improve the correlations. Thus, the combination of V_1, log $P_{(o/w)}$, and binding energy yielded a final regression line that was significant ($P = 0.007$), with 55.7% of the variance[3] explained by regression.

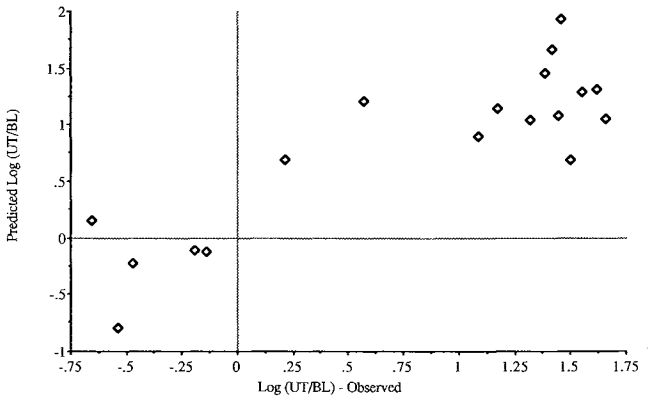

Fig. 3. Comparison between observed uveal tract-to-blood ratios at 5 min postdose and the values fitted by multiple linear regression analysis for 18 CGS compounds.

Figure 4 shows the observed and fitted values for the regression model at 96 hr postdosing. Unlike the 5-min data in Fig. 3, the model is more likely to predict incorrectly the quantity of drug-derived radioactivity in the uveal tract at this time point. Thus, quantitative prediction is not as feasible using the multiple linear regression approach. However, the qualitative expectation of a compound's presence after 96 hr can be estimated from the model, knowing either the volume of distribution of the particular compound or the estimated uveal tract-to-blood ratio for 5 min. The greater the value for either biologic parameter, the greater the likelihood that a new drug entity might also be retained after 96 hr.

DISCUSSION

The results of the present study show that early distribution into the uveal tract of the eye is primarily a function of four physicochemical parameters. When considered simultaneously, acid/base status, pK_a, binding energy, and log $P_{(o/w)}$ can be used to predict quantitatively the distribution of a new drug entity into the uveal tract of the eye. In contrast, the prediction of retention of radioactivity in the uveal tract

[3] Percentage of variance = [$R^2 * 100$].

Table IV. Stepwise Regression of log [1 + Drug Concentration in Uveal Tract] at 96 hr Postinjection Versus Physicochemical and Biological Characteristics: Final Equation, log [1 + (Concentration$_{drug}$UT)]$_{96\ hr}$ = 3.39×10^{-4} (V_1) + 0.194 [log $P_{(o/w)}$] − 0.009 (Binding Energy) − 0.736

Step No.	Variable entered	r, correlation coefficient	R^2 coefficient of determination	df[a]	F test	P[b]
1	V_1	0.700	0.491	1,20	19.26	≤0.0003
2	log $P_{(o/w)}$	0.730	0.532	2,19	10.82	≤0.0007
3	Binding energy	0.746	0.577	3,18	7.54	≤0.0018

[a] Degrees of freedom in numerator, denominator.
[b] Significance level set at $P \leq 0.05$.

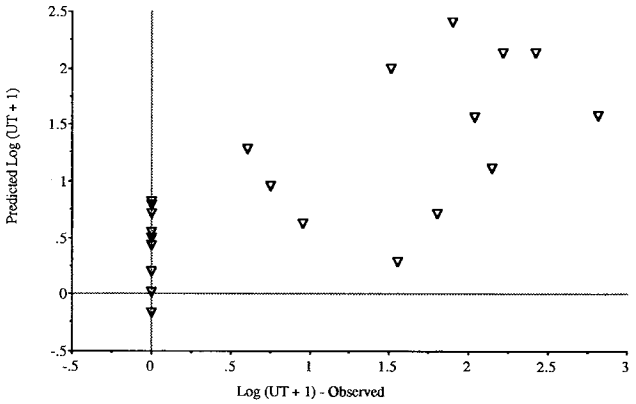

Fig. 4. Comparison between the observed uveal tract concentrations at 96 hr postdose and the values fitted by multiple linear regression analysis for 22 CGS compounds.

at 96 hr depends on the volume of distribution (V_1), log $P_{(o/w)}$, and binding energy.

The primary biological process influencing drug movement during the first 5 min postdose is distribution. The distributive process is influenced mainly by the physical and chemical characteristics of the particular drug entity, such as degree of ionization and lipophilicity (15). Furthermore, during the initial distributive phase, the role of metabolism and other processes of elimination is often minimal. Therefore, the use of the physicochemical characteristics of the parent drug would be appropriate for modeling the behavior of even total radioactivity at 5 min.

However, the correlation of the physicochemical parameters to drug retention at 96 hr, though significant, was still low, suggesting that, in addition to the volume of distribution of the compound, some other biological properties of drugs might play a role in determining retention in melanin-containing structures. The most notable factor which probably contributed to the low correlation for the 96-hr time point was metabolism. Whole-body autoradiography locates radioactive material without differentiation of unchanged drug from radioactive metabolites that may be present. At 5 min after an intravenous dose, most of the radioactive material is represented by the parent compound, but at 96 hr, metabolites will, in most instances, constitute a major portion of the body burden of radioactivity. Hence, a correlation analysis using the physicochemical properties of the parent drug may not satisfactorily describe the characteristics of the drug-derived material remaining in the uveal tract 96 hr after dosing. There is evidence for some of the 27 compounds that minimal metabolism occurred and that the radioactive material in the uveal tract was the parent compound.

Hence, in this case, the use of multiple linear regression analysis was quite useful in distinguishing which physicochemical characteristics of a compound or group of compounds contributed to melanin binding in pigmented rats *in vivo*. In a more generalized case, however, this approach can be employed not only to identify those tissues which might be most susceptible to toxic insults, but also, and possibly more importantly, to provide the information for optimal development of new drugs targeted for specific tissues and/or organs. Further investigation is currently ongoing using this approach to predict the distribution of new drug candidates into tissues such as the brain and gastric (glandular) mucosa.

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NOMENCLATURE

log $P_{(o/w)}$	Octanol/water partition coefficient
log K'_o	Temperature-corrected intercept for the capacity factor
log k'	Logarithm of the capacity factor
CGS	CIBA-GEIGY Summit
UT	Uveal tract
{UT + 1}	Uveal tract concentration + 1 at 96 hr
UT/BL	Uveal tract-to-blood level ratios at 5 min
V_1	Volume of distribution

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