RESEARCH ARTICLE

In silico quantitative structure pharmacokinetic relationship modeling of quinolones: Apparent volume of distribution

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The use of *in silico* approaches for successful prediction of pharmacokinetic properties of compounds during new drug discovery has been increasing exponentially. These *in silico* models, for the prognosis of absorption, distribution, metabolism and excretion (ADME), are invariably based on the implementation of quantitative structure pharmacokinetic relationship (QSPR) techniques. The current study was conducted to investigate QSPR for apparent volume of distribution (V_d) in man among 24 Quinolone drugs employing an extrathermodynamic approach. It is vital to predict the V_d value of various drug leads during drug discovery so that compounds with poor bioavailability can be eliminated and those with an acceptable metabolic stability can be identified. Analysis of several thousands of QSPR correlations developed in the present study revealed an extremely high degree of cross-validated coefficient (Q^2) using the leave-one-out method ($P < 0.001$). Logarithmic transformation tends to improve the correlations marginally ($R^2 = 0.936$) but the inverse transform resulted in a distinct improvement in the correlation ($R^2 = 0.994$). Electronic and topological parameters were found to primarily ascribe the variation in V_d. Overall, the diffusional interactions seem to play a major role in attributing V_d rather than the permeational ones.

Key words: *ADME prediction, pharmacokinetics, quantitative structure pharmacokinetic relationship, QSPR, fluoroquinolones*

INTRODUCTION

It is now duly recognized by the pharmaceutical industry that undesirable absorption, distribution, metabolism and excretion (ADME) of new drug candidates are the cause(s) of many clinical phase drug development failures. Nearly 40% of the drug candidates fail during the clinical trials owing to poor pharmacokinetic properties. This is an economic disaster as the failed drugs have been in the pipeline for several years with a huge expenditure of efforts, time and money invested in their development. Accordingly, it has been an earnest endeavor of the pharmaceutical scientists to identify such problems early during the drug delivery process and design new drug molecules with optimal pharmacokinetic and pharmacodynamic properties before their synthesis.

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Of late, the *in vitro* approaches have been widely practiced to investigate the ADME properties of new chemical entities.[1] More recently, *in silico* modeling has been investigated as a tool to optimize selection of the most suitable drug candidates for development. This novel approach of quickly predicting the ADME properties using computational means is of great importance because the experimental ADME testing is phenomenally expensive and arduous. Therefore, the use of computational models in the prediction of ADME parameters has been growing rapidly in drug discovery because of their immense benefits in throughput and early application of drug design.[2]

Apparent volume of distribution (V_d), a vital pharma cokinetic parameter characterizing the dispositional attributes of a drug, is simplistically a proportionality constant relating the plasma drug concentrations to the total amount of drug in the body.[3] Its magnitude provides a broad inkling as to how widely the drug gets distributed in the body. Also, V_d provides an excellent tool to correlate the physicochemical properties with the duration and intensity of action based on its distribution in the body. Depending on the degree of drug binding to

the plasma proteins and tissues, large variations are noticeable in the apparent volume of distribution of various drugs in man. An estimate of V_d is of paramount importance while selecting a drug candidate in therapeutics and while calculating its biological half life and clearance values.[4]

Traditionally, the V_d value of a drug candidate is obtained via *in vivo* studies, which tends to be quite arduous, time consuming and expensive. The *in silico* ADME modeling using the quantitative structure pharmacokinetic relationship (QSPR) method has been explored for predicting the V_d value of drug candidates in an efficient and cost-efficient manner.[5-8] The primary aim of these QSPR studies is to enable the drug designer to modify the chemical structure of a pharmacodynamically active drug in such a manner as to alter its pharmacokinetic properties without diminishing its pharmacodynamic potential.[9,10] The major advantage of QSPR, therefore, lies in the fact that once such a relationship is ascertained with an adequate statistical degree of confidence, it can be a valuable assistance in the prognosis of the behavior of new molecules even before they are actually synthesized.[11]

The key objective of the current study was to investigate *in silico* QSPR among various quinolone drugs for V_d. Quinolones were chosen for QSPR studies as this category of drugs is extensively used as antimicrobial agents in the treatment of serious infections. Also, quinolones consist of a significant number of drug compounds thoroughly investigated for their pharmacokinetic performance, particularly for V_d ($n = 24$). Further, the congeners in this class have many common pharmacokinetic characteristics, mechanism and degree of affinity with body tissues. Several descriptors like experimental values of log P, pKa, melting point, etc. of these drugs are available in the standard texts or journals.

MATERIALS AND METHODS

QSPR was conducted among quinolone drugs employing an extrathermodynamic multilinear regression analysis (MLRA) approach. The general steps for developing a QSPR model include data set selection, chemical structure entry, 3D structure generation and descriptor calculation, model construction that involves selection of descriptors and validation of the testing set using a Pentium dual core microprocessor (Intel, Santa Clara, USA) desktop (IBM, Bangalore, India) with 1 GB RAM and 160 GB hard disk drive. The computer peripherals included an HP Laser 1020 series printer and an HP Scanjet 2400 scanner.

Dataset selection

The reported values of V_d of the quinolone drugs in humans were taken from various literature sources.[3,12-15] In order to ensure that experimental variations in determining V_d do not significantly affect the quality of our datasets, only V_d values obtained from healthy adult males after oral administration were employed for constructing the dataset. A total of 24 quinolone drugs were selected and used as the dataset for this study. The V_d value of each of these compounds was also log-transformed ($\log V_d$) and inverse transformed ($1/V_d$) to normalize the data and to reduce the unequal error variance, respectively.

Molecular structure and descriptors

Various structural parameters were computed theoretically employing diverse computer software.

Descriptors calculated by Pallas 2.0

The values of structural descriptors, like log P, pK_a and log D of the various quinolones, were calculated using the software Pallas 2.0 (CompuDrug International Inc., Sedona, USA). The structures of the drugs were graphically drawn on the monitor with the help of a mouse. Suitable templates/rings were chosen, bonds were drawn and different heteroatoms were chosen from the periodic table provided in the software and incorporated into the structure. The rough graphical sketch representing the structural formula of the compound was transformed to its least-energy configuration. The name of the compound was entered to let the structure of the drug be stored under its assigned name in the software database. For the estimation of log P and log D, compounds from the database were selected, the software run for estimation of the desired descriptors and the results were stored as an MDL molfile.

Descriptors calculated by HyperChem

Log P, pKa, surface area and surface volume of various quinolones were calculated using the software HyperChem 8.0.5 (Hypercube Inc., Gainesville, USA). The structures of the drugs were graphically drawn on the monitor and the same procedure was followed as described above in descriptors calculated by Pallas 2.0 and, however, at the end in this case hin files were generated.

Parameters calculated by Dragon

The molfiles generated by the Chem 3D software pro v.3.5. (Cambridge Soft Corporation, Cambridge, MA, USA) were imported to Dragon 5.5 (Talete Srl, Milano, Italy). As many as 1497 diverse descriptors, viz constitutional, geometrical, topological, Whim 3D, electronic, etc., were calculated with the help of the Dragon software.

Parameters calculated by CODESSA

A large number of molecular descriptors were calculated with the help of the CODESSA 2.0 software (Semichem, Shawnee, Terrace, USA) also. First of all, a worksheet was made in an MS- Excel environment to load various molfiles into the software. The file was saved as a nondocument ASCII text file. The said text file consisted of a number of columns separated by blanks, each column containing data of one type, e.g. structure names, property values, file names, etc. Each line contained the same number of columns. The program then scans the file in order to determine the number of columns

and provides a column dialog box, where the type of data in each column and other parameters were specified. Before calculating the descriptors, the loaded structure was checked and necessary corrections were made. A "structure dialog box" was used to enter or change the structure name as well as names and type of files associated with the structure. Various classes of descriptors, viz constitutional, topological, geometrical and electronic descriptors, were selected for calculation using the "calculate descriptor" dialog box. Initially, the descriptors were computed for all the structures loaded into the software. Further, as and when any information was available about new congeners, those particular compounds were also selected for computation of the descriptors.

Multivariate statistical analysis

Attempts were made to correlate all the types of descriptors, viz lipophilic, constitutional, electrostatic, electronic, topological and steric, with the pharmacokinetic parameter V_d. The initial regression analysis was carried out using a heuristic analysis followed by the best multilinear regression and MLRA options of the CODESSA software.

In case of the heuristic method, a preselection of the descriptors was accomplished. All the descriptors were checked to ensure that the value of each descriptor was available for each structure with significant variation among these values. Descriptors, for which values were not available for every loaded structure in the data, were discarded. Thereafter, the one-parameter correlation equations for each descriptor were calculated. The number of descriptors in the starting set was further reduced by discarding if:

- The F-value for the one-parameter correlation with the descriptor is below 1.0.
- The r^2 value of the one-parameter equation is less than the assigned value of r^2_{min} (usually 0.1).
- The one-parameter *t*-value is less than the assigned value (usually 1.5).
- The multiparameter *t*-value is less than the assigned value (usually 1.95).
- The descriptors are highly intercorrelated with another descriptor ($r^2 > 0.65$).

Pharmacokinetic data of the V_d parameter, available for 24 quinolones, were analyzed limiting the descriptors:drug ratio to 1:4. The heuristic method yielded a list of the best 10 correlations, each with the highest values of R^2 and F ratio. Numerous attempts were carried out to obtain significant correlations for quinolones, some of which are shown in Table 1.

A set of important descriptors found to significantly ascribe the variation of V_d was constructed. Further, a search for the multiparameter regression with the maximum predicting ability was performed. A number of sets of descriptors were thus made and MLRA was performed with V_d. Regression plots of each correlation thus attempted were examined for linearity and coherence. Residual plots were also examined for randomization and absence of distinct patterns in order to eliminate chance correlations [Figure 1]. Logarithmic and inverse transformations of V_d were also carried out in order to screen the correlation with improved values of R^2 and/or F ratio [Figures 2 and 3]. Graphs were constructed using the MS-Excel software.

Validation of the testing set

Statistical significance of each correlation was determined on the basis of the value of the F-criterion and the magnitude of the cross-validated R^2, commonly represented as Q^2, calculated according to Equation no.1.

$$Q^2 = 1 - \frac{\sum (ypred - yobs)^2}{\sum (yobs - ymean)^2} \quad (1)$$

A model with good predictive performance will have a Q^2-value close to 1, models that do not predict better than merely chance alone can have negative values.

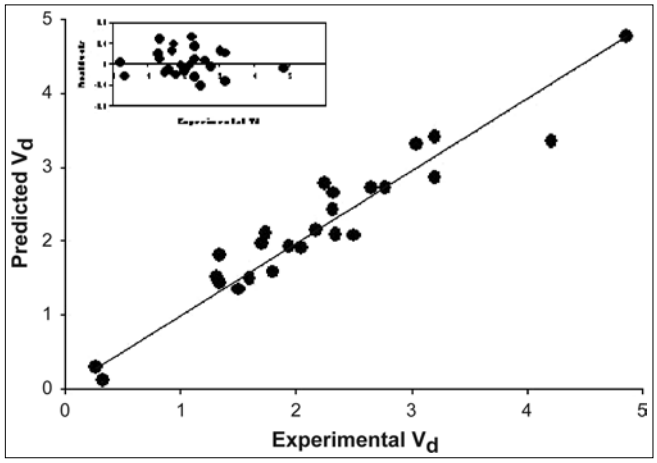

Figure 1: Linear correlation plot between the values of V_d as reported in the literature and those predicted using the multiparameter quantitative structure pharmacokinetic relationship for a series of 24 quinolones. The inset shows the corresponding residual plot

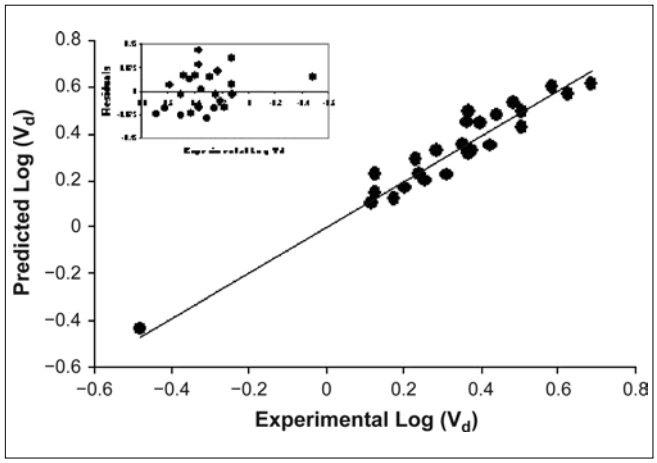

Figure 2: Linear correlation plot between the values of log transform of V_d as reported in the literature and those predicted using the multiparameter quantitative structure pharmacokinetic relationship for a series of 24 quinolones. The inset shows the corresponding residual plot

Table 1: Significant QSPR polynomial equations along with the statistical parameters for a series of 24 quinolones using volume of distribution (V_d) as the pharmacokinetic parameter

Equation	m	R^2	F	S^2	Q^2	*P* <
V_d = 11.844 − 14.807 ZXS/ZXR	1	0.4056	15.69	0.6617	0.3167	0.001
V_d = 9.7059 − 46.692 G3p	1	0.3421	11.96	0.7323	0.2494	0.005
V_d = 10.754 − 21.161 ABIC0	1	0.3144	10.55	0.7631	0.2079	0.005
V_d = −1.4795 + 1.4661 Hy − 43.836 QNmax	2	0.6178	17.78	0.4447	0.4985	0.001
V_d = 16.474 − 22.595 DBrel + 79.235 Qmin	2	0.5774	15.03	0.4918	0.4824	0.001
V_d = 20.311 + 1.6976 Hy − 7.9232 AIC0	2	0.5539	13.66	0.5191	0.4264	0.005
V_d = 3.7920 − 14.439 DBrel + 1.1949 Hy	2	0.5163	11.74	0.5629	0.3732	0.005
V_d = 9.2021 + 1.0180 Hy − 28.529 QNmax − 12.835 Dbrel + 53.047 Qmin	4	0.7585	15.71	0.3091	0.6321	0.001
V_d = − 22.598 + 1.1607 Hy − 17.305 QNmax − 26.942 SICO − 11.961 ZXS/ZXR + 40.921 FDI	5	0.8406	20.04	0.2148	0.7360	0.001
V_d = 18.376 − 55.204 ABIC0 + 1.4855 Hy + 38.732 FDI − 2.4072 SP02 − 85.728 G3e − 0.07325	6	0.9117	30.99	0.1255	0.8520	0.001
Log V_d = 0.83760 − 6.6523 Orel	1	0.4423	18.24	0.0302	0.0181	0.001
Log V_d = −0.59132 − 9.5439 Qnmax	1	0.3610	12.99	0.0346	0.1227	0.005
Log V_d = 1.5753 − 11.344 QNmax + 14.882 Qmin	2	0.6141	17.50	0.0219	0.4003	0.001
Log V_d = −4.6504 − 12.417 QNmax + 12.897 Qmin + 0.082391 HDcount + 2.9024 ARR + 4.1662 ASIC1 + 0.79735 DISPp	6	0.9355	39.56	0.0049	0.8811	0.001
Log V_d = 0.59314 − 10.422 QNmax + 8.1016 Qmin + 0.042772 HDcount − 0.07077 L1s + 0.30433 HACA − 2 − 0.10973 On	6	0.9286	39.03	0.0049	0.7979	0.001
1/V_d = −9.5313 + 64.718 G3e	1	0.6635	45.36	0.2489	0.4878	0.001
1/V_d = −7.7285 + 52.457 G3p	1	0.6496	42.65	0.2592	0.4455	0.001
1/V_d = −8.9993 + 14.900 ZXS/ZXR	1	0.6180	37.20	0.2826	0.3948	0.001
1/V_d = 0.65154 + 13.716 YZS/YZR + 79.158 QNmin	2	0.8581	66.51	0.1098	0.6974	0.001
1/V_d = −5.1946 + 27.332 G3m + 19.021 Orel	2	0.8486	61.67	0.1171	0.3622	0.001
1/V_d = −1.0565 + 9.9654 YZS/YZR + 62.701 QNmin + 14.384 G3m	3	0.9110	71.67	0.0721	0.7284	0.001
1/V_d = 15.706 + 9.9154 YZS/YZR + 85.225 QNmin + 27.228 G3u − 16.691 FDI − 3.2317 P2m − 0.00506 PNSA − 2	6	0.9941	174.68	0.0160	0.9182	0.001
1/V_d = 13.43 + 84.349 QNmin + 8.4451 YZS/YZR + 12.810 G3m − 13.24 FDI + 18.145 G3u − 137.13 MIB	6	0.9807	152.32	0.0183	0.9501	0.001

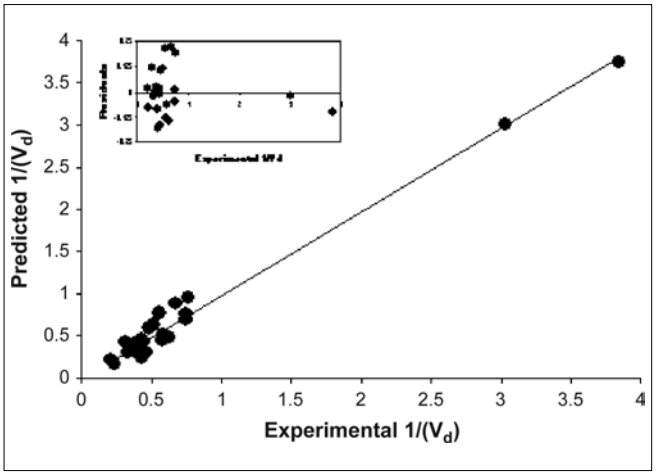

Figure 3: Linear correlation plot between the values of inverse transform of V_d as reported in the literature and those predicted using the multiparameter quantitative structure pharmacokinetic relationship for a series of 24 quinolones. The inset shows the corresponding residual plot

The F-values were computed according to Equation no. 2:

$$F = \frac{S_1^2}{S_2^2} \quad (2)$$

where, S_1 is the variance between the samples and S_2 is the variance within the samples.

The values of the computed F-ratio were compared with that of the critical values tabulated in the statistical texts and the levels of significance were discerned. The QSPR correlations found to be statistically significant were compiled from the CODESSA software and were stored as respective files under the extension of COD. The names of descriptors were conveniently coded using a WS-Macro program and the files were converted to an appropriate ASCII format using in-house developed program codes. These ASCII files were further converted into tabular formats in MS-Word.

RESULTS AND DISCUSSION

Variable QSPR results were obtained following the application of multivariate statistical analyses on quinolone drugs. Thousands of such correlation and regression analysis were attempted choosing all the possible combinations of available descriptors, each yielding an elaborate output. The concise results of only those correlations that were found to be statistically significant, usually at a 5% level or less, and/or those that have important applications have been taken into consideration.

The volume of distribution for a combined set of 24 quinolones showed significant dependence on the topological parameters and geometric parameters. The prominent descriptors explaining variation in V_d encompass the information contents, structured information contents, hydrophilic factor (Hy) and other parameters like shape profile no. 02 (SP02), 3st component symmetry directional WHIM index/weighted by atomic Sanderson electronegativities (G3e), 3st component symmetry directional WHIM index/ weighted by atomic polarizabilities (G3p), number of H atoms (Hn) and folding degree index (FDI). The electronic parameters like Max partial charge for N atom (Zefirov's PC) (QNmax) and Min partial charge for N atom (Zefirov's PC) (Qnmin) and geometrical parameters like XY Shadow (XYS) also yielded minor contributions toward improvement in relationships. Thus, overall, the diffusional interactions seem to play a pivotal role in attributing V_d rather than the permeational ones.

Logarithmic transformation tends to improve the correlations marginally ($R^2 = 0.936$) but inverse transforms resulted in a distinct improvement in the correlation ($R^2 = 0.994$). Dependence on the nature of the descriptors remained similar for log-transformed values. However, for the inverse transformed values, increased dependence on WHIM descriptors (like G3e, G3P, G3m) was noted.

Earlier studies[16-18] have correlated volume of distribution to lipophilicity. Our results in the current studies, on the contrary, show dependence of V_d more on topological and electronic parameters than on lipophilic parameters. It can be very well explained on the basis of the involvement of ionic bonding and van der Waal's interactions that play a major role in tissue and protein binding thus affecting the V_d.[19] The primary reason for the difference in the outcomes might be the involvement of numerous descriptors of a varied nature in our study vis-à-vis only a limited number of mainly lipophilic descriptors involved in the earlier reports.

CONCLUSIONS

Highly significant results on *in silico* prognosis of V_d ($P < 0.001$) attributed major variation to the electronic and topological descriptor, vouching the dependence on the diffusional interactions. Chance correlations, if any, were ruled out in the light of high magnitudes of cross-validated variance, i.e. Q^2, obtained in the current QSPR studies. Pharmacokinetic performance of a drug is known to be not merely a function of its physicochemical nature but of the biological system(s) too, like somatic, psychological, pathological environmental, nutritional, genetic, hereditary and diurnal status of the human subjects. This causes a great deal of plausible variation in pharmacokinetic profiles among the volunteers/patients undergoing the study. The literature values of the pharmacokinetic parameters taken up in the present investigations pertain to diverse subject populations hailing from different age groups, genders, races, nutritional and physical attributes, etc. studied in different geographical regions under different weather conditions. Considering these potentially high intersubject and intrasubject variations among the pharmacokinetic parameters, the currently established relationships assume much higher credibility. It seems highly probable that the *in silico* approaches will evolve rapidly, as did the *in vitro* methods during the last decade. Past experience with the latter could be helpful in avoiding repetition of similar errors and in taking the necessary steps to ensure effective implementation of the former.

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