7-Substituted-4-hydroxyquinoline-3-carboxylic Acids as Inhibitors of Dehydrogenase Enzymes and of the Respiration of Ehrlich Ascites Tumor Cells: Multivariate Analysis and Quantitative Structure-Activity Relationship for Polar Substituents

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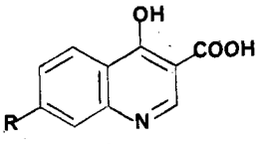
The inhibitory activities of a set of nine 7-substituted-4-hydroxyquinoline-3-carboxylic acids against three dehydrogenase enzymes and one whole cell system (Ehrlich ascites tumor cells) have been subjected to principal component analysis. The results clearly indicate that activity against the whole cell test system cannot directly be attributed to inhibition of the enzymes evaluated. The enzyme systems are reflected by the first component that can be identified with polar and steric parameters while hydrophobic effects are absent. The second component is entirely due to the inhibition of ascites cell respiration that depends primarily on hydrophobicity.

4-Hydroxyquinoline-3-carboxylic acids (HQCA) have been studied in several laboratories with the objective of developing inhibitors of dehydrogenase enzymes as potential antineoplastic agents.[1-8] Recently a set of 7-substituted-4-hydroxyquinoline-3-carboxylic acids (1) was designed[7,8] to encompass a very broad range in octanol/water partition coefficients (log P) to allow delineation of the relationship between hydrophobicity and cell membrane transport as reflected by the ability of selected congeners to inhibit the respiration of Ehrlich ascites tumor cell suspensions. The same set of compounds was evaluated for inhibition of three respiratory enzymes, rabbit muscle lactate dehydrogenase (M_4-LDH), pig heart cytoplasmic malate dehydrogenase (s-MDH), and pig heart mitochondrial malate dehydrogenase (m-MDH).[7,8] The



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objective was to establish quantitative structure-activity relationships (QSAR) in order to facilitate the design of selective and potent inhibitors of aerobic (m-MDH) and of anaerobic (M_4-LDH) cellular respiration. Although the effects of variation in physicochemical properties of the HQCA were successfully elucidated, the QSAR for the tumor cell system did not appear to be directly related to the QSAR for the three isolated enzyme systems. Closer examination of the reported data,[7,8] either by simple inspection of the biological activities or by plotting of partition coefficients against dehydrogenase enzyme and ascites activity, shows that the quinolines appear to fall into two clusters. One cluster, possessing low activity, contains the 13 more polar, nonaromatic substituents while the second cluster includes 18 aromatic substituents that introduce significant steric bulk and high hydrophobicity. It seems possible that, when conducting correlation analyses on the entire data set, the aromatic substituents may have concealed electronic and/or polar effects exerted by the smaller, polar substituents. Moreover, the two clusters, when combined, might have clouded interrelationships between the tumor cell system and the isolated enzyme systems. The problem was also complicated by the fact that not all congeners were active against all four biological test systems.

Consequently, we have selected the nine small congeners that do exhibit measurable activity against all systems, providing a data matrix for more detailed examination. This offers a distinct advantage in that the problem can now be treated as a multivariate one from the viewpoint of the biological activities, thus allowing the performance of factor and principal component analysis to assess relationships among the four activities. In addition, the principal components may be identified with physicochemical molecular parameters.

Methods

Table I summarizes the biological data while Table II contains substituent parameter values for the nine congeners employed in the analyses. The syntheses, biological activities, and π value determination have been previously reported.[7,8]

Principal Component Analysis. Principal component analysis (PCA) is a powerful method to decipher the number and nature of basis properties behind a data matrix (Table I) obtained from measurements of a set of objects (compounds; rows of the data matrix) in a number of systems (biological tests; columns of the data matrix). Basically, this analysis is a mathematical method of describing and reproducing the variables (pI_{50} values in the different tests) of the data matrix by means of a new set of "abstract" variables, the principal components (for details, see ref 9), on the basis of a linear model made up from a sum of products each comprising an object and a system component according to

$$y_{ij} = \sum_{k=1}^{r} a_{ik}x_{kj} + a_{i0} + \epsilon_{ij} \quad (1)$$

where $y_{ij} = pI_{50}$ value measured for the jth object (compound) in the ith system (biological test), ϵ_{ij} = residuals (experimental + model error), r = number of relevant components necessary to reproduce the data within the ϵ_{ij}, a_{ik} = ith element of the kth system component (characterizes the kth property of the ith system), x_{kj} = jth

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(1) Baker, B. R.; Bramhall, R. R. *J. Med. Chem.* **1972**, *15*, 230.
(2) Baker, B. R.; Bramhall, R. R. *J. Med. Chem.* **1972**, *15*, 233.
(3) Baker, B. R.; Bramhall, R. R. *J. Med. Chem.* **1972**, *15*, 235.
(4) Baker, B. R.; Bramhall, R. R. *J. Med. Chem.* **1972**, *15*, 237.
(5) Baker, B. R.; Bramhall, R. R. *J. Med. Chem.* **1972**, *15*, 937.
(6) Yoshimoto, M.; Hansch, C. *J. Med. Chem.* **1976**, *19*, 71.
(7) Shah, K. J.; Coats, E. A. *J. Med. Chem.* **1977**, *2*, 1001.
(8) Coats, E. A.; Shah, K. J.; Milstein, S. R.; Genther, C. S.; Nene, D. M.; Roesener, J.; Schmidt, J.; Pleiss, M.; Wagner, E.; Baker, J. K. *J. Med. Chem.* **1982**, *25*, 57.
(9) Franke, R.; Dove, S.; Kuehne, R. *Eur. J. Med. Chem.* **1979**, *14*, 363.

0022-2623/85/1828-0447$01.50/0

Table I. Biological Activity and Object Components for 7-Substituted-4-hydroxyquinoline-3-carboxylic Acids

no.	R	m-MDH[a]	s-MDH[b]	LDH[c]	ascites[d] obsd	ascites[d] pred[e]	x_{1j}[f]	x_{2j}[g]
2	SO_2CH_3	3.18 (3.14–3.22)[h]	3.36 (2.96–3.78)	3.39 (3.05–3.71)	2.75 (2.57–3.04)	2.67	0.625	0.023
3	OH	3.31 (3.19–3.47)	3.02 (2.37–3.77)	2.95 (2.76–3.14)	3.04 (2.67–4.17)	3.09	0.121	−0.021
4	Cl	2.44 (2.38–2.51)	2.33 (2.25–2.35)	2.74 (2.53–2.91)	3.84 (3.68–4.15)	3.55	−1.167	0.311
5	COOH	2.97 (2.74–3.52)	2.89 (1.50–3.76)	2.91 (2.53–3.29)	2.24 (2.18–2.30)	2.14	−0.036	−0.626
6	SO_3^-	2.67 (2.43–3.21)	2.51 (2.30–2.72)	3.06 (2.91–3.20)	2.88 (2.71–3.18)	1.60[e]	−0.471	−0.173
7	SO_2NH_2	3.02 (2.99–3.05)	3.16 (3.01–3.30)	3.24 (3.07–3.41)	2.47 (2.40–2.54)	2.65	0.352	−0.274
8	$COCH_3$	3.04 (3.03–3.05)	3.04 (2.93–3.25)	3.25 (2.94–3.53)	3.10 (2.90–3.48)	3.12	0.170	0.153
9	NO_2	2.72 (2.59–2.91)	2.92 (2.91–2.93)	3.02 (2.91–3.12)	3.24 (3.14–3.36)	3.42	−0.309	0.114
10	$N(CH_3)_2$	3.32 (3.29–3.35)	3.43 (2.76–4.07)	3.50 (3.39–3.62)	3.33 (3.24–3.44)	3.43	0.716	0.492

[a] Log $1/I_{50}$ in moles/liter for pig heart mitochondrial malate dehydrogenase (ref 7). [b] Log $1/I_{50}$ for pig heart cytoplasmic malate dehydrogenase (ref 8). [c] Log $1/I_{50}$ for rabbit muscle lactate dehydrogenase (ref 8). [d] Log $1/I_{50}$ for Ehrlich ascites cell respiration (ref 7). [e] Predicted by eq 9; compound 6, SO_3^- omitted. [f] First object component. [g] Second object component. [h] 95% confidence intervals.

Table II. Parameters Used in Multiple Regression Analysis

no.	R	π[a]	MR[b]	L[c]	B_4[c]	μ[d]
2	SO_2CH_3	−1.39	1.349	4.37	3.15	4.123
3	OH	0.06	0.285	2.74	1.93	5.371
4	Cl	0.55	0.603	3.52	1.80	3.130
5	COOH	−2.80	0.693	3.91	2.66	4.011
6	SO_3^-	−4.76	1.038	3.50	2.70	2.103
7	SO_2NH_2	−1.36	1.228	3.82	3.07	4.379
8	$COCH_3$	−0.39	1.118	4.06	2.93	3.653
9	NO_2	−0.40	0.736	3.44	2.44	1.097
10	$N(CH_3)_2$	1.10	1.555	3.53	2.80	5.942

[a] Calculated from apparent log P values (ref 7). [b] Molar refractivity, scaled by 0.1. (ref 15). [c] Taken from ref 15. [d] Dipole moments calculated in this study.

element of the kth object component (characterizes the kth property of the jth compound), and a_{i0} = mean of the ith column of the data matrix.

The system as well as the object components are calculated from the eigenvalues and eigenvectors of the correlation matrix[10] with use of the program PCANEW.[11] In this program, the number, r, of relevant components is evaluated with the help of Malinowski's indicator function[12,13] which passes either through a minimum at the correct r or shows, at least, a distinct (significant) increase only at a value of $r + 1$.

When the relevant principal components have been extracted, the principal component analysis as such is essentially complete. At this stage, however, no interpretation of the result is possible since the components are only abstract mathematical solutions for data reproduction. The object components are therefore correlated in the next step with the original variables, y_{ij} (columns of the data matrix). The corresponding correlation coefficients, r_{ik}, characterize the contribution of the kth principal component to the ith variable. If the same basic effect operates in different biological test systems, this will be reflected by high correlations with the same principal component. Differences between test systems are, on the other hand, characterized by high values of r_i in different principal components. A unique system usually affords an additional principal component. The relatedness of the tests can also be visualized from a plot of the systems in a coordinate system of the first two principal components, provided that these represent a sufficient part of the data variance. To make the correlation structure more obvious, the coordinates are represented by the elements of the eigenvectors of the correlation matrix that correspond to

Table III. Correlation Matrix of Molecular Parameters

	π	L	B_4	μ	MR
π	1				
L	−0.533	1			
B_4	−0.460	0.796	1		
μ	0.154	−0.122	0.160	1	
MR	0.054	0.631	0.796	0.289	1

standardized test system components. In this way the biological tests are grouped with respect to their similarity or, in other words, the similarity of basic effects operating in them. Up to this point it is not possible to clarify what these effects are. For this purpose the object components are identified with physicochemical parameters by multiple regression analysis with the components as dependent and the parameters as independent variables. The following parameters were used: hydrophobic substituent constant π, STERIMOL parameters L and B_4, which characterize the length and maximal width of substituents,[14] respectively, molar refractivity,[15] net charges for selected atoms of the quinoline ring, and the global dipole moment μ obtained from quantum chemical calculations. These parameters, except for the atomic charges that did not make significant contributions to any of the correlations considered, are summarized in Table II, and Table III shows the corresponding correlation matrix. It should be noted from Table III, that significant covariance between MR, L, and B_4 does exist as might be expected as these are all steric parameters. This precludes any attempt to combine these parameters in a single relationship. Fortunately, μ is not covariant with other parameters and π does not correlate with MR. Thus it is possible to distinguish electronic, steric, and lipophilic influences in the analysis. For reasons to be discussed later, a Hansch analysis was also performed

(10) Harman, H. H. "Modern Factor Analysis", 2nd ed.; University of Chicago Press: Chicago, 1970.
(11) Dove, S. "PCANEW—Ein Programm zur Ermittlung und Identifikation von Hauptkomponentan"; Institute of Drug Research, Berlin, 1980.
(12) Malinowski, E. R. *Anal. Chem.* **1977**, *49*, 606.
(13) Malinowski, E. R. *Anal. Chem.* **1977**, *49*, 612.
(14) Verloop, A.; Hoogenstraaten, W.; Tipker, J. In "Drug Design"; Ariens E. J., Ed.; Academic Press: New York, 1976; Vol. 3, p 165.
(15) Hansch, C.; Leo, A. "Substituent Constants for Correlation Analysis in Chemistry and Biology"; Wiley: New York, 1979.

Table IV. Results of Principal Component Analysis for the Four Test Systems

i	test system	a_{i0}	a_{i1}	a_{i2}	r_{i1}[a]	r_{i2}[b]	r_{1+2}[c]
1	m-MDH	2.963	0.469	0.029	0.91	0.03	0.92
2	s-MDH	2.962	0.596	0.135	0.97	0.12	0.98
3	LDH	3.118	0.362	0.225	0.87	0.30	0.92
4	ascites	2.988	−0.383	1.262	−0.47	0.87	0.99

[a] Correlation coefficient between the ith test system (ith column of the data matrix) and the first object component, x_1. [b] Correlation with the second object component, x_2. [c] Multiple correlation with both object components.

with the experimental data of Ehrlich ascites inhibition directly.

Some problems arose with the SO_3^- substituent since it has to be assumed to be in its uncharged form (SO_3H) for a reliable calculation of the dipole moment. Since this is not very likely under the conditions of the tests considering the pK_a of this group, all equations involving the dipole moment were calculated with and without the SO_3^--substituted compound. This compound was also omitted from the relationships with B_4 since a B_4 value of the SO_3H group is not available. As the results will show, the only effect of omitting this substituent from the correlations with the first object component is that electronic effects become somewhat less overt while the general QSAR picture does not change. In the correlation with the second component, omission of the SO_3^--substituted compound leads to the elimination of an apparently artificial π^2 term.

Calculation of Atomic Net Charges and Dipole Moments. Atomic net charges and the global dipole moment of the molecules were calculated by the semiempirical CNDO/2 method in its standard parameterization.[16] The structure of the quinoline molecule was optimized under the constraints of planarity and fixed carbon–hydrogen bond length (R_{C-H} = 0.108 nm = constant). For this purpose a program[17] that uses curvilinear internal coordinates[18] was employed. The structures of the substituents attached to the quinoline moiety were evaluated from standard geometry data.[16] In order to get a reliable (invariant) result for the dipole moment of the SO_3-substituted compound, this substituent had to be assumed to be in its protonated, uncharged form (SO_3H).

Results

Two components are necessary to reproduce the data within ϵ_{ij} accounting for, respectively, 69% and 22% of the data variance. This follows from the eigenvalues of the correlation matrix (λ_1 = 2.752, λ_2 = 0.873, λ_3 = 0.312, λ_4 = 0.064) and from Malinowski's indicator function IND, which does not change significantly up to k = 2 (IND_0 = 0.02, IND_1 = 0.02, IND_2 = 0.03, IND_3 = 0.09). The x_{kj} are given in Table I, and Table IV summarizes the a_{i0} and a_{ik} as well as the correlation coefficients, r_{ik}, between the object components and the original variables (columns of the data matrix). The correlation coefficients show that the first component reflects enzyme inhibition in vitro, whereas the second one is afforded by the ascites system. It thus becomes evident that the ascites test is completely separate from the three enzyme tests such that these do not model ascites respiratory inhibition and do not provide information about the latter. The same conclusion can be drawn from Figure 1, where the four biological tests are represented as points in the space spanned by the standardized system components.

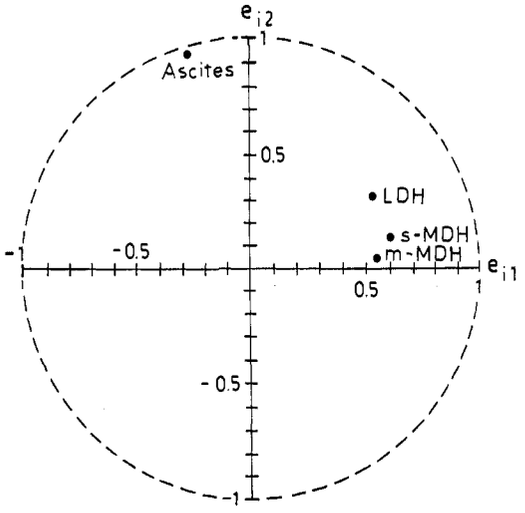

Figure 1. Plot of biological system components a_{i1} and a_{i2} from Table IV, on standardized scales e_{i1} and e_{i2}.

Obviously, the first object component, x_1, may be regarded as an average expression for the inhibition of all three enzymes with only a small contribution from the ascites system. Correlating x_1 with physicochemical parameters of the compounds yields the relationships given in eq 2–5, where n is the number of data points, r the correlation coefficient, s the standard deviation, and p the critical probability of error; the terms in brackets give the 90% confidence intervals.

$$x_1 = \underset{p = 0.03}{0.90\ (\pm 0.61)}\ B_4 - 2.28\ (\pm 1.61) \quad (2)$$

$$n = 8,\ r = 0.761,\ s = 0.419$$

$$x_1 = \underset{p = 0.12}{0.24\ (\pm 0.26)}\ \mu - 0.90\ (\pm 1.09) \quad (3)$$

$$n = 8,\ r = 0.592,\ s = 0.521$$

$$x_1 = \underset{p = 0.06}{0.25\ (\pm 0.22)}\ \mu - 0.93\ (\pm 0.86) \quad (4)$$

$$n = 9,\ r = 0.638,\ s = 0.483 (SO_3^- \text{ included})$$

$$x_1 = \underset{p = 0.05}{0.20\ (\pm 0.16)}\ \mu + \underset{p = 0.02}{0.81\ (\pm 0.46)}\ B_4 - 2.85\ (\pm 1.30) \quad (5)$$

$$n = 8,\ r = 0.902,\ s = 0.305$$

Equations 2–5 show that enzyme inhibitory potency depends on both steric and electronic effects with a slight dominance of the former. Potency increases with increasing dipole moment or electron-donating power of the substituents and with increasing substituent width.[19] Adding further parameters or squared terms to eq 5 does not result in a substantial or physically interpretable improvement so that the inclusion of other terms is not justified. In particular, no relation in which π makes a

(16) Pople, J. A.; Beveridge, D. L. "Approximate Molecular Orbital Theory"; McGraw-Hill: New York, 1970.
(17) Scharfenberg, P. "BIENE 2—Ein Programm zur Bestimmung der Gleichgewichtsgeometrie grosser Molekuele"; Institute of Drug Research, Berlin, 1975.
(18) Scharfenberg, P.; Sauer, J. *Int. J. Quant. Chem.* **1980**, *18*, 1309 and references therein.
(19) Everse, J.; Zoll, E. C.; Kahan, L.; Kaplan, N. O. *Bioorg. Chem.* **1971**, *1*, 207.

significant contribution could be found.

For the second component, the following relations were obtained:

$$x_2 = 0.26\ (\pm 0.08)\ \pi + 0.17\ (\pm 0.11) \quad (6)$$
$$p = 0.0001$$
$$n = 8,\ r = 0.931,\ s = 0.137$$

$$x_2 = 0.13\ (\pm 0.09)\ \pi + 0.14\ (\pm 0.18) \quad (7)$$
$$p = 0.02$$
$$n = 9,\ r = 0.735,\ s = 0.240\ (SO_3^-\ \text{included})$$

No relationships with significant contributions of steric or electronic parameters could be found. The inclusion of the SO_3^- derivative diminishes the goodness of fit (compare eq 6 and 7). Addition of a π^2 term to eq 7 does improve the correlation coefficient but is not statistically significant and is best regarded as an artifact.

The picture thus far obtained may be summarized as follows: the first component attributable to the isolated enzymes represents electronic and steric effects while the second component related to the ascites test only reflects substituent hydrophobicity. By means of a special target rotation of the object components onto the relationships obtained for x_1 and x_2 as "test vectors", it would, in principle, be possible to replace the abstract object components in eq 1 by steric, electronic, and hydrophobic substituent constants so that a set of QSARs is obtained. Because of the special data structure in the present case where the second component is practically irrelevant for the three enzymes, this would, however, not add information in comparison with eq 2–5. For the ascites test, the situation is a little different since here a small contribution of the first component (about 22% of the data variance) in addition to that of the second component is present as follows from the correlation coefficient r_{42} in Table IV. We therefore decided not to perform a global target rotation but rather to apply a Hansch analysis directly to the experimental data of ascites inhibition alone. The SO_3^--substituted derivative was omitted from this analysis because of the peculiar behavior of this substituent detected in the correlations with the second component (eq 6 and 7). The following relationships were obtained:

$$pI_{50} = 0.36\ (\pm 0.15)\ \pi + 3.21\ (\pm 0.20) \quad (8)$$
$$p = 0.004$$
$$n = 8,\ r = 0.883,\ s = 0.257$$

$$pI_{50} = 0.39\ (\pm 0.12)\ \pi - 0.12\ (\pm 0.001)\ \mu + 3.71\ (\pm 0.43) \quad (9)$$
$$p = 0.001 \qquad p = 0.06$$
$$n = 8,\ r = 0.949,\ s = 0.190$$

If the SO_3^- derivative is included, the fit of eq 9 becomes much poorer ($r = 0.568$), and as with the second object component, an artificial π^2 term is afforded ($r = 0.819$).

As was to be expected from eq 6 and 7, hydrophobicity dominates in the inhibition of Ehrlich ascites respiration, but a marginal electronic effect (reflected by the contribution of the first component to the ascites test) also exists. Equation 9 is completely different from the relationships obtained for the first component representing the isolated enzymes (eq 2–5) since no steric effect is present and the electronic effect, μ, is of the opposite direction. Thus, eq 9 again indicates that none of the three isolated enzymes is a primary target in the inhibition of Ehrlich ascites respiration by the compounds considered.

Discussion

Multivariate data analysis is a powerful tool in all cases where systems and objects are varied simultaneously. A clear picture of the whole data structure and the relatedness of the systems as well as of the number and physical nature of the underlying basic effects can be obtained. Although one certainly would like to have more data points (more objects investigated) and larger ranges of activity covered, the results clearly show that enzyme inhibition and inhibition of Ehrlich ascites respiration are not directly connected so that none of the enzymes can be the sole target for the latter. The three enzymes, on the other hand, are very similar in that their inhibition is governed by the same basic influences as expressed by the first component. As eq 2–5 show, these influences can be labeled as steric and polar effects, while hydrophobic interactions are absent. This is in keeping with earlier QSAR results for the inhibition of dehydrogenases by the title compounds, where the influence of variations in the 7-position on inhibitory potency was shown to be related with molar refractivity of the substituents.[6,7] Molar refractivity as a composite of substituent bulk and polarity represents both effects. It is clearly more advantageous and leads to a more straightforward interpretation if these effects are separated as in eq 2–5. If the first component is identified with molar refractivity instead of B_4 and μ, a much poorer although statistically significant relationship is obtained (eq 10).

$$x_1 = 0.92\ (\pm 0.82)\ MR - 0.82\ (\pm 0.83) \quad (10)$$
$$p = 0.07$$
$$n = 8,\ r = 0.670,\ s = 0.470$$

High values of the dipole moment are connected with high negative charges at the oxygen atoms of the 3-carboxyl group. Equations 3–5 are, therefore, consistent with the view that hydroxyquinoline carboxylates may interact with the enzymes via a mechanism similar to that observed for ternary complex formation between the oxidized substrate, coenzyme NADH, and the enzyme.[19] The carboxylic group of the HQCA could bind at the cationic arginine site of the dehydrogenase, which appears responsible for substrate carboxyl binding.

Equations 2–5 cannot directly be used for predictive purposes since they only describe and identify the first component and not the original pI_{50} values directly. It can be concluded, however, that substituents that produce a large dipole moment and, at the same time, possess sufficient width will afford the highest activity. This explains why the analogues with hydrogen or fluorine in the 7-position are inactive since both substituents are nonpolar and very small. More difficult to explain is the fact that no activity could be measured for the methoxy derivative. It might be that this compound is simply not soluble enough in order to reach the concentration range necessary to produce measurable enzyme inhibition.

The existence of one principal component reflecting enzyme inhibition implies a similar mechanism of binding in all three enzymes. It seems, however, that this is no longer true if the substituents in the 7-position become very large and lipophilic.[8] In the case of MDH such substituents seem to bind in a hydrophobic pocket[8] that is too far removed from the quinoline nucleus to be contacted by the small substituents considered in this study.

In contrast to the enzymes, inhibition of Ehrlich ascites respiration primarily depends on hydrophobicity. Whether the π term in eq 9 reflects transport or hydrophobic binding to an as yet unknown receptor site cannot be decided. In the light of the a_B- rule,[20] it is tempting to speculate, however, that this term might reflect hydro-

(20) Franke, R.; Kuehne, R. *Eur. J. Med. Chem.* **1978**, *13*, 399.

phobic interactions with the surface of a hydrophobic binding region since π-term slopes of about 0.5 are typical for such an event. If the substituents become very hydrophobic, however, the linear relationship with lipophilicity changes to a parabolic one,[8] indicating that at least for such substituents hydrophobic influences are more likely connected with transport processes.

It is evident from the results of this study that 4-hydroxyquinoline-3-carboxylic acids, containing relatively small substituents, interact in a very similar manner with the three dehydrogenase enzymes and that significant differences among the enzymes are only observed if one explores binding sites at greater distances from the quinoline nucleus. Since the analysis clearly does not reflect the observed activities of larger, nonpolar substituents, it appears that such substituents afford a shift in binding during the course of interacting at more unique sites on mitochondrial malate dehydrogenase in particular. More importantly, the principal component analysis has demonstrated that the inhibition of ascites cell respiration by the quinolines incorporating nonaromatic substituents does not result from inhibition of lactate dehydrogenase or the malate dehydrogenases as the primary targets, thus indicating the involvement of additional or alternative sites of action. Irrespective of the implications for the specific data evaluated in this investigation, the potential utility of multivariate techniques such as principal component analysis as tools for assessing the interrelatedness of multiple biological test systems has been further substantiated.

Selective Uptake of a Toxic Lipophilic Anthracycline Derivative by the Low-Density Lipoprotein Receptor Pathway in Cultured Fibroblasts

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N-(*N*-Retinoyl)-L-leucyldoxorubicin 14-linoleate (r11-DOX), a new lipophilic derivative of doxorubicin, was synthesized and incorporated into low-density lipoprotein (LDL). The drug–LDL complex contained 100–200 drug molecules/LDL particle. When cultured normal human fibroblasts were incubated with ^{125}I-LDL-incorporated drug, there was a perfect correlation between the cellular uptake plus degradation of ^{125}I-LDL and the cellular drug accumulation. The presence of excess native LDL inhibited the cellular uptake and degradation of ^{125}I-LDL and the drug accumulation to the same extent. In contrast, methylated LDL, which does not bind to the LDL receptor, did not alter the cellular uptake and degradation of ^{125}I-LDL nor did it alter the drug accumulation. When LDL receptor-negative fibroblasts from a patient with the homozygous form of familial hypercholesterolemia were incubated with the drug–^{125}I-LDL complex, cellular drug accumulation was very low. The drug–LDL complex inhibited the growth of cultured normal human fibroblasts. The drug incorporated into methylated LDL was much less toxic. These findings suggest that r11-DOX incorporated into LDL is delivered to cells selectively by the LDL receptor pathway. This might be of value in the treatment of leukemia, since we have previously found that leukemic cells exhibit higher LDL receptor activity than white blood cells and bone marrow cells from healthy subjects.

The main problem in cancer chemotherapy is not lack of activity but of selectivity. Thereby, toxic effects on normal cells limit the possibilities to treat patients. A possible way of reducing the undesired effects of antineoplastic drugs and concentrating the toxic effects to the malignant cells could be to link a potent anticancer agent to a carrier with a high affinity for the malignant cells. We have focused our attention on the possibility of using low-density lipoprotein (LDL) as a drug carrier in the treatment of leukemia.

LDL is the major cholesterol-carrying lipoprotein in human plasma. The spherical LDL particles with a diameter of 220 Å contain a lipid core of about 1500 cholesteryl ester molecules surrounded by a polar shell of free cholesterol, phospholipids, and protein.[1] Human cells express cell-surface receptors for LDL.[1] Once bound to its receptor, LDL is internalized and degraded in lysosomes. The lipid core of LDL yields unesterified cholesterol, which is used for membrane synthesis, whereas the protein part of LDL is degraded to amino acids.[1]

The reason why LDL is of special interest as a carrier for cytotoxic drugs is that leukemic cells isolated from patients with acute myelogenous leukemia have much higher LDL receptor activities (measured as the high-affinity degradation of ^{125}I-LDL) than normal white blood cells and nucleated bone marrow cells.[2] Gynecologic cancer cells also possess high LDL receptor activity both when assayed in monolayer culture and in membrane preparations from tumor-bearing nude mice.[3] Recently, an enhanced receptor-mediated uptake of LDL by tumor tissue in vivo was demonstrated in an animal model.[4]

The free and esterified cholesterol of LDL can be extracted with heptane and the particles can be reconstituted with exogenous hydrophobic compounds.[5] Such reconstituted LDL binds to the LDL receptor with the same affinity as native LDL and is internalized and degraded intracellularly. We took advantage of this fact and incorporated a very lipophilic cytotoxic agent, *N*-(trifluoroacetyl)adriamycin 14-valerate (AD 32) into LDL.[6] *N*-(Trifluoroacetyl)adriamycin 14-valerate is poorly soluble in water and has been withdrawn from further clinical trials because of lung toxicity due to the vehicle used to administer it—a mixture of ethanol and castor oil.[7] When white blood cells were incubated with the *N*-(trifluoroacetyl)adriamycin 14-valerate–LDL complex, cells with high LDL-receptor activity accumulated more drug than

(1) Brown, M. S.; Goldstein, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 3330.
(2) Vitols, S.; Gahrton, G.; Öst, Å.; Peterson, C. *Blood* **1984**, *63*, 1186.
(3) Gal, D.; Ohashi, M.; MacDonald, P. C.; Buchsbaum, H. J.; Simpson, E. R. *Am. J. Obstet. Gynecol.* **1981**, *139*, 877.
(4) Welsh, J.; Calmar, K. C.; Glegg, J.; Stewart, J. M.; Packard, C. J.; Morgan, H. G.; Shephard, J. *Clin. Sci.* **1982**, *63*, 44, abstract.
(5) Krieger, M.; Smith, L. C.; Andersson, R. G.; Goldstein, J. L.; Kao, Y.; Pownall, H. J.; Gotto, A. M., Jr.; Brown, M. S. *J. Supramol. Struct.* **1979**, *10*, 467.
(6) Vitols, S.; Gahrton, G.; Peterson, C. *Cancer Treat. Rep.* **1984**, *68*, 515.
(7) Blum, R. H.; Garnick, M. B.; Israel, M.; Canellos, G. P.; Henderson, I. C.; Frei, E. *Cancer Treat. Rep.* **1979**, *63*, 919.

0022-2623/85/1828-0451$01.50/0