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Short Communication

Lipophilic character of cardiac glycosides: correlation between R_M values and acute toxicity data in different animal species

G. L. BIAGI*, M. C. GUERRA, A. M. BARBARO and A. SAPONE
Istituto di Farmacologia, Università di Bologna, Bologna (Italy)
and
M. RECANATINI
Dipartimento di Scienze Farmaceutiche, Università di Bologna, Bologna (Italy)
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ABSTRACT

The R_M for a new series of cardiac glycosides were calculated by means of some of the ΔR_M values previously derived from another series of compounds. The experimental or calculated R_M values of both series of derivatives were correlated with the acute toxicity data (log $1/C$). The slopes of the linear equations for cats, dogs, guinea-pigs and frogs are very close, showing that the dependence of toxicity on the lipophilic character is the same in these animal species.

INTRODUCTION

The R_M values of cardiac steroids have been shown to represent a reliable measure of their lipophilicity [1–3]. In a previous paper [3] we reported the experimental and calculated R_M values for a series of cardiac steroids. The existence of good correlations between chromatographic parameters and *n*-octanol–water partition coefficients has been demonstrated [1–3]. In particular, a number of ΔR_M values were calculated, accounting for the lipophilic contribution of substituent groups either in the steroid skeleton or in the sugar side-chain. The ΔR_M values allowed the calculation of the R_M values of several compounds, which were included in the above correlations. The aim of that work was to collect a set of lipophilicity indices with a view to performing a quantitative structure–activity relationship (QSAR) study of cardiac steroids, as such studies are scarce.

Davydov *et al.* [4], Jinno [5] and Davydov [6] showed a linear relationship between reversed-phase high-performance liquid chromatographic (HPLC) retention

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data of cardiac glycosides and acute toxicity in cats. More recently, Dzimiri and co-workers [2,7] pointed out the influence of the lipophilic character, as expressed by chromatographic parameters, on the ATPase inhibition and the inotropic action on guinea-pig isolated left atria. The purpose of this work was to extend the set of available R_M values of cardiac glycosides in order to study the relationships between R_M values and acute toxicity data [4–6].

EXPERIMENTAL

The thin-layer chromatographic technique has been described previously [3]. Glass plates were coated with silica gel G (E. Merck, Darmstadt, Germany). A slurry of silica gel G was prepared with 0.09 *M* sodium hydroxide solution. A non-polar stationary phase was obtained by impregnating the silica gel layer with silicone DC 200 (Applied Science Labs., State College, PA, USA). The mobile phase was aqueous buffer (sodium acetate–Veronal buffer, 1/7 *M* at pH 7.2), alone or mixed with various amounts of acetone.

The previously reported ΔR_M values [3] were used in order to calculate the R_M values of the compounds listed in Table I. The lipophilic character of the parent structures is expressed by experimental R_M values and the ΔR_M values were used only for the substituent groups. The previously published R_M values of cardiac glycosides and the newly calculated values are reported in Table II. The acute toxicity of cardiac glycosides is due to their arrhythmogenic action causing cardiac arrest.

The classical method for determining the toxicity of cardiac glycosides is based on the continuation of intravenous infusion until cardiac systolic arrest occurs in anaesthetized cats, guinea-pigs, dogs, rabbits or pigs [8]. The smallest dose necessary to produce cardiac arrest in 60–90 min in 100% of the population is determined and the so-called MLD (minimum lethal dose) is calculated per kilogram body weight.

Different kinds of assay methods are used for the determination of lethal doses in non-anaesthetized frogs, mice and rats [8]. In frogs the principle of the assay method depends on the occurrence of cardiac systolic arrest in 1 h, brought about by the cumulative effect of successive increasing doses injected into the lymphatic sac. In mice and rats individual increasing doses are administered i.v. or i.p. After an observation period of 3–14 days, the median lethal dose (LD_{50}) is calculated statistically.

In this work we used the cat MLDs listed by Gisvold [9], Baumgarten [10] and Davydov *et al.* [4,6] or in the NIOSH registry [11]. They are reported in Table II as $\log(1/C)$ values, where C is the MLD expressed in 10^{-6} *M*/kg. In a similar way, Table II reports the $\log(1/C)$ values calculated from the lethal doses measured in guinea-pigs, dogs, frogs, mice and rats by means of the above assay methods [8,10–16].

RESULTS AND DISCUSSION

Jinno [5] examined the data of Davydov *et al.* [4] and showed a good linear relationship between the lipophilic character of sixteen out of a series of seventeen cardiac glycosides and their acute toxicity in cats. The equation was recalculated using the original HPLC $\ln V$ values of Davydov *et al.* [4] and their biological data transformed into log $1/C$ values:

$$\log(1/C) = 1.103\ (\pm 0.042) - 0.161\ (\pm 0.013)\ \ln V \quad (1)$$
$$(n = 16;\ r = 0.958;\ s = 0.060;\ F = 156.0;\ P < 0.005)$$

TABLE I

CALCULATION OF R_M VALUES OF CARDIAC GLYCOSIDES

No.	Compound			Calculation of R_M value: parent structure $(R_M)^a$ ± substituent group(s) $(\Delta R_M)^b$
	Generic name	Genin	Side-chain at C-3-β-OH	
1	Digoxigenin monoglucoside	Digoxigenin	D-Glucose	Digoxigenin (0.98) + glucosyl (−0.18)
17	Acetylneriifolin	Digitoxigenin	L-Thevetose + acetyl	Neriifolin (2.14) + acetyl (0.35)
19	Somalin	Digitoxigenin	D-Cymarose	Digitoxigenin (1.93) + cymarosyl (0.43)
20	Echubioside	Digitoxigenin	D-Cymarose + D-glucose	Digitoxigenin (1.93) + cymarosyl (0.43) + glucosyl (−0.18)
21	Echujin	Digitoxigenin	D-Cymarose + 2 D-glucose	Digitoxigenin (1.93) + cymarosyl (0.43) + 2 glucosyl (−0.36)
18	Diacetylneriifolin	Digitoxigenin	L-Thevetose + 2 acetyl	Neriifolin (2.14) + 2 acetyl (0.70)
8	Digitoxigenin monoglucoside	Digitoxigenin	D-Glucose	Digitoxigenin (1.93) + glucosyl (−0.18)
26	Deslanoside B	Gitoxigenin	3 D-Digitoxose + D-glucose	Lanatoside B (3.12) − acetyl (0.35)
30	Lanatoside E	16-Formylgitoxigenin	3 D-Digitoxose + D-glucose + acetyl	16-Formylgitoxin (2.50) + glucosyl (−0.18) + acetyl (0.35)
34	Hongheloside A	Oleandrigenin	D-Cymarose	Oleandrigenin (1.58) + cymarosil (0.43)
35	Hongheloside C	Oleandrigenin	D-Cymarose + D-glucose	Oleandrigenin (1.58) + cymarosyl (0.43) + glucosyl (−0.18)
36	Honghelin	Oleandrigenin	L-Thevetose	Oleandrigenin (1.58) + thevetosyl (0.21)
32	Urechitoxin	Oleandrigenin	L-Oleandrose + D-glucose	Oleandrin (2.34) + glucosyl (−0.18)
47	Strophanthidin monoglucoside	Strophanthidin	D-Glucose	Strophanthidin (0.94) + glucosyl (−0.18)
41	Convalloside	Strophanthidin	L-Rhamnose + D-glucose	Convallatoxin (1.29) + glucosyl (−0.18)
43	Cheirotoxin	Strophanthidin	D-Gulomethylose + D-glucose	Strophanthidin (0.94) + gulomethylosyl (0.35)[c] + glucosyl (−0.18)
53	Glucoscillaren A	Scillarenin	L-Rhamnose + 2 D-glucose	Proscillaridin (2.15) + 2 glucosoyl (−0.36)
55	Lanatoside D	Gitaloxigenin	3 D-Digitoxose + D-glucose + acetyl	Lanatoside A (3.38) + 16-OH(−0.19) + 12-OH(−1.03)

[a] Experimental R_M values; see ref. 3.

[b] See ref. 3.

[c] As gulomethylose is an isomeric form of rhamnose, the ΔR_M value of the latter was used; see ref. 3.

TABLE II

R_M VALUES AND ACUTE TOXICITY DATA FOR CARDIAC GLYCOSIDES

No.	Compound	R_M	Acute toxicity [log (1/C)[a]]					
			Cat	Dog	Guinea-pig	Frog[h]	Mouse (i.p.)[j]	Rat (i.v.)[j]
1	Digoxigenin monoglucoside	0.80	0.74[b]	–	–	–	–	–
2	Digoxin	2.01	0.53[b]	0.59[g]	−0.01[i]	–	−0.84	−1.50
3	α-Acetyldigoxin	2.27	0.36[c]	–	−0.22[g]	–	–	–
4	β-Acetyldigoxin	2.38	0.35[c]	–	−0.23[i]	–	–	–
5	Lanatoside C	2.19	0.63[b]	–	−0.02[i]	–	−0.85	−1.48
6	Deslanoside C	1.86	0.62[b]	–	–	–	–	–
7	β-Methyldigoxin	2.48	0.48[d]	–	–	–	–	–
8	Digitoxigenin monoglucoside	1.75	0.65[b]	–	–	–	–	–
9	Digitoxigenin monodigitoxoside	2.23	0.27[e]	–	–	–	–	–
10	Digitoxigenin bisdigitoxoside	2.65	0.26[e]	–	–	–	–	–
11	Digitoxin	3.18	0.36[b]	0.18[g]	−0.33[i]	−0.76	−0.71	−0.74
12	α-Acetyldigitoxin	3.54	0.26[b]	–	−0.45[i]	–	–	–
13	β-Acetyldigitoxin	3.53	0.22[c]	–	–	–	–	–
14	Lanatoside A	3.38	0.43[b]	–	−0.05[g]	–	−1.31	−1.22
15	Evomonoside	2.28	0.27[c]	–	–	–	–	–
16	Neriifolin	2.14	0.43[c]	–	–	–	–	–
17	Acetylneriifolin	2.49	0.58[c]	–	–	–	–	–
18	Diacetylneriifolin	2.84	0.02[c]	–	–	–	–	–
19	Somalin	2.36	0.25[b]	–	–	–	–	–
20	Echubioside	2.18	0.37[c]	–	–	–	–	–
21	Echujin	2.00	0.45[c]	–	–	–	–	–
22	Gitoxin	3.00	0.29[b]	–	−0.31[c]	−1.04	–	–
23	α-Acetylgitoxin	3.35	0.20[c]	–	–	–	–	–
24	β-Acetylgitoxin	3.33	0.13[c]	–	–	–	–	–
25	Lanatoside B	3.12	0.40[b]	–	−0.57[g]	–	–	–
26	Deslanoside B	2.77	0.23[b]	–	–	–	–	–
27	Pentaacetylgitoxin	4.59	–	–	–	–	−0.81	−1.33
28	16-Acetylgitoxin	2.93	0.19[b]	–	–	–	−0.92	−1.30
29	16-Formylgitoxin	2.50	0.05[c]	–	–	–	–	–
30	Lanatoside E	2.67	0.23[c]	–	–	–	–	–

31	Oleandrin	2.34	0.46[c]	–	–	−0.59	–	–
32	16-Desacetyloleandrin	2.44	0.25[b]	–	–	–	–	–
33	Urechitoxin	2.16	0.31[c]	–	–	–	–	–
34	Honghelosíde A	2.01	0.17[c]	–	–	–	–	–
35	Honghelosíde C	1.83	0.31[c]	–	–	–	–	–
36	Honghelin	1.79	0.40[b]	–	–	–	–	–
37	Cymarin	1.34	0.62[b]	0.41[h]	–	−0.33	−1.34	−1.56
38	K-Strophanthin-β	1.02	0.74[b]	0.81[h]	0.03[c]	−0.08	−0.69	−1.29
39	K-Strophanthoside	0.99	0.67[b]	–	0.23[c]	–	–	–
40	Convallatoxin	1.29	0.84[b]	–	0.12[c]	–	−1.26	−1.46
41	Convalloside	1.11	0.51[b]	–	–	–	–	–
42	Desglucocheirotoxin	1.29	0.74[c]	–	–	–	–	–
43	Cheirotoxin	1.11	0.77[b]	–	–	–	–	–
44	Olitoriside	1.28	0.71[f]	–	–	–	–	–
45	Helveticoside	1.21	0.74[g]	–	−0.18[g]	–	−1.16	−2.00
46	Erysimoside	1.03	0.64[c]	–	–	–	–	–
47	Strophanthidin monoglucoside	0.76	0.99[b]	–	–	–	–	–
48	Cymarol	1.19	0.74[b]	–	–	–	–	–
49	Convallatoxol	1.11	0.79[c]	–	–	–	−1.73	−2.00
50	Ouabain	0.53	0.81[c]	1.07[g]	0.36[i]	−0.20	−1.27	−1.53
51	Proscillaridin	2.15	0.53[b]	–	−0.08[i]	–	–	–
52	Scillaren A	1.97	0.54[g]	0.12[h]	0.21[c]	–	–	–
53	Glucoscillaren A	1.79	0.70[c]	–	–	–	–	–
54	Peruvoside	1.68	0.56[c]	–	−0.11[g]	–	–	–
55	Lanatoside	2.16	0.39[c]	–	–	–	–	

[a] where $C = M \times 10^{-6}$/kg.
[b] Ref. 9.
[c] Ref. 10.
[d] Ref. 8.
[e] Ref. 12.
[f] Ref. 4.
[g] Ref. 11.
[h] Ref. 15.
[i] Ref. 13.
[j] Ref. 16.

For the same series of compounds, the R_M values in Table II allowed the calculation of a similar equation:

$$\log(1/C) = 1.010\ (\pm 0.044) - 0.211\ (\pm 0.020)\ R_M \quad (2)$$
$$(n = 15;\ r = 0.945;\ s = 0.069;\ F = 108.0;\ P < 0.005)$$

As the R_M value of corelborin-π was not available in the present chromatographic system, eqn. 2 is based on only 15 compounds.

When considering a larger series of cardiac glycosides and different animal species (Table II), eqns. 3–6 were calculated:

Cat:
$$\log(1/C) = 0.951\ (\pm 0.053) - 0.234\ (\pm 0.024)\ R_M \quad (3)$$
$$(n = 54;\ r = 0.805;\ s = 0.136;\ F = 96.0;\ P < 0.005)$$

Guinea-pig:
$$\log(1/C) = 0.346\ (\pm 0.102) - 0.209\ (\pm 0.044)\ R_M \quad (4)$$
$$(n = 17;\ r = 0.771;\ s = 0.162;\ F = 22.0;\ P < 0.005)$$

Dog:
$$\log(1/C) = 1.069\ (\pm 0.217) - 0.322\ (\pm 0.116)\ R_M \quad (5)$$
$$(n = 6;\ r = 0.812;\ s = 0.241;\ F = 7.72;\ P < 0.05)$$

Frog (*Rana temporaria*):
$$\log(1/C) = 0.083\ (\pm 0.137) - 0.307\ (\pm 0.064)\ R_M \quad (6)$$
$$(n = 6;\ r = 0.923;\ s = 0.156;\ F = 23.1;\ P < 0.01)$$

Although the correlation coefficient in eqn. 3, calculated with 54 compounds, is lower than that in eqn. 2, based on only 15 compounds, it is remarkable that the intercept and slope are similar in the two equations. The exclusion of the four most deviating compounds, *i.e.*, 14, 18, 29 and 34, would increase the correlation coefficient to 0.853. In a similar way, on excluding compounds 14, 45 and 52 from eqn. 4 the correlation coefficient increases to 0.935. In any event, the correlation coefficients in eqns. 3–6 are reasonable when it is considered that one is dealing with data from different laboratories for intact animals.

However, rather than the correlation coefficient for each equation it is more interesting here that the slopes of eqns. 2–6 are very similar. Although great care must be taken in discussing eqns. 5 and 6 on the basis of only six compounds, the similarity of the slopes of eqns. 3–6 shows that the dependence on the lipophilic character is very similar for the four animal species. The closeness of the slope of eqn. 6 to those of eqns. 3–5 is probably due to the fact that the assay method for the frog closely resembles the classical i.v. procedure used in cats, guinea-pigs and dogs. It can be noted that the slopes are negative. As it is well known that generally the relationship between biological activity and lipophilicity is parabolic, one must conclude that in the present instance one is dealing with the descending branch of a parabola. On the other hand, less lipophilic cardiac glycosides are not known.

As regards the intercepts, eqns. 3–6 show that the cat and dog are equally more

susceptible than the guinea-pig and the frog is much less sensitive to this kind of drug. The toxicity data used in calculating eqns. 2–6 were taken from several compilations. Therefore, one could argue that strain and seasonal differences, state of health, diet, etc., might influence the acute toxicity. However, the fact that similar linear relationships were found seems to indicate that lipophilicity plays a basic role, which is not masked by the above sources of variation.

Mice and rats are generally considered to be of little use for measuring the effects of cardiac glycosides because it is always uncertain whether death is due to cardiac arrest or to some extracardiac action, and paralyses and/or convulsions may occur [8]. The only set of homogeneous data on the effects of cardiac steroids in mice and rats seems to be that reported by Foerster *et al.* [16]. However, the acute toxicity data listed in Table II did not show any significant correlation with the R_M values (r = 0.433 in mice; r = 0.570 in rats). Here one should consider also the lipophilic requirements necessary for penetration into the central nervous system.

CONCLUSIONS

The role of lipophilic character in determining the biological activity of cardiac glycosides was questioned by Repke [17]. On the other hand, experimental evidence for a relationship between lipophilicity and *in vivo* and *in vitro* activity has been presented [2,4–7]. The present results confirm that lipophilicity significantly affects the acute toxicity of these compounds, as previously found by Davydov *et al.* [4,6] and Jinno [5]. It is interesting that eqn. 2, calculated with only fifteen compounds, is very similar to eqn. 3 with 54 derivatives. Another important point is that the same relationship holds also for other animal species, such as the guinea-pig, dog and frog, strongly suggesting a similar mechanism leading to the toxic effect. This aspect shows the importance of comparative studies, where the QSAR approach can be very useful in ascertaining the mechanism of action [18]. The lack of any significant relationship for mice and rats depends on the fact that in these animals the acute toxicity of cardiac glycosides is also due to their action on the central nervous system. Finally, this work shows again the usefulness of R_M and ΔR_M values in QSAR studies.

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