Ticrynafen Effect on Warfarin Protein Binding in Human Serum

Keyphrases □ Ticrynafen—effect on warfarin protein binding, human serum □ Protein binding—warfarin to serum protein, effect of ticrynafen, humans □ Warfarin—serum protein binding, effect of ticrynafen, humans □ Antihypertensive agents—ticrynafen, effect on warfarin protein binding, human serum □ Anticoagulants—warfarin, serum protein binding, effect of ticrynafen, humans

To the Editor:

Ticrynafen[1] [2,3-dichloro-4-(2-thienylcarbonyl)-phenoxyacetic acid] is a new antihypertensive, diuretic, and uricosuric agent (1). About 99.5% of this weak acid is bound to human serum proteins in the therapeutic drug concentration range (2). Serious drug interactions between ticrynafen and the coumarin anticoagulants ethyl biscoumacetate and acenocoumarol have been reported, with anticoagulant potentiation and hemorrhages (3–5). It has been suggested that these interactions resulted from anticoagulant displacement from serum protein binding sites by ticrynafen and that this might also occur with warfarin (2–5).

We determined the ticrynafen effect on warfarin protein binding in human serum over a wide range of ticrynafen concentrations. Blood was obtained from two healthy adult male donors, serum was separated and pooled, and racemic ^{14}C-warfarin (2 μg/ml) was added. Ticrynafen[2] was dissolved in 50 μl of ethanol, and pH 7.4 sodium phosphate buffer (0.134 *M*) was added to yield 5 ml of solution containing 10–200 μg of ticrynafen/ml. Ticrynafen-free buffer solutions contained the same concentration of ethanol. Plasma containing warfarin was dialyzed at 37° to equilibrium against these solutions. Warfarin was then extracted from both phases, separated from impurities and degradation products by TLC, and assayed by scintillation spectrometry as previously described (6, 7).

The results are shown in Table I. Warfarin, in the absence of ticrynafen, was 99.288% bound (results of three dialysis experiments were 99.265, 99.297, and 99.301%). Ticrynafen (10–200 μg/ml) had no apparent effect on warfarin serum protein binding. Essentially identical results were obtained when both warfarin and ticrynafen were added to serum (without ethanol), and these serums were dialyzed against buffer only. The warfarin concentration used in this study was in the upper therapeutic concentration range (8, 9); the concentrations of ticrynafen ranged from therapeutic (~10–40 μg/ml) to far above therapeutic concentrations (1, 2). Since both drugs are extensively protein-bound weak acids, displacement effects may occur at even higher concentrations of either compound or in diluted plasma or diluted albumin solutions.

The results indicate that warfarin will not be significantly displaced from serum protein binding sites by ticrynafen under the usual therapeutic conditions. They do not rule out other interactions such as inhibition of warfarin metabolism, direct effects of ticrynafen on the blood coagulation process, and effects of ticrynafen metabolites.

Table I—Effect of Ticrynafen on Warfarin Protein Binding in Human Serum [a]

Ticrynafen Concentration, μg/ml	Warfarin Free Fraction[b] × 100	Warfarin Free Fraction Ratio[c]
0	0.712	1.00
10	0.688	0.97
20	0.655	0.92
30	0.710	1.00
40	0.771	1.08
50	0.695	0.98
100	0.751	1.05
200	0.725	1.02

[a] Determined by equilibrium dialysis at 37°. Initial warfarin concentration was 2 μg/ml. [b] Mean of two or three determinations. [c] Free fraction divided by free fraction in the absence of ticrynafen.

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Comparison of Antineoplastic Activity of Aminoethylaminoanthraquinones and Anthracycline Antibiotics

Keyphrases □ Antineoplastic activity—aminoethylaminoanthraquinones, doxorubicin, daunorubicin, and cardiotoxicity □ Antineoplastic agents—aminoethylaminoanthraquinones, doxorubicin, daunorubicin, and cardiotoxicity □ Aminoethylaminoanthraquinones—antineoplastic activity and cardiotoxicity, compared to doxorubicin and daunorubicin □ Cardiotoxicity—evaluated, aminoethylaminoanthraquinones, doxorubicin, daunorubicin

To the Editor:

The anthracycline antibiotics doxorubicin and daunorubicin are among the most important antineoplastic agents studied in recent years. Both antibiotics demon-

[1] SKF-62698, Selacryn.
[2] Supplied by Smith Kline & French Laboratories, Philadelphia, Pa.

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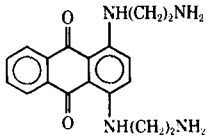
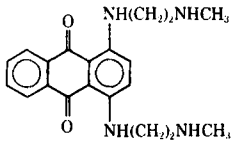
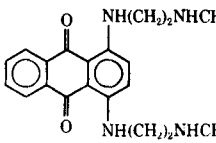
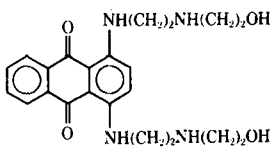
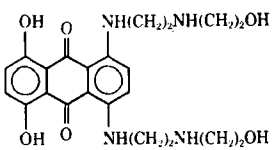
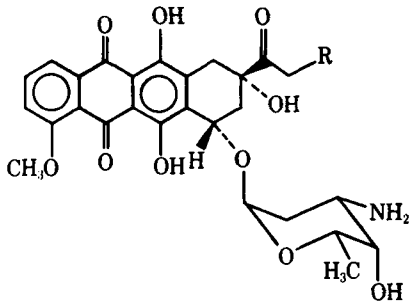
Table I—Antineoplastic Activity of Anthracycline Antibiotics and 1,4-Bis(substituted aminoethylamino)anthraquinones in Mice

Agent	NSC Number	L-1210 Leukemia			P-388 Leukemia			B16 Melanoma			C6 Colon			P-388/Doxorubicin (PA)		P-388/Daunorubicin (PB)	
		Dose, mg/kg	T/C, %	Cures	Dose, mg/kg	T/C, %	Cures	Dose, mg/kg	T/C, %	Cures	Dose, mg/kg	T/C, %	Cures	Dose, mg/kg	T/C, %	Dose, mg/kg	T/C, %
Doxorubicin hydrochloride	123127	4	291	2/8	2	254	2/8	6	189	3/10	10.5	205		10	87	6	92
		2	251	1/8	1	250		3	412	5/10	8.1	148	1/10	6	106	3.6	105
		1	129		0.5	177		1.5	412	9/10	6.3	197	1/10	3	102	2.16	105
		0.5	134					0.75	210	3/10							
								0.5	158	1/10							
Daunorubicin hydrochloride	82151	2	145		4	105		1	285	4/6	4	128		8	107	4.8	117
		1	126		2	224		0.5	285	3/6	2	124		4.8	121	2.88	117
		0.5	120		1	234		0.25	188					2.88	114		
					0.5	191		0.13	142								
					0.25	194											
	281246	25	272		16	243	1/8	8	218	4/10	16	227	1/10	21.6	87	21.6	87
		12.5	227		8	203		4	210	2/10	8	213	1/10	13	106	13	93
		6	156		4	194					4	175					
					2	185											
	291923	8	185		12.5	200	2/6	8	231	1/10	8	225	2/10	13	93	13	90
		4	150		4	175	1/6	2	230	2/10	4	215	2/10				
		2	146		2	183	1/6	1	150	1/10	2	219	1/10				
		1	134														
	276740	16	130		16	166	1/6	16	158		16	169		21.6	100	21.6	101
		8	128		8	174		8	157	1/10	8	166		13	93	13	102
		4	122		4	157		4	127		4	145	1/10				
	196473	16	250	3/6	25	517	7/8	8	280	6/10	8	286		21.6	104		
		8	220	2/6	16	416		4	248		4	215	2/10	13	105		
		4	157		6.25	189	2/8	2	186		2	139					
					3.2	239	3/8										
	279836	18	308	5/8	2	280	5/6	8	303	7/10	6	305	4/10	3.6	150	3.6	131
		10.8	261	1/8	1	450		2	317	7/10	3	324	7/10	2.16	135	2.16	134
		4	230	2/6	0.5	351	3/8	1	222	3/10	1	365	8/10			1.3	131
		2	223	2/6	0.25	280	2/6	0.5	262	8/10	0.5	365	6/10				
		1	274	2/6							0.25	359	4/10				
		0.25	196														



doxorubicin: R = OH
daunorubicin: R = H

strated inhibitory activity against a wide spectrum of experimental animal neoplasms and human cancers, including leukemias, lymphomas, and several solid tumors. These drugs possess some serious toxicities, of which the most troublesome is cardiotoxicity. The latter is characterized by a delayed and insidious cardiomyopathy with ECG abnormalities, cyanosis, dyspnea, and, in some cases, irreversible congestive heart failure (1). In spite of some studies indicating that preadministration of vitamin E, edetate disodium, 1,2-di(3,5-dioxopiperazin-1-yl)propane, *cis*-diamminedichloroplatinum, or ubiquinone$_{10}$ may relieve the cardiotoxicity (1–6), it remains a grave concern.

In 1970, the presence of a common N—O—O triangular feature among a number of antileukemic compounds, including doxorubicin and daunorubicin, was postulated (7). Based on this report, Adamson (8) proposed replacing the amino sugar portion of these antibiotics, believed to cause their cardiotoxicity, with amino-containing functions having the nitrogen atom placed at a proper spatial distance from the oxygen atoms of the aglycone portion. This arrangement would, hopefully, produce antineoplastic compounds with minimal cardiotoxicity.

In connection with a study of various amino- and hydroxyquinones as potential antineoplastic agents (9–12), Adamson's suggestion prompted the synthesis and structure–activity analysis of a number of bis(substituted aminoalkylamino)anthraquinones (13). Recent test data (14, 15) revealed that the biological activity profile of these compounds resembles that of doxorubicin and daunorubicin. In addition, most of these aminoanthraquinones were cross-resistant to the doxorubicin- and daunorubicin-resistant leukemia P-388 sublines (Table I), indicating antineoplastic action similar to the naturally occurring antibiotics. Perhaps due to a lipophilicity change of the ring-hydroxylated derivative, 1,4-dihydroxy-5,8-bis[[2-(hydroxyethyl)amino]ethyl]amino-9,10-anthracenedione, which may modify the cellular transport mechanism, a low level of antileukemic activity of this anthracenedione against the doxorubicin- and daunorubicin-resistant P-388 sublines was retained.

Table II—Minimum Cumulative Cardiotoxic Dose

Agent	NSC Number	P-388 Leukemia Optimum Dose in Mice, QD 1–9, mg/kg ip	Minimum Cumulative Cardiotoxic Dose in Rats, mg/kg ip/duration	Ratio
Daunorubicin	82151	9.73	14	1.44
Doxorubicin	123127	10.69	11	1.03
1,4-Bis[2-[(2-hydroxyethyl)amino]ethyl]amino-9,10-anthracenedione diacetate	287513	137.5	300	2.18
1,4-Dihydroxy-5,8-bis[[(2-hydroxyethyl)amino]ethyl]amino-9,10-anthracenedione	279836	8.3	18	2.17

Preliminary evaluation of the cardiotoxicity of the anthracenedione was conducted in rats (16, 17). The minimal cumulative cardiotoxic dose of this anthracenedione was 18 mg/kg. (It was somewhat less cardiotoxic than doxorubicin, whose minimal cumulative cardiotoxic dose was ~11 mg/kg.) The margin of safety (Table II) of the two anthracenediones, 1,4-bis[2-[(2-hydroxyethyl)amino]ethyl]amino-9,10-anthracenedione diacetate and 1,4-dihydroxy-5,8-bis[[(2-hydroxyethyl)amino]ethyl]amino-9,10-anthracenedione, was slightly larger than that of the two anthracyclines (daunorubicin hydrochloride and doxorubicin hydrochloride). The margin of safety is the ratio of the minimal cumulative cardiotoxic dose to the optimum therapeutic cumulative dose in the P-388 leukemia model.

Even though some interesting investigations on the syntheses of daunorubicin and doxorubicin have been conducted, the main supplies of these antineoplastic drugs still come from natural sources. The ease of synthesis of the aminoanthraquinones, their relatively low cardiotoxicity level, and their biological activity resemblance to the anthracycline antibiotics indicate that 1,4-dihydroxy-5,8-bis[[2-(hydroxyethyl)amino]ethyl]amino-9,10-anthracenedione and related compounds merit further oncological studies.

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Conformation, Partition, and Drug Design

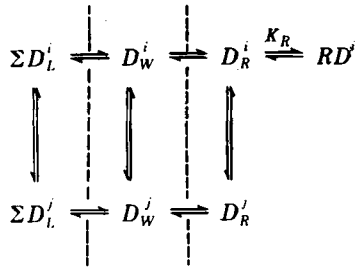
Keyphrases □ Conformations—drug, biological activity, fraction of conformer and partition coefficient □ Structure-activity relationships—drug conformers, biological activity, fraction of conformer and partition coefficient □ Partition coefficients—drug conformers, structure-activity relationships, biological activity

To the Editor:

Current interest in drug conformation rests on the postulate that a single preferred conformer binds to the receptor productively. If this conformer can be identified, it can then be fixed, or at least its population can be enhanced, by chemical means. In most areas of interest in drug design, however, drug–receptor complexes are not available for detailed molecular study, so the active conformation must be inferred. The usual procedure has been to seek correlations between conformation, determined experimentally or theoretically for a drug series, and biological response.

Until recently, the dominant conformer was assumed to be the biologically active agent (1). While there have been problems of agreement on the form of the dominant conformer (2), the widely variable responses that can occur even when the physical conformation is unambiguous (3) have encouraged the idea that a minor conformer may sometimes be responsible for activity (4–7). Thus, besides an interest in the nature of the active conformation, there is an interest in the fraction of active conformer in solution and in how it varies with chemical structure (5, 6).

An entirely different approach to drug design is based on multiparameter correlations of biological response with partition coefficients and other physical properties. This



Scheme I—Distribution of drug D *as active conformer* D^i *and inactive conformer(s)* D^j *between the aqueous phase* W, *the lipoidal loss phases* L, *the receptor phase* R, *and the receptor surface.*

quantitative structure–activity relationship approach (8) is used routinely by medicinal chemists in drug design (9). These two different approaches are almost invariably pursued in isolation. The purpose of this paper is to discuss the relation between them.

A flexible molecule behaves as many different molecules, depending on conformation. Different conformations have their own physical, chemical, biological, and thermodynamic properties. Of particular use is the concept of the micropartition coefficient, defined as the partition coefficient attaching to an individual conformer.

Consider a drug supplied as a single dose to a multiphase system (Scheme I). Regions of similar partition coefficient constitute a single phase even if anatomically separate. The model comprises an aqueous phase (volume V_W) and a receptor phase (volume V_R), together with N nonaqueous loss phases (devoid of receptors) with volumes V_L (L = 1–N). If biological activity resides in a single conformer D^i, its equilibrium concentrations in the various phases are $[D_W^i]$, $[D_R^i]$, and $[D_L^i]$, giving fractions of active conformer:

$$f_W^i = \frac{[D_W^i]}{[D_W]} \qquad \text{(Eq. 1a)}$$

$$f_R^i = \frac{[D_R^i]}{[D_R]} \qquad \text{(Eq. 1b)}$$

$$f_L^i = \frac{[D_L^i]}{[D_L]} \qquad \text{(Eq. 1c)}$$

Equilibration of the drug between the receptors and the receptor phase is defined by a binding constant:

$$K_R = \frac{[RD^i]}{[R][D_R^i]} \qquad \text{(Eq. 2)}$$

where $[R]$ and $[RD^i]$ are the concentrations of free receptors and productive drug–receptor complexes, respectively.

Suppose that drug distribution is much faster than degradation or elimination. Suppose also that the biological response is directly proportional to receptor coverage $[RD^i]$, that receptors are identical and independent, and that a negligible fraction of the total drug is bound to receptors. Receptor flexibility and the mechanism and kinetics of drug binding are outside the scope of this discussion. If the total dose is S, then:

$$S = V_W[D_W] + V_R[D_R] + \sum_{L=1}^{L=N} V_L[D_L] \qquad \text{(Eq. 3)}$$

$$S = [D_W]\left\{V_W + V_R P_R + \sum_{L=1}^{L=N} V_L P_L\right\} \qquad \text{(Eq. 4)}$$

where P_R and P_L are partition coefficients. The relationship $[D_W] = [D_R]/P_R$, taken with Eqs. 1 and 2, allows $[D_W]$ to be substituted, giving:

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