Biochemical Pharmacology. Vol. 27, pp. 1881–1882.

0006-2952/78/0715-1881$02.00/0

Studies on the mode of interaction of 4'-epi-adriamycin and 4-demethoxydaunomycin with DNA

(*Received* 24 *November* 1977; *accepted* 3 *March* 1978)

There is currently intense interest in the development of analogues of the antitumour antibiotics adriamycin and daunomycin [1, 2] in order to improve upon these drugs and especially to overcome their cardiotoxicity. 4'-Epi-adriamycin and 4-demethoxydaunomycin are two derivatives which are stated to have improved properties on experimental systems. The most significant property of 4'-epi-adriamycin is that it has no deleterious effect in cultured heart cells at 50 times the concentration at which adriamycin, 3 hr after administration, causes a halving in the beating rate [3]. In all other respects it is equivalent to adriamycin: it has equivalent activity against experimental tumours *in vivo* [3] and is equivalent as an inhibitor of RNA polymerase and DNA polymerase 1 *in vitro* [4]. The second compound, 4-demethoxydaunomycin, is remarkable in that it shows the same antitumour activity as daunomycin but at only 1/10th of the dose [5]. It has therefore a 10-fold increase in potency over the parent drug. Furthermore it shows a significantly greater degree of oral absorption than the parent drug[6]. All studies to date indicate that the two analogues bind to DNA in a similar manner to the parent drugs [4, 6, 7] and so, by inference, they intercalate into the DNA helix. This study presents further evidence to show that 4'-epi-adriamycin and 4-demethoxydaunomycin are equivalent to the parent drugs in their affinity for DNA, and to substantiate that they do intercalate into the DNA helix.

The binding of analogues of adriamycin and daunomycin to DNA can be monitored by the changes which occur in the absorption spectrum of the drug. Consequently, solutions of adriamycin, 4'-epi-adriamycin, daunomycin and 4-demethoxydaunomycin (about 5×10^{-6} M in 0.008 M Tris, 0.05 M NaCl buffer, pH 7.0) were prepared to contain about 5×10^{-5} M DNA. The DNA was added from a master solution which was assayed for DNA using the figure $\epsilon(P)_{260} = 6600$. The DNA–drug ratio (10:1) was such that there was an excess of intercalation sites, so that an intercalating drug would be fully bound. The spectra were compared to those from solutions containing no DNA. On binding to DNA, 4'-epi-adriamycin shows a 11 nm bathochromic shift in the λmax of the absorption peak at longest wavelength and there is a 48 per cent decrease in extinction at 480 nm. The corresponding values for adriamycin are a 11 nm shift and a 49 per cent decrease in extinction. Similarly, 4-demethoxydaunomycin on binding to DNA shows a 9 nm shift in the λmax of the absorption peak at longest wavelength, and a 47 per cent decrease in extinction at 480 nm; the figures for daunomycin being a 13 nm shift and a 40 per cent decrease in extinction. The magnitude of the changes shown by the analogues suggests a mode of binding similar to that of the parent drugs, namely intercalation. One feature of the intercalative mode of binding is that intercalated drug molecules are removed from the aqueous environment to a hydrophobic region and so will be unaffected by changes in pH. Basification of a drug–DNA mixture can therefore be used to examine the nature of the binding of anthracycline antibiotics. If a drug is not removed to a hydrophobic region, a bathochromic shift will be seen due to ionisation of the phenolic groups. Solutions of each drug (about 5×10^{-6} M) and DNA (about 5×10^{-5} M) were therefore prepared in pH 7.0 and pH 9.6 buffer, the DNA–drug ratio being such that all of an intercalating drug would be bound to DNA. For all four drugs the spectra were found to be identical in both buffers, in contrast to solutions containing no DNA where a change in pH from 7.0 to 9.6 gave a marked bathochromic shift in λmax (visible region of the spectrum). This strongly implicates intercalation as the mode of binding of the two analogues.

The change in extinction on binding to DNA can be used to determine the affinity of a drug for DNA providing an isosbestic point is shown. To test for the presence of an isosbestic point, six solutions were prepared for each drug, containing the same concentration of drug (about 5×10^{-5} M in 0.008 M Tris, 0.05 M NaCl buffer pH 7.0) and increasing amounts of DNA so that in the final solution the DNA–drug ratio was at least 15:1. Spectra were recorded over the range of maximum absorption in the visible region of the spectrum. Similar to the parent drugs, both analogues showed an isosbestic point, and so it was possible to use the spectrophotometric-titration method to determine the affinity constant (K) and number of sites (n) for the interaction of each drug with DNA. For each drug, three solutions each of 3 ml and initial concentration 5×10^{-5} M in 0.008 M Tris, 0.05 M NaCl buffer, pH 7.0, were titrated with DNA (total addition 1.0 ml of a 2.75×10^{-3} M solution of DNA in buffer in 22 aliquots of between 20 and 100 μl). The absorption of the solutions was monitored at the λmax of the unbound drug in the visible region of the spectrum against a blank containing an equal amount of DNA. The data were then fitted to a two-site binding model by non-linear regression analysis, with cumulative volume of DNA as the independent variable, by the method described elsewhere [8]. The values of K and n are quoted in Table 1 alongside values from other studies in which a direct comparison has been made between the analogue and its parent drug. The values obtained for the derivatives are very similar to those for the parent drugs, confirming earlier studies (Table 1) that the derivatives have an affinity for DNA similar to that of the parent drugs. It is important to note that differing buffers, differing experimental methods and differing methods of analysis have been used in the three studies reported in Table 1, and it is only valid to compare values obtained within one particular study.

The effect of the four drugs on the stabilisation of the DNA helix was next examined. Solutions were prepared containing drug (1.5×10^{-5} M) and DNA (1.5×10^{-4} M) in 0.003 M Tris, 0.018 M NaCl buffer, pH 7.0. The T_m was determined using a Pye-Unicam SP-1800 spectrophotometer with temperature programmer and the ΔT_m calculated by subtraction of the T_m in absence of drug. The values obtained are given in Table 1: clearly the analogues stabilise the DNA helix in a manner very similar to the parent drugs. This is consistent with their similar affinities for the DNA. No increased stabilisation by 4-demethoxydaunomycin compared to daunomycin was found, as had previously been reported [6].

All the above data suggest that the derivatives have a qualitatively and quantitatively similar mode of DNA-binding to that of the parent drugs. Final confirmation was obtained from fluorescence polarisation studies. Solutions of drug were prepared, assayed spectropho-

Table 1. Binding parameters and ΔT_m for the interaction of adriamycin, 4'-epi-adriamycin, daunomycin and 4-demethoxydaunomycin with DNA

Drug	$K M^{-1} \times 10^{-6}$	n	ΔT_m	Reference
Adriamycin	1.9*	0.25*	16.25†	This work
	0.4‡	0.18‡		[7]
	4.8§	0.18§	14.5‖	[4]
4'-Epi-adriamycin	1.9*	0.27*	15.0†	This work
	0.4‡	0.18‡		[7]
	2.2§	0.24§	12.5‖	[4]
Daunomycin	1.3*	0.20*	14.25†	This work
	3.3¶	0.18¶	13.0**	[6]
4-Demethoxydaunomycin	0.9*	0.21*	12.8†	This work
	2.4¶	0.20¶	17.0**	[6]

*0.008 M Tris, 0.05 M NaCl, pH 7.0: spectrophotometric titration.
†0.003 M Tris, 0.018 M NaCl, pH 7.0: drug–DNA ratio, 1:10.
‡0.1 M Tris, 0.15 M NaCl, pH 7.0: equilibrium dialysis.
§0.01 M Tris, 0.1 M NaCl, pH 7.0: spectrofluorimetric titration.
‖0.01 M Tris, pH 7.0: drug–DNA ratio, 1:10.
¶0.01 M Tris, 0.1 M NaCl, 0.5 mM EDTA, pH 7.0: spectrofluorimetric titration.
**0.01 M Tris, 0.01 M NaCl, pH 7.0: drug–DNA ratio, 1:10.

tometrically and diluted to approximately 2.5×10^{-6} M. Titration with DNA was then performed in an analogous manner to the spectrophotometric titration, continuing until 50:1, DNA–drug ratios were reached. Fluorescence polarisation was monitored using a Perkin-Elmer MPF-3L fluorescence spectrophotometer in the ratio mode with the cell maintained at 20°, the usual correction being made to account for spurious polarisation due to the instrument. In both cases the analogues mimicked the parent drugs. In solutions containing no DNA the fluorescence of the drugs is depolarised ($p = 0.10$) when irradiated with polarised light, due to Brownian motion. On titration with DNA this depolarisation effect was progressively reduced until at DNA–drug ratios of 50:1 the fluorescent light was significantly polarised (for adriamycin $p = 0.44$, for 4'-epi-adriamycin $p = 0.35$, for daunomycin $p = 0.32$ and for 4-demothoxydaunomycin $p = 0.29$). This indicates that the motion of the drugs is markedly reduced on binding to DNA, and the values of p obtained are indicative of intercalation; if any of the drugs were binding externally then the degree of polarisation would remain similar to the unbound drug ($p = 0.10$) [9]. In all four cases the fluorescence of the drug was markedly quenched on binding to DNA whereas this does not occur when the drug is externally bound on a nucleic acid [9].

In conclusion, the interactions of 4'-epi-adriamycin and 4-demethoxydaunomycin with DNA are qualitatively and quantitatively similar to the interactions of the parent drugs. The increased potency in experimental systems and the improved oral absorption of 4-demethoxydaunomycin must therefore be due to the increase in lipophilic nature on removal of the 4-methoxy group. This emphasises the importance of considering the cellular and disposition properties *in vivo* when designing anthracycline analogues. The molecular basis for the apparently reduced cardiotoxic effect of 4'-epi-adriamycin compared to adriamycin is not yet clear but it is certainly not due to a difference in the interaction with DNA.

Acknowledgement—We wish to thank Dr. F. Arcamone, Farmitalia, Milan for samples of adriamycin, 4'-epi-adriamycin, daunomycin and 4'-demethoxydaunomycin.

Department of Pharmacy, University of Manchester, Manchester M13 9PL, U.K.

TERENCE W. PLUMBRIDGE
JEFFREY R. BROWN

REFERENCES

1. D. W. Henry, in *Cancer Chemotherapy*, p. 15 (Ed. A. C. Sartorelli) American Chemical Society, Washington (1976).
2. F. Arcamone, *Lloydia* **40**, 45 (1977).
3. F. Arcamone, S. Penco, A. Vigevani, S. Redaelli, G. Franchi, A. Di Marco, A. M. Casazza, T. Dasdia, F. Formelli, A. Necco and C. Soranzo, *J. Med. Chem.* **18**, 703 (1975).
4. A. Di Marco, A. M. Casazza, R. Gambetta, R. Supino and F. Zunino, *Cancer Res.* **36**, 1962 (1976).
5. A. Di Marco, A. M. Casazza and G. Pratesi, *Cancer Treatment Reps.* **61**, 893 (1977).
6. F. Zunino, R. Gambetta, A. Di Marco, G. Luoni and A. Zaccara, *Biochem. biophys. Res. Commun.* **69**, 744 (1976).
7. E. Arlandini, A. Vigevani and F. Arcamone, *Il Farmaco, Ed. Sci.* **32**, 315 (1977).
8. T. W. Plumbridge, L. J. Aarons and J. R. Brown, *J. Pharm. Pharmac.* **30**, 69 (1978).
9. T. W. Plumbridge and J. R. Brown, *Biochim. biophys. Acta* **479**, 441 (1977).