Biochemical Pharmacology, Vol. 45, No. 7, pp. 1536–1539, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

Lack of discrimination between DNA ligases I and III by two classes of inhibitors, anthracyclines and distamycins

(*Received* 4 *June* 1992; *accepted* 18 *December* 1992)

Abstract—We have measured the effects of eight distamycin and two anthracycline derivatives on polynucleotide joining and self-adenylating activities of human DNA ligase I and rat DNA ligases I and III. All test drugs show good inhibitory activity against the three enzymes in the poly[d(A-T)] joining assay. Several distamycins also inhibit the DNA-independent self-adenylation reaction catalysed by the human enzyme and, to a lesser extent, by rat DNA ligases. These results confirm that anthracyclines and distamycins express their inhibitory action against DNA joining activities mainly via specific interactions with the substrate, and suggest that the three test DNA ligases utilize similar, if not identical, mechanisms of recognition and interaction with DNA–drug complexes. Our findings also indicate that distamycins have a greater affinity for human DNA ligase I than for rat enzymes, suggesting that, in this respect, rat DNA ligase I is more similar to rat DNA ligase III than to human DNA ligase I.

Multiple forms of DNA-joining activities have been described for higher eukaryotes [1–3]. The major DNA ligase activity in proliferating mammalian cells is DNA ligase I which shares several catalytic properties with prokaryotic enzymes: (1) the covalent binding of a molecule of AMP (from ATP or NAD) [1, 4], (2) the requirement for adjacent 3′-OH and 5′-PO_4 termini in duplex DNA to generate a new phosphodiester bond [4], (3) the capacity of unwinding supercoiled DNA in a topoisomerase-like reaction in the presence of AMP [5, 6], and (4) the sensitivity of the enzyme DNA complex to EDTA, giving rise to single strand breaks [5, 6]. Two minor forms of DNA ligase have been described that are dissimilar from DNA ligase I with regard to its physical and catalytic properties such as substrate specificity [2], cofactor stringency and inability of catalysing the AMP-dependent DNA relaxation [3–7].

In an attempt to increase our knowledge of the structural and catalytic properties of different DNA ligases we tested the ability of several drugs to inhibit different mammalian DNA ligase activities. Our results show that although the rat liver enzymes have different biochemical properties, they are similarly inhibited by test compounds suggesting that by this criterion, rat liver DNA ligase I is more similar to rat liver DNA ligase III than to human DNA ligase I.

Results

The inhibitory effects of drugs shown in Fig. 1 on DNA-joining and self-adenylating activities of human DNA ligase I [8] were tested in the poly[d(A-T)] circularization assay [9] and in the presence of [α-^{35}S]ATP [10], respectively (Table 1). The most striking features observed were: (a) the generalized efficacy of all distamycins in inhibiting the joining activity; (b) the presence of two distamycin derivatives very active in preventing the enzyme adenylation; (c) the lack of correlation between the inhibitory effects against the two test human DNA ligase I activities. In an attempt to find additional differences to discriminate between known DNA ligases, test drugs were also challenged against the DNA-joining and the self-adenylating activities of DNA ligase I and III purified as describe previously [3]. As shown in Table 1 distamycins inhibit to the same extent as rat DNA ligase I and III and human enzyme, while there is a noticeable difference between rat and human enzymes by the inhibition of the self-adenylating activity. The importance of drug–substrate interaction for the inhibition of polynucleotide joining activity of DNA ligases was evaluated on three different substrates: the poly[d(A-T)] [9], the poly(dA).oligo(dT)$_{16}$ and the poly(A).oligo(dT)$_{16}$ [11]. The results, presented in Table 2, clearly indicate that the alternating co-polymer is the best substrate to reveal the inhibition of the DNA-joining activity. The hybrid ribo-deoxyribo substrate, which can be utilized only by the form III [7], is evidently not recognized by the drugs.

Discussion

In the case of the DNA-joining activities the values of inhibition of the three enzymes indicate that distamycins are a family of good inhibitors of DNA ligases and suggest that drug–DNA complexes are recognized by means of similar mechanisms. The results obtained with the different substrates {poly[d(A-T)], poly(dA).oligo(dT)$_{16}$ and poly(A).oligo(dT)$_{16}$} are in agreement with the binding specificity of distamycin A for the three substrates {$\Delta\varepsilon$ ($M^{-1}cm^{-1}$) 17, 10 and 1.4 for poly[d(A-T)], poly(dA).poly(dT) and poly(A).poly(dT), respectively} [12]. These observations strongly suggest that a specific drug–DNA interaction is at the base of the joining activity inhibition by test drugs [9, 13, 14]. The homogeneity of distamycin behaviour excludes an influence by (a) the number of pyrrole units, (b) the presence of the strong basic group on the side chain, (c) the presence of a specific N-terminal group. However, the inhibitory effect could be related to the presence of a common group mimicking the effect of the amino group of anthracyclines [14] or to the stabilization of the B structure induced by distamycin analogues. On the contrary, the effect on the self-adenylating activity of the three enzymes shows a huge variation in inhibitory potency (>40-fold). In addition, while rat forms I and III show little sensitivity and a very similar pattern of inhibition, the human enzyme differs from them and is confirmed to be the DNA ligase activity most sensitive to distamycin derivatives [12]. Since the adenylation reaction takes place in the absence of DNA substrate these results should reflect a direct drug–protein interaction. Therefore our observations suggest that, at least from this point of view, there exists a greater similarity between the two forms of rat DNA ligases.

Table 1. Effects of test drugs on human DNA ligase I and rat DNA ligases I and III

	Compound	M_r	ε (M^{-1} cm^{-1})	Wavelength (nm)	Human DNA ligase I		Rat DNA ligase I		Rat DNA ligase III	
					Joining activity* ID_{50} (drug/bp)	Self-adenylation ID_{50} (μM)	Joining activity* ID_{50} (drug/bp)	Self-adenylation ID_{50} (μM)	Joining activity* ID_{50} (drug/bp)	Self-adenylation ID_{50} (μM)
I	Distamycin A	481.52	30,000 37,000	237 303	0.023 ± 0.004	240 ± 45	0.032 ± 0.008	>500	0.033 ± 0.007	>500
II	FCE24517 (296)	693.60	54,616	314	0.067 ± 0.015	26 ± 4	0.067 ± 0.015	110 ± 14	0.083 ± 0.015	125 ± 4
III	FCE25217 (373)	856.21	28,413 33,520	241 306	0.047 ± 0.009	30 ± 6	0.067 ± 0.009	60 ± 8	0.108 ± 0.010	70 ± 10
IV	FCE24558 (291)	620.66	34,291 45,446	242 308	0.117 ± 0.017	75 ± 10	0.050 ± 0.011	>500	0.060 ± 0.017	>500
V	FCE24662 (307)	737.09	30,707 40,441	243 316	0.068 ± 0.015	170 ± 35	ND	Ineff.	ND	Ineff.
VI	FCE24561 (294)	560.01	26,936 32,877	241 306	0.062 ± 0.011	300 ± 56	0.050 ± 0.010	>500	0.030 ± 0.007	>500
VII	ICE25946 (437)	867.17	34,847 53,018	240 312	0.022 ± 0.02	>1000	0.033 ± 0.007	>500	0.040 ± 0.009	>500
VIII	FCE25238 (377)	587.84	28,413 33,520	241 306	0.136 ± 0.023	>1000	ND	Ineff.	ND	Ineff.
IX	Doxorubicin.HCl	579.99	13,080	480	0.055 ± 0.006	690 ± 50	0.075 ± 0.018	>500	0.078 ± 0.017	>500
X	3′-Deamino-4′-deoxy-4′-epiamino-4-demethoxy-daunorubicin.HCl	533.97	13,080	482	0.170 ± 0.045	65 ± 17	ND	75 ± 19	ND	75 ± 17

* Measured on poly[d(A-T)].
ND, not done.
Ineff., ineffective or slightly stimulating.

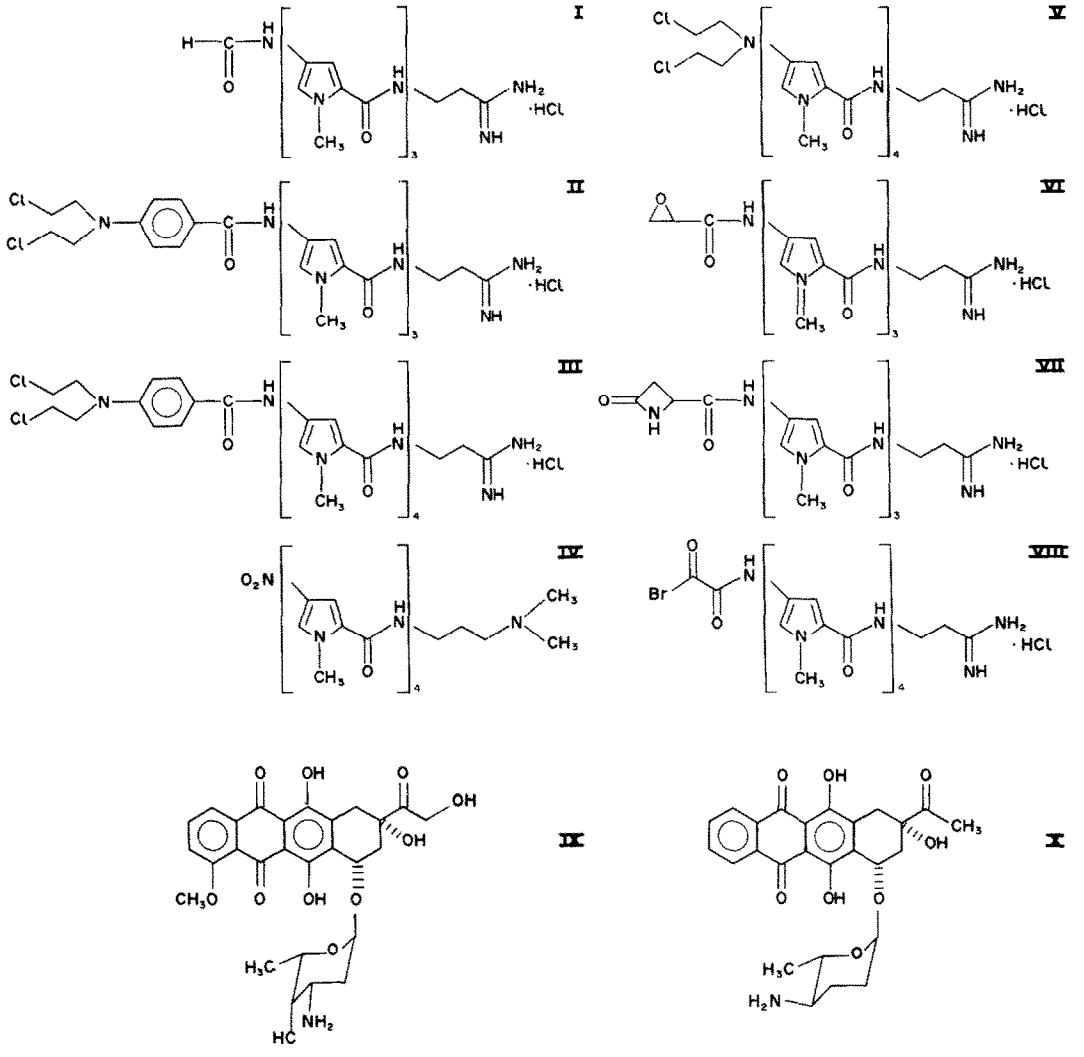

Fig. 1. Structural formula of drugs studied. Anthracycline and distamycin derivatives (HPLC grade) were kindly supplied by Dr A. Suarato and Dr N. Mongelli, Farmitalia-Carlo Erba (Milan, Italy). Stock solutions of drugs were prepared in dimethyl sulphoxide or methanol. The organic solvent in the assay never exceeded 1%. Drug concentrations were determined on the basis of extinction coefficients reported in Table 1.

Table 2. Role of the substrate on the inhibition by drugs of polynucleotide joining activities

	Rat DNA ligase I joining activity ID_{50} (drug/bp)		Rat DNA ligase III joining activity ID_{50} (drug/bp)		
Compound	poly[d(A-T)]	poly(dA)	poly[d(A-T)]	poly(dA)	poly(A)
I	0.032 ± 0.008	0.15 ± 0.03	0.033 ± 0.007	ND	ND
II	0.067 ± 0.015	1.15 ± 0.25	0.083 ± 0.015	ND	ND
IV	0.050 ± 0.011	ND	0.060 ± 0.017	ND	>10
IX	0.075 ± 0.018	0.98 ± 0.18	0.078 ± 0.017	1.05 ± 0.21	>10

ND, not done.

Acknowledgements—The authors are grateful to M. T. Chiesa for her technical assistance. This work was supported by grants of the Associazione Italiana per la Ricerca sul Cancro (AIRC) and of the PF "Ingegneria Genetica" of the CNR. A.M. was supported by a short-term EMBO fellowship.

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