Biochemical Pharmacology, Vol. 24, pp. 309–311. Pergamon Press, 1975. Printed in Great Britain.

The inhibition *in vitro* of DNA polymerase and RNA polymerases by daunomycin and adriamycin

(*Received* 28 *January* 1974; *accepted* 18 *June* 1974)

Adriamycin is a 14-hydroxy derivative of daunomycin. This structural modification has resulted in improved therapeutic effectiveness for adriamycin over daunomycin in a spectrum of animal tumor systems and in clinical trials [1]. It has been suggested that these compounds exert their cytotoxic activity by inhibition of nucleic acid synthesis [2]. Earlier studies [3, 4] indicated that both daunomycin and adriamycin bind to DNA. As expected, daunomycin interferes with the template properties of DNA [5–7].

In an attempt to determine whether the observed differential tumor susceptibility has also a biochemical basis, studies were undertaken to compare these antitumor antibiotics as inhibitors of nucleic acid synthesis *in vitro*.

The inhibition of the *M. lysodeikticus* DNA polymerase and *E. coli* RNA polymerase at various concentrations of antibiotics is shown in Fig. 1. Under conditions used, the rate of incorporation in control samples was nearly linear for the first 7–10 min, in the RNA polymerase reaction and for the first 12–15 min, in the DNA polymerase reaction. At an Mg^{2+} concentration of 6 mM, daunomycin inhibited calf thymus DNA-directed DNA synthesis and RNA synthesis to a similar extent. The requisite concentrations for 50 per cent inhibition of RNA and DNA polymerase reactions were 1×10^{-5} and $1{\cdot}8 \times 10^{-5}$ M, respectively, in these experiments. As the extent of inhibition of *E. coli* RNA polymerase by daunomycin is sensitive to the Mg^{2+} concentration in the *in vitro* assay system [7], we compared the results of inhibition experiments conducted at the same Mg^{2+} concentration. In addition, as the inhibitory effect of the drug appeared to be dependent on the antibiotic/DNA nucleotide ratio in the reaction mixture [7], we used similar template concentrations (80 and 100 μM calf thymus DNA, in the DNA polymerase and RNA polymerase system, respectively). When the inhibition data are compared, the percent inhibition is represented as a function of the antibiotic/DNA nucleotide molar ratio. Under the same conditions, adriamycin resembles daunomycin by inhibiting DNA and RNA synthesis about equally (Fig. 1). However, adriamycin is significantly more effective than daunomycin in inhibiting DNA synthesis and transcription. The effects of these anthracycline antibiotics on different nucleic acid polymerizing reactions are summarized in Table 1. However, it should be emphasized that different DNA polymerase preparations have somewhat different sensitivity to daunomycin (or adriamycin) inactivation.

Further studies were carried out to compare the effect of these antibiotics on the RNA synthesis directed by denatured DNA. The sensitivity of calf thymus DNA to daunomycin was changed drastically when this DNA was denatured (Fig. 2A). A 7-fold higher concentration of daunomycin was required to inhibit transcription of the denatured form by 50 per cent than was required to inhibit transcription of the native form. At the lower daunomycin level of 5–10 μM, no inhibition was observed; indeed, a slight stimulation was reproducibly observed. That the transcription of native DNA is more effectively inhibited than the transcription of denatured DNA is consistent with the physical

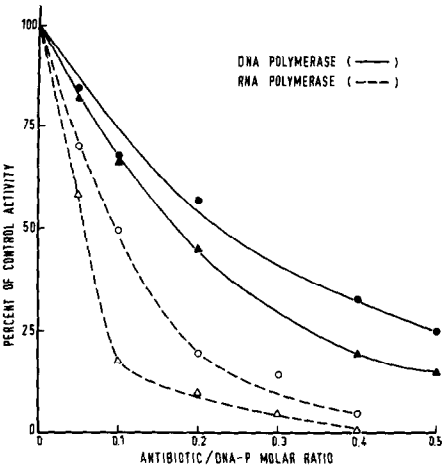

Fig. 1. Effect of daunomycin and adriamycin on DNA and RNA synthesis directed by calf thymus DNA. In the assay for RNA synthesis the reaction mixture contained 0·06 M Tris–HCl buffer (pH 7·9), 0·01 M KCl, 0·006 M $MgCl_2$, 0·015 M β-mercaptoethanol, 0·4 mM each of ATP, GTP, CTP and [^{3}H]UTP (10 Ci/mol), 100 μM calf thymus DNA. The reaction was initiated by the addition of 3·4 μg of *E. coli* RNA polymerase to the complete incubation mixture (final vol. 0·2 ml). DNA synthesis reaction mixtures (0·25 ml) contained 0·06 M Tris–HCl buffer (pH 7·4), 0·006 M $MgCl_2$, 0·010 β-mercaptoethanol, 0·4 mM each of dATP, dGTP, dCTP, [^{3}H]dTTP (10 Ci/mole), 80 μM activated native calf thymus DNA [15]. The reaction was initiated by the final addition of 0·5 unit of *M. lysodeikticus* DNA polymerase. The concentrations of all substrates and template (or primer-template) used were saturating in all reactions. Both RNA and DNA syntheses were terminated after 10 min incubation and the acid-insoluble material was measured [7]. The control assay for RNA synthesis (dashed line) incorporated 0·215 nmole of [^{3}H]UMP and that for DNA synthesis (solid line) incorporated 0·360 nmoles of [^{3}H]dTMP. The designations used are: (●, ○), daunomycin; (▲, △), adriamycin. *E. coli* RNA polymerase and *M. lysodeikticus* DNA polymerase were obtained from Miles Laboratories. ^{3}H-labeled nucleotides were purchased from New England Nuclear; unlabeled nucleotides from Boehringer Mannheim. Antibiotics were supplied by Farmitalia; solutions in water were freshly prepared immediately before use.

Table 1. Comparison of the effects of daunomycin and adriamycin on the activities of *M. lysodeikticus* DNA polymerase and *E. coli* RNA polymerase

Antibiotic	Concentration (μM) required for 50% inhibition of	
	DNA polymerase	RNA polymerase
Adriamycin	14	6
Daunomycin	18	10

data [4]. The affinity of the drug for denatured calf thymus DNA was much lower. As expected from the structural similarity of daunomycin and adriamycin, denatured DNA was less sensitive to inactivation by adriamycin (Fig. 2B) than native DNA. However, when equivalent molar concentrations of the drugs were compared, adriamycin inhibited the RNA synthesis directed by denatured DNA to a greater extent than does daunomycin; indeed, only 2-fold higher concentrations of adriamycin was required for 50 per cent inhibition of RNA synthesis with denatured DNA as template than with native DNA.

Studies on RNA polymerase activity of rat liver nuclei were undertaken in an attempt to find differences in the inhibitory effects caused by daunomycin and adriamycin. The regulation of gene expression (via transcription) in eukariotic cells appears to involve multiple forms of RNA polymerase [8]. On the basis of preferential stimulation, determination of Mg^{2+}- and $Mn^{2+}/(NH_4)_2SO_4$-activated RNA polymerase activity can be assumed to approximate ribosomal and messenger RNA synthesis, respectively. The inhibitory effects of daunomycin and adriamycin on RNA polymerase activities assayed in whole nuclei are compared in Fig. 3. In this figure, the effect of actinomycin D is shown for the purpose of comparison. As expected, daunomycin, a less potent inhibitor of the bacterial RNA polymerase [5] than actinomycin D, is also less potent than actinomycin D in inhibiting both Mg^{2+}-and $Mn^{2+}/(NH_4)_2SO_4$-activated RNA polymerase activities in isolated rat liver nuclei. In this system, daunomycin has very little effect. Factors affecting daunomycin and adriamycin inhibition have been not determined. Therefore, no definitive conclusions may be drawn

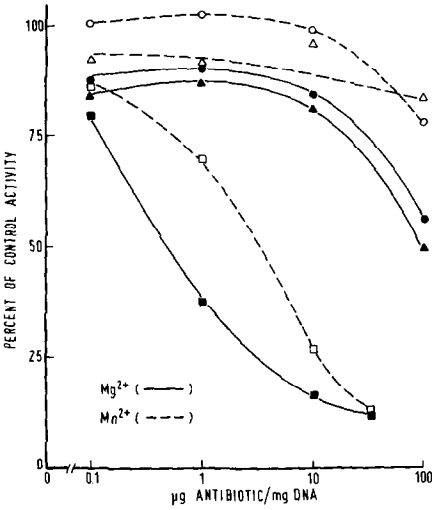

Fig. 3. Comparison of the inhibitory effect of daunomycin, adriamycin and actinomycin D on RNA polymerase activities of whole nuclei, assayed with Mg^{2+} or with Mn^{2+} plus ammonium sulfate. Nuclei were isolated from rat livers by the procedure described by Widnell and Tata [16] and suspended in 0·25 M sucrose, 1 mM $MgCl_2$ at a concentration of approx. 2·5 mg DNA/ml. Assay conditions also were based on those used by Widnell and Tata [8]. The assay mixture for the Mg^{2+}-activated enzyme was at 37° for 20 min in a final volume of 0·5 ml containing 100 mM Tris–HCl (pH 8·5), 10 mM DDT, 5 mM $MgCl_2$, 0·6 mM each of ATP, GTP, CTP; 0·06 mM UTP containing 2·5 μCi [^{3}H]UTP and 0·1 ml of nuclear suspension. The assay mixture for the $Mn^{2+}/(NH_4)_2SO_4$-stimulated enzyme was at 37° for 40 min, and contained all components required for the Mg^{2+}-dependent reaction, except that Mg^{2+} was replaced by $MnCl_2$ (4 mM) and $(NH_4)_2SO_4$ was included at a final concentration of 0·4 M. The buffer was 100 mM Tris–HCl (pH 7·5). Control incorporation: 484 pmoles UMP incorporated/mg DNA, for the Mg^{2+} activated enzyme (solid line); 2695 pmoles UMP incorporated/mg DNA for the $Mn^{2+}/(NH_4)_2SO_4$ activated enzyme (dashed line). (●, ○), daunomycin; (▲, △), adriamycin; (■, □), actinomycin D.

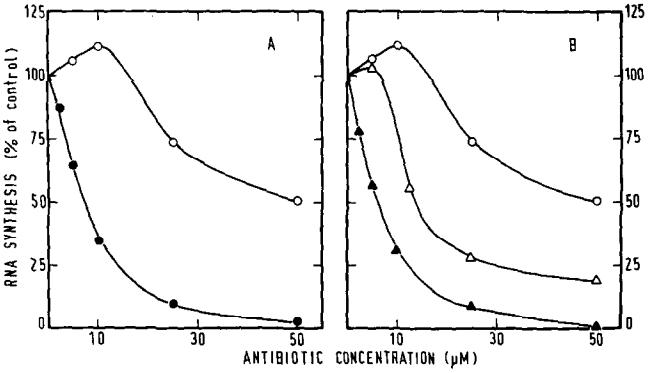

Fig. 2. Effect of daunomycin and adriamycin on transcription of native and denatured calf thymus DNA. RNA synthesis was measured in standard assay mixtures (Fig. 1). RNA synthesis was terminated after 5 min incubation. Calf thymus DNA was prepared as described [4] and was denatured by heating to 100° for 10 min and rapidly cooling in ice. (A) Effect of daunomycin on the transcription of native (●) and denatured DNA (○). (B) Effect of adriamycin on the transcription of native (▲) and denatured DNA (△).

from these experiments. However, that Mg^{2+}-activated RNA polymerase activity is more effectively inhibited by daunomycin than $Mn^{2+}/(NH_4)_2SO_4$-activated enzyme is consistent with the preferential inhibition of ribosomal RNA synthesis by this drug [9, 10]. In a similar manner, the Mg^{2+}-activated RNA polymerase activity is more susceptible to inhibition by adriamycin than is $Mn^{2+}/(NH_4)_2SO_4$-activated polymerase. However, at all concentrations tested, both RNA polymerases are significantly more sensitive to adriamycin than to daunomycin.

These data taken together with that showing interaction of adriamycin with DNA [4] suggest that the mechanism of action of adriamycin is similar to that of daunomycin. Daunomycin and adriamycin appear to act as inhibitors of both DNA and RNA synthesis. By inhibiting DNA and RNA synthesis to a similar extent, these drugs differ from actinomycin D and nogalamycin which preferentially inhibit RNA polymerase [5, 11]. Similar behaviour was observed in nucleic acid metabolism of mammalian cells in culture [12, 13]. Daunomycin inhibits both DNA and RNA metabolism to the same degree.

Under all conditions used, it is found that adriamycin is more effective than daunomycin in inhibiting DNA synthesis and transcription. The extent of inhibition of these drugs is consistent with the effect of the thermal transition temperature (Tm) of calf thymus DNA [4]. At a drug to DNA-P molar ratio of 0·1, the increase in Tm (ΔTm) produced by daunomycin was 13·4° compared with 14·8° produced by adriamycin.

It has been reported that in intact L1210 cells, daunomycin is apparently a more potent inhibitor of nucleic acid metabolism than adriamycin [13, 14]. Meriwether and Bachur [13] reported significant differences in cellular uptake of daunomycin and adriamycin. This probably plays a significant role in the inhibition of nucleic acid synthesis by these drugs in L1210 cells *in vitro*. After 1 hr incubation, the inhibition of DNA and RNA metabolism by daunomycin was significantly greater than with adriamycin. When incubations were continued for 2 hr at the higher drug levels (5 μM), the inhibitory effect of adriamycin was similar to that of daunomycin. However, when the cells were incubated with 5 μM antibiotics, at 2 hr the daunomycin uptake was approximately twice that of adriamycin.

These results are consistent with our observations indicating that the actual inhibitory effectiveness of adriamycin is greater than that of daunomycin.

Division of Experimental Oncology B,
National Cancer Institute,
Via Venezian, 1
20133 *Milano, Italy*

FRANCO ZUNINO
ROMOLO GAMBETTA
AURELIO DI MARCO

REFERENCES

1. A. Di Marco and L. Lenaz, in *Cancer Medicine* (Eds. J. F. Holland and E. Frei, III), p. 826. Lea & Febiger, Philadelphia (1973).
2. A. Di Marco, in *Antibiotics* (Eds. D. Gottlieb and P. D. Shaw). Vol. 1, p. 190. Springer, Berlin (1967).
3. E. Calendi, A. Di Marco, M. Reggiani, B. Scarpinato and L. Valentini, *Biochim. biophys. Acta* **103,** 25 (1965).
4. F. Zunino, R. Gambetta, A. Di Marco and A. Zaccara, *Biochim. biophys. Acta* **277,** 489 (1972).
5. K. Koschel, G. Hartmann, W. Kersten and H. Kersten, *Biochem. Z.* **344,** 76 (1966).
6. D. C. Ward, E. Reich and I. H. Goldberg, *Science N.Y.* **149,** 1259 (1965).
7. F. Zunino, A. Di Marco, A. Zaccara and G. Luoni, *Chem. Biol. Interactions* **9,** 25 (1974).
8. C. C. Widnell and J. R. Tata, *Biochim. biophys. Acta* **123,** 478 (1966).
9. L. E. Crook, K. R. Rees and A. Cohen, *Biochem. Pharmac.* **21,** 281 (1972).
10. K. Danø, S. Frederiksen and P. Hellung-Larsen, *Cancer Res.* **32,** 1307 (1972).
11. B. K. Bhuyan and C. G. Smith, *Proc. nat. Acad. Sci. U.S.A.* **54,** 566 (1965).
12. A. Rusconi and A. Di Marco, *Cancer Res.* **29,** 1507 (1969).
13. W. D. Meriwether and N. R. Bachur, *Cancer Res.* **32,** 1137 (1972).
14. J. J. Wang, D. S. Chervinski and J. M. Rosen, *Cancer Res.* **32,** 511 (1972).
15. A. Schlabach, B. Fridlender, A. Bolden and A. Weissbach, *Biochem. biophys. Res. Commun.* **44,** 879 (1971).
16. C. C. Widnell and J. R. Tata, *Biochem. J.* **22,** 313 (1964).