Biochemical Pharmacology, Vol. 26, pp. 1953–1954. Pergamon Press, 1977. Printed in Great Britain.

Effect of Daunomycin, Adriamycin and its congener AD 32 on the activity of DNase I from bovine pancreas

(*Received* 22 *February* 1977; *accepted* 14 *March* 1977)

The anthracycline antibiotics Adriamycin (AM) and Daunomycin (DM) (Fig. 1) are clinically effective cancer chemotherapeutic agents [1]. Intercalation with DNA and subsequent inhibition of nucleic acid synthesis have been generally credited with a major role in their cytotoxic mode of action [2]. However, inhibited tumor cell proliferation has been reported under conditions in which no effect on nucleic acid biosynthesis was observed [3]. Therefore other effects of these drugs, such as induction of DNA breaks or interference with membrane function, could also be important determinants of their biochemical mode of action [3–7, 14].

Recently a new analogue of AM, *N*-trifluoroacetyladriamycin-14-valerate (AD 32) (Fig. 1), was shown to have better antitumoral activity than the parent compound [8, 9]. In this paper we present evidence that while AM and DM modify DNase I activity, probably after binding to DNA, AD 32 does not interfere at all with this enzyme activity.

The three drugs used, AM, DM and AD 32, were kindly donated by Farmitalia, Milan. DNase I, DN-CS from bovine pancreas and DNA from calf thymus type V were purchased from Sigma. AM and DM were dissolved in distilled water at an initial concentration of 1 mg/ml. AD 32 was dissolved in saline containing 10% Tween 80, at an initial concentration of 6 mg/ml.

The enzymatic reactions were carried out according to Kunitz [10]. 2.5 ml of 8.33 mM acetate buffer, pH 5.0 contained 83.4 mM $MgSO_4$ and a final DNA concentration ranging from 5×10^{-10} M to 5×10^{-9} M, 0.5 ml of DNase corresponding to 0.8 mg of protein were added. The formation of free nucleotides was followed at 260 nm with a model 25 Beckman spectrophotometer. In our experimental conditions, Tween 80 caused no interference with DNase activity. Inhibition was studied by adding to the cuvette one of the three compounds under study at final concentrations ranging from 0.6×10^{-10} M to 1.3×10^{-5} M; then 0.5 ml of DNase solution was added. The IC_{50} values, i.e. the concentration of inhibitors causing 50 per cent inhibition, were obtained by plotting the percentage of inhibition as a function of inhibitor concentration

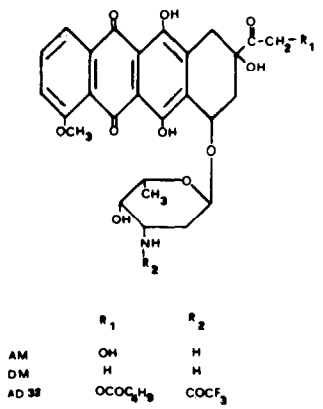

Fig. 1. Structure of Adriamycin (AM), Daunomycin (DM) and *N*-trifluoro-acetyl-Adriamycin-14-valerate (AD 32).

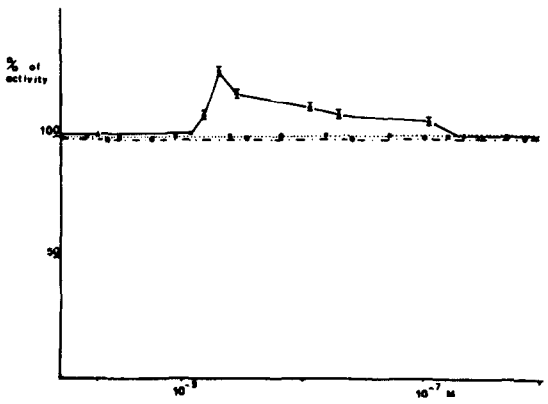

Fig. 2. Effect of Adriamycin (▲), Daunomycin (■) and AD 32 (●) on DNAse I activity. Each point represents the mean ± S.E. of four different determinations.

on semilogarithmic paper. K_m, V_{max} and K_i values were estimated according to the Woolf Plot, with a Hewlett Packard HP 69 computer. The type of inhibition and K_i values were determined using a concentration of inhibitor corresponding to its IC_{50}.

Figure 2 shows the activation of DNase I by AM at concentrations ranging from 1.80×10^{-9} M to 0.61×10^{-7} M. Maximum activation occurs at 3.08×10^{-9} M and corresponds to about 128 per cent of the original activity. DM and AD32 did not show any activation in the same range of concentrations.

Figure 3 shows the inhibition of DNase caused by AM and DM. The IC_{50} for AM and DM were respectively 43.2×10^{-7} M and 6.03×10^{-7}. AD32 did not inhibit the enzyme up to a concentration of 5×10^{-5} M.

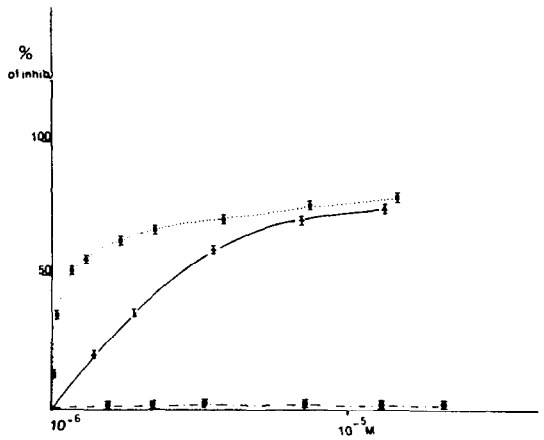

Fig. 3. Inhibitory effect of Adriamycin (▲), Daunomycin (■) and AD 32 (●) on DNAse I activity. Each point represents the mean ± S.E. of four different determinations.

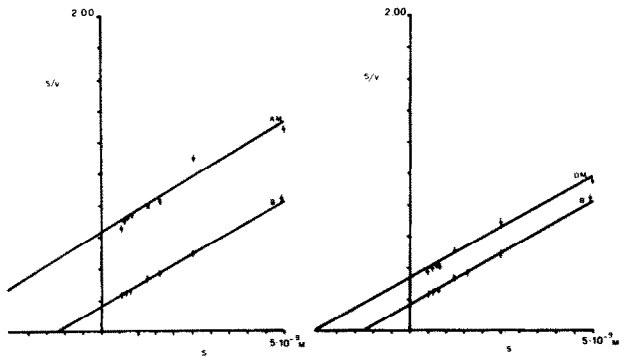

Fig. 4. Woolf Plot analysis of DNAse I inhibition by Adriamycin (AM) and Daunomycin (DM). B = kinetic curves in absence of inhibitor. The K_i values are respectively 16.3×10^{-7} and 5.4×10^{-7} M. All the experiments were replicated 4 times.

Figure 4 shows the Woolf Plot for AM and DM. The inhibition is of a competitive nature for both drugs. K_i values are respectively 16.3×10^{-7} M and 5.4×10^{-7} M.

The free basic glycosidic amino group in the sugar moiety of the anthracycline molecule appears to be a prerequisite for intercalation and subsequent inhibition of DNA polymerase [2, 11–14]. AD32, the *N*-trifluoroacetyl derivative of AM which does not bind to DNA [15], did not affect DNase activity. Thus it is tempting to speculate that intercalation may be necessary for inhibition of DNase. Lee and Byfield [7] recently reported that exposure of tumoral cells to AM resulted in DNA breaks and they suggested that activation of a nuclease might play a role in the induction of DNA fragmentation. In the present study slight though significant DNase activation was in fact observed at AM concentrations below 0.6×10^{-7} M, whereas neither DM nor AD32 had this effect. It remains to be clarified how relevant this effect is to the cytotoxic mode of action of AM. The biochemical mechanism of action of type I bovine pancreas DNase employed as a model for these studies resembles that of human serum nuclease, both enzymes yielding oligonucleotides with 5'-nucleotide terminals [16, 17]. AM-DNA complexes have been claimed to give better antineoplastic activity [18–21]. The interaction of AM with the enzyme systems which degrade the AM-DNA complex may conceivably be critical in determining bioavailability of the drug.

Acknowledgement—This work was supported by NIH, Contract NIH-N01-CM 23242.

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