Biochemical Pharmacology, Vol. 37, No. 9, pp. 1803–1804, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00
Pergamon Press plc

A NOVEL TARGET IN DNA METABOLISM FOR CYTOTOXIC DRUGS

Giovanni Ciarrocchi, Alessandra Montecucco, Guido Pedrali-Noy and Silvio Spadari

Istituto di Genetica Biochimica ed Evoluzionistica, CNR, Pavia

INTRODUCTION

It has been proposed that the stabilization of the "cleavable complex" between DNA and topoisomerase II might be somehow at the bases of the antitumor properties of different classes of antineoplastic drugs such as the Vinca antimitotics and those generally interacting with DNA (1-2). However a correlation between the induction of all the possible products of interaction between drugs and DNA topoisomerase II, the double- and single-stranded breaks and DNA-protein crosslinks, and cytotoxicity is mainly observed with the non-DNA binding epipodophillotoxins (3). We have recently shown that a certain number of anthracyclines, including doxorubicine, in addition to increasing the cleavable complex fraction, are indeed also inhibitors of the relaxing activity of human DNA topoisomerase II (4). Therefore we find very difficult to believe that the inhibitory properties of molecules which are so different one from the other, rely on characteristics different for each kind of molecule and not on the main property they share: the ability to alter the DNA structure. We have also considered that many DNA binding drugs show some kind of antibiotic activity. On these bases, we have undertaken a search for an alternative target of intercalating drugs. The topic of this presentation is the description of a new possible target for such drugs.

MATERIALS AND METHODS

Circle-ligation assay: Nicked circular substrate preparation and assay conditions were as described by Depew and Wang (5).
Cohesive- and blunt-ends ligation assays: Substrates were obtained by restriction of pAT153 DNA with EcoRI and NruI, respectively. Assays conditions were those suggested by Bethesda Research Laboratory for bacteriophage T4 ligase or elsewere described for the human enzyme (6).

RESULTS AND DISCUSSION

Ethidium bromide was initially utilized as model system. In fact ethidium is a very well studied intercalating agent, with known trypanocide activity, no antitumor properties and is able to generate topoisomerase II cleavable complexes, but only at high concentration. Any target sensitive to ethidium bromide and more sensitive to anthracyclines would be a possible candidate as alternative target for antitumor drugs with DNA-binding properties.
We found that ethidium bromide behaves as a good reversible inhibitor of bacteriophage T4 DNA ligase. This property is clearly shown in Fig. 1. The inhibition of ligation of nicked circular DNA molecules is detected as an increment of the nicked material which migrates slowlier than the ligated products. The covalently closed molecules have in fact acquired all the typical topological properties of cccDNA, including a different mobility on agarose gel. We also found that ethidium bromide inhibits the ligation of substrates containing either cohesive or blunt ends and also in these cases

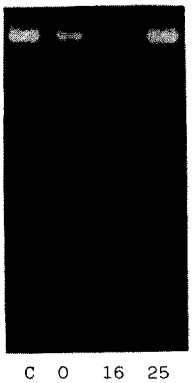
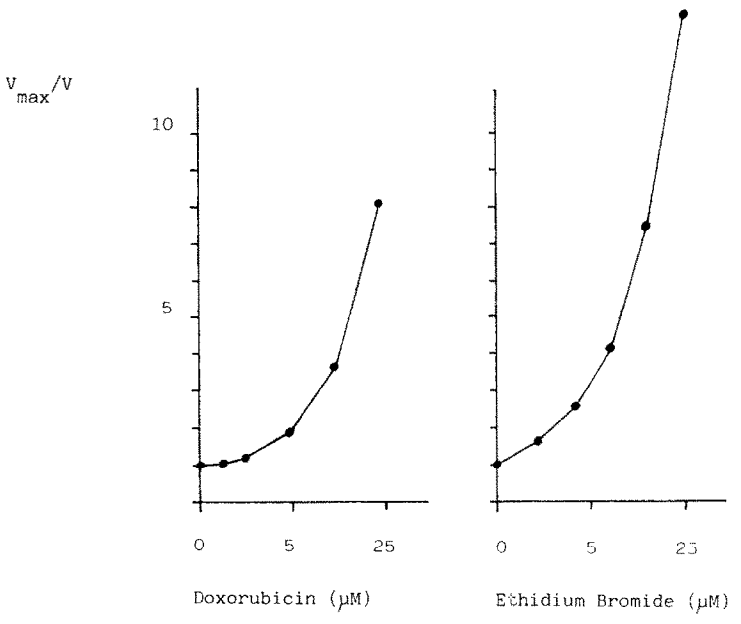


Fig. 1

100% inhibition can be observed. In all these cases the inhibition of ligation is dose-dependent. Since these assays of ligation are simple to run but difficult to quantitate, we have also utilized the assay of circularization of labelled poly(dAT) (Fig. 2). In this way we showed that the V_{max}/V is not a straight line. This might result from the limited dimension of the substrate but more probably is a reflection of the non-linear binding process of intercalating drugs. When doxorubicin was tested against T4 DNA ligase it proved it was found to be a more potent inhibitor than ethidium (Fig. 2). Also 4'-6-diamidino-2-phenylindole (DAPI), a non-intercalating drug but with DNA-binding and trypanocide properties, proved very effective in preventing DNA ligation. When the human DNA ligase (7) was tested, it was found to behave exactly as the bacteriophage T4 enzyme, for what the inhibitory properties are concerned. We have then investigated the possible correlations between the observed inhibition of DNA ligases and DNA topoisomerases. We have then hypothesized a similarity in the mode of action of both classes of enzymes, in contrast with what described for the E. coli DNA topoisomerase I and the DNA ligase (8). We have therefore tested the ability of both bacteriophage T4 and human DNA ligases to relaxe supercoiled DNA in the presence of AMP. In this way we have discovered that ATP dependent ligases clearly behave as AMP-dependent nicking-closing enzymes.
We believe that our observations are in support of the hypotesis that DNA ligase migth be an alternative target for many cytotoxic drugs which reversibly bind to DNA. In addition, we are confident that a new model of action for DNA ligases will be of foundamental help in defining the mode of inhibition of DNA topoisomerase II by the same drugs.

Fig. 2



ACKNOWLEDGEMENTS

This work was supported by Progetto Finalizzato "Oncologia" and Progetto Strategico "Aspetti chimici e fisici dei sistemi biologici" of the Consiglio Nazionale delle Ricerche to G. Ciarrocchi. A.M. was supported by the "Fondazione A.Buzzati Traverso".

REFERENCES

1) G.L. Chen, L. Yang, T.C. Rowe, B.D. Halligan, K.M. Tewey and L.F. Liu, J. Biol. Chem. **259**, 13560 (1985)
2) Y. Pommier, J.K. Minford, R.E. Schwartz, L.A. Zwelling and K.W. Khon, Biochemistry **24**, 6410 (1985)
3) W.E. Ross and M.O. Bradley, Biochem. Biophys. Acta **654**, 129 (1981)
4) S. Spadari, G. Pedrali-Noy, F. Focher, A. Montecucco, T. Bordoni, C. Geroni, G. Ventrella, F. Arcamone and G. Ciarrocchi, Anticancer Res. **6**, 935 (1986)
5) R.E. Depew and J.C. Wang, Proc. Natl. Acad. Sci. USA **72**, 4275 (1975)
6) P. Modrich and I.R. Lehman, J. Biol. Chem. **245**, 3626 (1970)
7) S. Spadari, G. Ciarrocchi and A. Falaschi, Eur. J. Biochem. **22**, 75 (1971)
8) I.R. Lehman, Science **186**, 790 (1974)