Vol. 117, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1983 Pages 1-5

A SPECIFIC INHIBITOR OF TYPE I DNA-TOPOISOMERASE OF TRYPANOSOMA CRUZI : DIMETHYL-HYDROXY-ELLIPTICINIUM

Setha DOUC-RASY, Alain KAYSER and Guy RIOU

Laboratoire de Pharmacologie Moleculaire
Institut Gustave Roussy
94805 VILLEJUIF CEDEX - FRANCE

Received September 16, 1983

Summary. 2-6 dimethyl-9-hydroxyellipticinium inhibited the relaxation of supercoiled DNA by the type I topoisomerase of T. cruzi. Since DNA relaxed in the presence of an intercalating drug prior to electrophoresis became supercoiled when the ligand was removed, we analysed the topoisomerisation in gels containing another ligand, chloroquine. The inhibition which is reported here, concerning a type I topoisomerase, is of an exceptional efficiency.

Ellipticine derivatives are intercalating ligands which affect topoisomerisation of DNA (1). This prompted us to assay the effect of an intercalating drug, 2-6-dimethyl-9-hydroxy-ellipticinium[1] (2) on Trypanosoma cruzi type I topoisomerase activity. Inhibition of the relaxing activity was not readily detectable by gel electrophoresis, since supercoiled DNA which was relaxed in the presence of an intercalating drug returned to the supercoiled state when the drug was removed. To allow relaxed DNA to be distinguished from unreacted DNA, electrophoretic analyses were performed in a buffer containing chloroquine (1). Di-CH_3-OH-El^m proved to be a potent inhibitor of T. cruzi type I topoisomerase. Homologous enzymes of other trypanosomatidae (T. equiperdum, Herpetomonas samuelpessoai) and of mammalian cells were by far less sensitive.

Topoisomerases are likely to be involved in every aspect of DNA replication and function (3)(4). They were characterized in nuclear extracts of eukaryotic cells. Type I topoisomerases introduce transient breaks in one strand, and topoisomerases II in both strands of DNA (5). This allows supercoils to be removed from circular molecules. Inhibition by drugs of the

Abbreviation. [1] 2-6 dimethyl-9-hydroxyellipticinium : Di-CH_3-OH-El^m

0006-291X/83 $1.50

activity of various topoisomerases could provide useful differences between them. Inhibitors of type II topoisomerases have been reported : nalidixic acid, oxolinic acid, novobiocin and coumermycin are well known inhibitors of Escherichia coli DNA-gyrase. Eukaryotic enzymes are more or less inhibited by some of these drugs ; anyway, type I enzymes are not. Other compounds were tested : some ellipticine derivatives were recently shown to inhibit type II rather than type I topoisomerase of rat liver (1). Drug sensitivity has obvious importance in the study of host-parasite systems, especially when a target enzymatic process of the parasite is more sensitive than the corresponding one of the host. We report that type I topoisomerase of T. cruzi was highly susceptible to a particular ellipticine derivative.

METHODS

T. cruzi Tehuantepec strain and Herpetomonas samuelpessoai were cultured in LIT medium and collected in the exponential phase of growth. T. equiperdum, Institut Pasteur strain, was obtained from the blood of infected rats. Liver was from male Wistar rat. Vero cells were provided by Dr. Berneman, Institut Pasteur. VX_2 carcinoma is a transplantable tumor derived from a tumor induced in domestic rabbit by the cottontail rabbit papillomavirus. VX_2 cells were prepared by Dr. G. Orth (Institut Pasteur) (7). Human ovarian carcinoma cells were provided by Dr. J. Benard after cultivation and heterotransplantation in nude mice. Type I topoisomerases were purified from nuclei as previously described (8). Briefly, nuclei were lysed in NaCl 1M and nucleic acids precipitated by 6 % polyethylene glycol 6000. The supernatant was loaded onto a hydroxylapatite column. Active fractions were further purified by phosphocellulose chromatography. Type I topoisomerase fractions were pooled, dialysed against a conservation buffer (50 % w/v glycerol, 0.1 M Tris-HCl pH 7.5, 10 mM mercapto-ethanol, 0.5 mM dithriothreitol, 0.1 mM EDTA) and stored at -20°C. The specific activities of our enzyme preparations were comprised between 10^5 and 10^6 units/mg of protein. One unit relaxes half of the supercoiled DNA under the conditions of the assays.

Topoisomerase activity was tested on DNA (15 µg) from bacteriophage fd purified in a gradient of CsCl-ethidium bromide. The reaction mixture contained Tris-HCl (pH 7,9) 10 mM, KCl 50 mM, $MgCl_2$ 10 mM, dithiothreitol 0.5 mM, EDTA 0.5 mM, serumalbumin 15 µg/ml. The total volume was 20 µl. Incubation was at 30° for 30 min. Reaction was stopped by addition of sodium dodecyl sulfate. Electrophoresis was performed in 50 mM Tris-phosphate (6.06 g Tris, 2.85 g 85 % PO_4 per liter) containing 1 mM EDTA and 18 µM chloroquine phosphate. The buffer was recirculated. The gels contained 1 % agarose. Electric field was 1.4 V/cm and duration 18 hrs. Di-CH_3-OH-El^m was supplied by Dr. J.B. Le Pecq (Laboratoire de Physicochimie Macromoléculaire, Institut Gustave Roussy, Villejuif). DNA was preincubated for 15 min. with the drug in the reaction mixture, then enzyme (2 units) was added.

RESULTS AND DISCUSSION

When assaying DNA-unwinding ligands as potential inhibitors, one has to take into account the variations of supercoiling they can induce. When enzyme

action proceeded in a medium which contained an ellipticine derivative, DNA was nevertheless supercoiled as it got subsequently rid of the ligand. This made electrophoresis in standard agarose gels an unsuitable means to test for enzyme inhibition. We were able to differentiate DNA relaxed in a medium containing ellipticine from unreacted supercoiled DNA by use of gels containing chloroquine. Chloroquine itself is an unwinding agent which had been used in electrophoresis buffers to separate topoisomers by Shure et al (6). In gels containing 18 μM chloroquine, unreacted DNA appeared in a medium position, between highly supercoiled and open circular molecules. Reacted DNA migrated as more or less supercoiled molecules, depending on the concentration of ellipticine in the reaction mixture. DNA treated by enzyme in the absence of ellipticine was first relaxed, then positively supercoiled during its migration in the gel, due to unwinding by chloroquine. The relaxed topoisomers obtained in the presence of little ellipticine were less affected by chloroquine, and migrated less rapidly than molecules relaxed without ellipticine. In the presence of more ellipticine, topoisomers became negatively supercoiled when analysed into the gel. When ellipticine concentration was further increased, highly supercoiled species were formed, which migrated at maximal speed. However, if the distribution of topoisomers of native DNA reappeared, then inhibition of the relaxation had occurred.

The inhibition by di-CH_3-OH-El^m of two enzymes is presented on the figure. Lanes 1-11 refer to the type I topoisomerase of a human ovarian carcinoma. 0.75 μM of di-CH_3-OH-El^m allowed relaxation to occur (lane 3) ; 1.5, 3, 15, 30 μM were not inhibitory (lanes 4-5-6-7) ; 60 μM was partially inhibitory (lane 8) ; 90 and 120 μM were totally inhibitory (lanes 9-10) since the distribution of DNA was the same as that of the controls (lanes 1 and 11). Lanes 12-17 refer to the type I topoisomerase (8) of T. cruzi. 0.75 μM of di-CH_3-OH-El^m allowed relaxation to occur (lane 13) ; 1.5 μM and higher concentrations were inhibitory (lanes 14-15-16 in which the distribution of lane 1 reappeared).

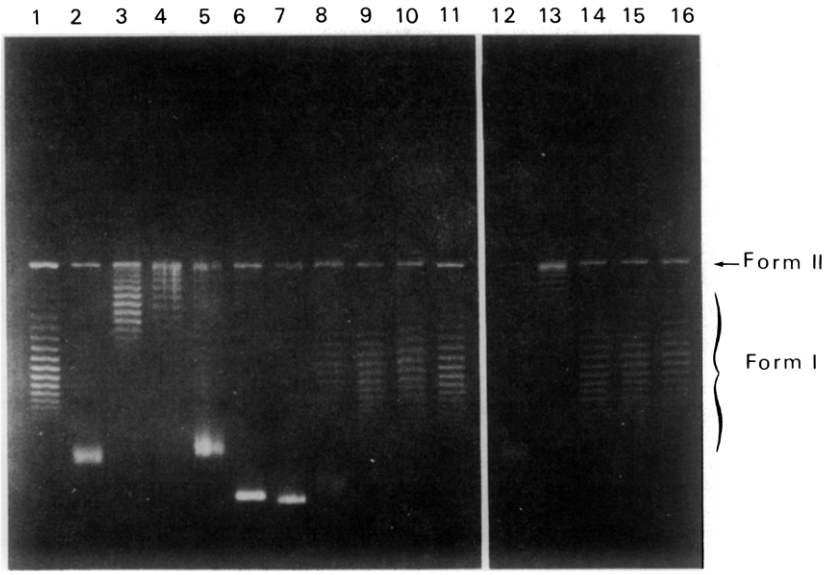

Figure - Inhibition of type I topoisomerases by di-CH_3-OH-$E1^m$.
DNA from bacteriophage fd was preincubated for 15 min. with di-CH_3-OH-$E1^m$, then enzyme (2 units) was added. [1] fd DNA. [2] + enzyme of a human ovarian carcinoma. [3]-[10] + 0.75, + 1.5, + 3, + 15, + 30, + 60, + 90, + 120 µM di-CH_3-OH-$E1^m$. [11] fd DNA. [12] + enzyme of T. cruzi. [13]-[16] + 0.75, + 1.50, + 3.75, + 7.5 µM di-CH_3-OH-$E1^m$.

Inhibitory concentrations of di-CH_3-OH-$E1^m$ were estimated from the preceding experiments to be 1.5-3 µM for T. cruzi enzyme and 90 µM for the human carcinoma. The last value, as shown in the table, is representative of similar experiments with cells from two species of trypanosomatidae (Trypanosoma equiperdum, Herpetomonas samuelpessoai) and from mammals. We prepared the topoisomerases of rat liver, of a cell line from the green monkey (Vero), of a carcinoma induced in the domestic rabbit by the cottontail rabbit papillomavirus (Vx_2) (8) and of a human ovarian carcinoma. All inhibitory concentrations were in the range 60 - 120 µM : see the table.

Some ellipticine derivatives were previously shown to be trypanocidal in vitro (9)(10). As a step to the investigation on therapeutic value of di-CH_3-OH-$E1^m$ in Chagas disease, we are testing for its trypanocidal activity in cultures of T. cruzi.

Table - Inhibition of type I DNA topoisomerases by di-CH_3-OH-El^m

Origin of topoisomerases	Inhibitory concentration (μM)
T. cruzi	1.5-3
T. equiperdum	60
Herpetomonas samuelpessoai	120
Rat liver	90
Vero (kidney of African green monkey)	90
Vx_2 carcinoma	60
Human ovarian carcinoma	90

ACKNOWLEDGMENTS

We are indebted to Dr. G. Orth (Institut Pasteur) for providing us with VX_2 cells, to Dr. J. Benard of this laboratory for providing us with Vero and human carcinoma cells, and to Dr. Dat Xuong, who supplied di-CH_3-OH-El^m. This work was supported by the grant N° 82-1025 from INSERM (France).

REFERENCES

1. **DOUC-RASY, S., MULTON, E., KAYSER, A. and RIOU, G.** (1983) C.R. Acad. Sci. Paris **296**, 899 904.

2. **PAOLETTI, C., LE PECQ, J. B., DAT-XUONG, N., JURET, P., GARNIER, H., AMIEL, J. L. and ROUESSE, J.** (1980) in Recent Results in Cancer Research, Mathe, G. and Muggia, F. M., Ed. (Springer Verlag, Heidelberg) pp.107-123.

3. **GELLERT, M.** (1981) Ann. Rev. Biochem. **50**, 879-910.

4. **COZZARELLI, N.R.** (1980) Science **207**, 953-960.

5. **WANG, J.C. and LIU, L.F.** (1979) in Molecular Genetics, J.H. Taylor, Ed. (Academic Press, New York) Part III pp. 65-88.

6. **SHURE, M., PULLEYBLANK, D. E. and VINOGRAD, J.** (1977) Nucleic Acids Res. **4**, 1183-1205.

7. **FAVRE, M., JIBARD, N. and ORTH, G.** (1982) Virology, **119**, 298-309.

8. **RIOU, G., GABILLOT, M., DOUC-RASY, S., KAYSER, A. and BARROIS, M.** (1983) Eur. J. Biochem **134**, 479-484.

9. **BENARD, J., DAT-XUONG, N. and RIOU, G.** (1975) C.R. Acad. Sci. Paris, **280**, 1177-1180.

10. **BENARD, J. and RIOU, G.** (1976) in Biochemistry of parasites and host-parasite relationships, Van den Bossche, H., Ed. (Elsevier North Holland biomedical press, Amsterdam) pp 477-484.