serum free fatty acid concentration which, as reported [18], blocks phospholipid methyltransferase Whether the high serum levels of free fatty acids are responsible for the impaired phospholipid methyltransferase response to glucagon in diabetes remains to be determined

Acknowledgements—I M and C M are fellows of Fundacion Conchita Rabago and I V is a fellow of Fundacion Jimenez Diaz This work was supported by grants from CAICYT, FISS and Europharma

Metabolismo Nutricion y Hormonas Fundacion Jimenez Diaz, and Instituto de Investigaciones Biomedicas, C S I C Reyes Catolicos 2, 28040 Madrid, Spain

CARMEN CABRERO
ISABEL MERIDA
PABLO ORTIZ
ISABEL VARELA
JOSE M MATO

REFERENCES

1 B Akesson, *Biochim biophys Acta* **218**, 57 (1970)
2 J M Mato in *Progress in Protein–Lipid Interactions*, Vol. 2, Chap 8 pp 267–302 Elsevier, Amsterdam (1986)
3 J D Johnson and W E Cornatzer, *Proc Soc exp Biol Med* **131**, 474 (1969)
4 C N Corder and R K Kalkhoff, *J Lab clin Med* **73**, 551 (1969)
5 D R Hoffman, J A. Haning and W E Cornatzer, *Proc Soc exp Biol Med* **167**, 143 (1981)
6 C Sharma, R Manjeshwar and S Weinhouse, *J biol Chem* **238**, 3840 (1963)
7 S Alemany, I Varela, J F Harper and J M Mato *J. biol Chem* **257**, 9249 (1982)
8 P K Chiang and G L Cantoni, *J biol Chem* **252**, 4506 (1977)
9 J. G Castano, S Alemany, A Nieto and J M Mato *J biol Chem* **255**, 9041 (1980)
10 D Marin Cao, V Alvarez Chiva and J M Mato, *Biochem J* **216**, 675 (1983)
11 A Guranowski, J A Montgomery G L Cantoni and P K Chiang, *Biochemistry* **20**, 110 (1981)
12 I Garcia Castro, J M Mato, G Vasanthakumar W P Weismann, E Schiffmann and P K Chiang, *J biol Chem.* **258**, 4345 (1983)
13 W H G Wemer, H Rey and Z Wichingen, *Analyt Chem* **252**, 224 (1970)
14 A Schuller, J Moscat, E Diez, J C Fernandez-Checa, F Gavilanes and A M Municio, *Hepatology* **5**, 36 (1985)
15 J A Duerre in *Biochemistry of S-adenosylmethionine and Related Compounds* (Eds E Usdin, R T Borchardt and C R Creveling), pp 595–602 Macmillan Basingstoke (1982)
16 F Audubert and D E Vance *J biol Chem* **258**, 10695 (1983)
17 G A Miura, J R Santangelo, R K Gordon and P K Chiang, *Analyt Biochem* **141**, 161 (1984)
18 E Audubert, S Pelech and D E Vance, *Biochim biophys Acta* **792**, 348 (1984)

Biochemical Pharmacology Vol 35, No 13, pp 2264–2267, 1986
Printed in Great Britain

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd

Interaction between ellipticine derivatives and circular supercoiled DNA as revealed by gel electrophoresis. Possible relationship with the mechanisms of cytotoxicity

(*Received* 7 *August* 1985, *accepted* 11 *December* 1985)

Certain ellipticine derivatives are DNA intercalating cytotoxic agents with antitumoral activity against experimental tumors [1] and in clinical cases [2] Their mechanisms of action have been related to their DNA affinity as measured by competition with ethidium bromide [1], to their ability to be oxidized and to generate electrophilic intermediates [3] and lastly to their capacity to inhibit topoisomerase II activity [4] However, no relationship between cytotoxic activity and the above mentioned biochemical properties has been demonstrated for various substituted compounds Two possibilities should, therefore, be taken into consideration (1) modifications in different regions of the ellipticine molecule induce different series of compounds with different mechanisms of action, and (2) some biochemical property of the ellipticine structure, related to its cytotoxic effect, remains unknown In this paper, we report the ability of different substituted derivatives of ellipticine to modify the electrophoretic migration of circular supercoiled DNA This effect is due to unknown properties of the DNA–ellipticine binding, which may be related to the mechanism of cytotoxicity

Materials

The ellipticine derivatives were synthesized by Dr Dat-Xuong (Gif/Yvette), Dr Viel (Châtenay-Malabry) and Dr Lesco (Villejuif) PM2 supercoiled DNA was either prepared according to the method of Espejo [5], or purchased from Boehringer (Grenoble, France)

Methods

(1) *Electrophoresis* Sample preparation a PM2 stock solution was diluted in 1 mM EDTA, 10 mM sodium phosphate (pH 7) for (i) drug interaction, (ii) optical density measurements DNA (3.7×10^{-3} M in bases) was mixed at 4°, with ellipticine derivatives ($5–25 \times 10^{-6}$ M) Before loading the samples into the gel slots, the solution was mixed with a six-fold concentrated solution of 0.25 mg/ml bromphenol blue and 40% sucrose, as previously described [6]

Electrophoresis was carried out for 18 hr in an 0.8% agarose horizontal gel (Model H1, BRL, Rockville, MD) in Gary's buffer (36 mM Tris, 30 mM Na_2HPO_4, 1 mM EDTA, pH 8) at 60 V A photograph of the ethidium bromide stained gel was taken under u v excitation A densitometric scanning of the DNA bands on the negative of this photography was then carried out with the Joyce Loebl apparatus

The width of the DNA bands was evaluated by measuring the bases of the densitometric peaks The DNA migration was obtained by measuring the distance between the slot and the peak of the densitometric DNA band

(2) *Cytotoxic effects on L1210 cells in culture* The experimental protocol has been previously reported [7] The cells were exposed to increasing concentrations of drug during 48 hr and incubated in a 5% CO_2 atmosphere at 37° The drugs were dissolved in dimethylsulfoxide (1% final) The cell concentration was determined with a ZBI Coulter Counter The cytotoxicity was evaluated by measuring the drug concentration which decreases by 50% the L1210 cell growth rate after 48 hr All experiments were performed within a period of 8 mo

Results and discussion

The electrophoretic migration of PM2 circular DNA was studied when DNA and ellipticine derivative were both present in the gel slot ($0.13 <$ drug/base < 0.65) The

major observation was a broadening of the supercoiled DNA band (cf Fig 1) as compared with that of a control DNA unmixed with drug before electrophoresis The importance of the effect depended on the type of ellipticine derivative A second observation was a decrease in electrophoretic mobility, roughly correlated with the band broadening effect Maximum effects were obtained with $2CH_3$-$6CH_3$-9OH-ellipticine broadening of the DNA band, 280%, decrease in electrophoretic mobility 25% 7-OH-ellipticine and 6-isopentyl-ellipticine had no effect When the agarose was mixed with the same active concentration of $2CH_3$-$6CH_3$-9OH-ellipticine before addition of DNA in the gel slot and electrophoresis, the broadening of the DNA band could no longer be observed, indicating that the latter effect was due to a non steady state of the drug–DNA relationship Depending on the derivative studied, ellipticines have either one positive charge, or a fraction of a positive charge The bound molecules, therefore, lower the negative DNA charge However, their known affinity for double-stranded DNA cannot explain the observed range of effect (for instance, the affinity for derivatives 5 and 25 is 1×10^5/M while that for derivatives 7 and 20 is $1\,2 \times 10^6$/M) Among other possibilities one may consider a two-step explanation to account for the observed effects on DNA migration (1) the binding of ellipticine on some DNA sites is very stable and has not

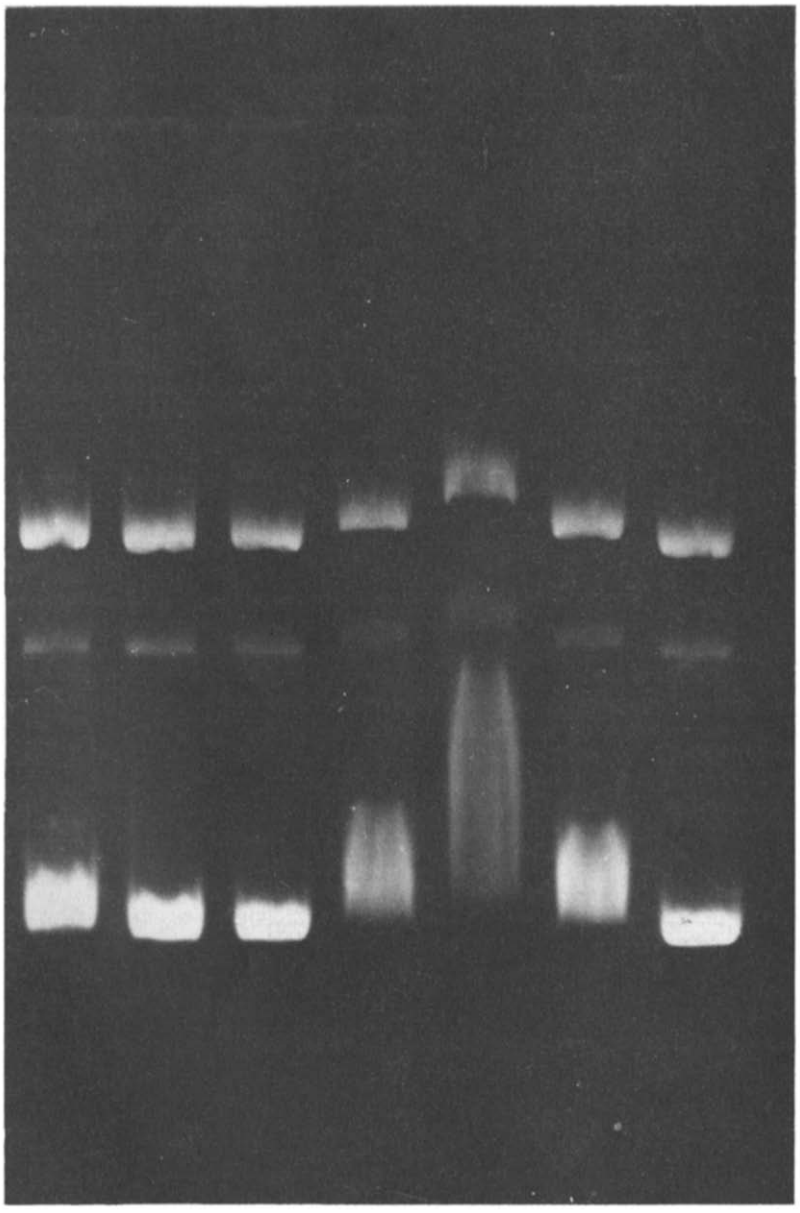


Fig 1 Effect of six ellipticine derivatives on the electrophoretic migration of PM2 DNA Electrophoresis was carried out in agarose gel, as described in Methods The different DNA bands are, from top to bottom open circular, linear, supercoiled circular DNA The ellipticine derivatives are used at a concentration of $2\,5 \times 10^{-5}$ M According to the classification of Table 1 the different compounds tested are, from right to left no drug, 5, 1, 8, 23, 21, 20

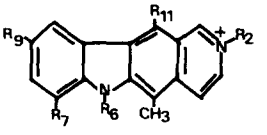
been revealed by the techniques employed to measure the affinity constant and (2) the number of bound molecules is different depending on the linking number

We have described above the observed effect with $2CH_3$-$6CH_3$-9OH-ellipticine This compound is active on L1210 leukemia [7]. We have, therefore, beeen led to investigate the relationship between the broadening of the supercoiled DNA band by ellipticine derivatives and their antitumoral activity on L1210 leukemia cells We have studied 23 derivatives, whose activity on L1210 leukemia cultured cells was known (cf Table 1) We have measured the broadening effect on the supercoiled DNA band, in relation to the drug concentration in the electrophoretic slot (cf Materials and Methods) Thirteen compounds induced a concentration dependent effect The broadening extent decreases from compound 1 to compound 13, but cannot easily be quantified by one figure (cf Fig 2) These compounds belong to class I Compounds 5 and 6 are not very cytotoxic but it should be noticed that they induce a broadening of DNA at a higher dose (cf Fig 2) Further studies are undertaken to correlate the initial slope of broadening curves with cytotoxicities Class II compounds either induce a weak, non dose dependent broadening between 5×10^{-6} M and 25×10^{-6} M or do not modify the DNA migration Table 1 shows (1) the classification according to electrophoretic parameters (I or II), (2) the concentration which inhibits by 50% the growth of cultured L1210 cells after 48 hr (ID_{50}) The ID_{50} of 13 derivatives among the 23 tested, is lower or equal to 0 15 μM Such a cytotoxic effect is related to a significant antitumoral effect on murine L1210 leukemia [7] Eleven of these 13 derivatives belong to class I It had previously been shown that the cytotoxicity of ellipticine derivatives on cultured L1210 cells was roughly correlated to the antitumor activity on murine L1210 leukemia [7] It is not, therefore, surprising that, among class I derivatives, only one (No 6) is not efficient *in vivo* on L1210 leukemia ([7] and unpublished data), whereas only one (No 18) is efficient among class II derivatives

The modifications of DNA electrophoretic parameters of DNA by antibiotic agents with antitumoral properties have previously been found to be related to their antitumoral activities [8] Our study confirms this observation with an additional set of antitumoral agents

The positions on the ellipticine molecule most often used for substitution are 2, 6, 9 CH_3 in position 6, and OH in position 9 are required to get a wide broadening effect It has already been shown that OH in position 9 increases the antitumoral activity of ellipticine derivatives [7] This effect was supposed to be related to oxidation reactions [3] However, our results indicate that OH in position 9 modifies the binding properties of the ellipticine molecule on purified DNA One may, therefore, suggest that revers-

Table 1 Comparison of the broadening of circular supercoiled PM2 DNA bands during electrophoresis with the cytotoxic activities against cultured L1210 leukemia cells



	R_2	R_6	R_9	R_{11}	R_7	TYPE	ID 50 (μM)	
1	CH_3	CH_3	OH	CH_3	H	I	0.089	
2	C_2H_5	CH_3	OH	CH_3	H	I	0151	
3	H	CH_3	OH	CH_3	H	I	0 076	
4	X_1	H	OH	CH_3	H	I	0 085	CH_2-CH_2-OH
5	H	H	CH_3O	CH_3	H	I	1 80	
6	H	CH_3	CH_3O	CH_3	H	I	1 12	
7	C_2H_5	H	OH	CH_3	H	I	0 076	
8	X_2	H	OH	CH_3	H	I	0 070	CH_2-CH_2-N⟨⟩
9	CH_3	H	OH	CH_3	H	I	0.112	
10	H	H	OH	CH_3	H	I	0.115	
11	X_3	H	OH	CH_3	"	I	0 062	OH CH_2-CH-CH_2-OH
12	X_6	H	OH	CH_3	"	I	0 081	CH_2-CH_2-CH_2-OH
13	X_4	H	OH	CH_3	"	I	0 050	CH_2-CH_2-N-C_2H_5 C_2H_5
14	CH_3	CH_3	H	CH_3	"	II	0.640	
15	CH_3	H	H	CH_3	"	II	1 03	
16	H	H	Br	'	"	II	4 12	
17	H	H	NO_2	"	"	II	1 87	
18	X_5	H	OH	'	"	II	0 128	C_2H_4-N⟨CH_2-CH_2 O, CH_2-CH_2⟩
19	H	'	H	"	'	II	0 145	
20	H	"	NH_2	'	"	II	0 851	
21	H	"	F	CH_3	"	II	2.83	
22	H	"	H	H	H	II	12.2	
23	H	H	H	CH_3	OH	II	11 9	

" Indicate a vertical continuity X_i substituents are on the right hand side

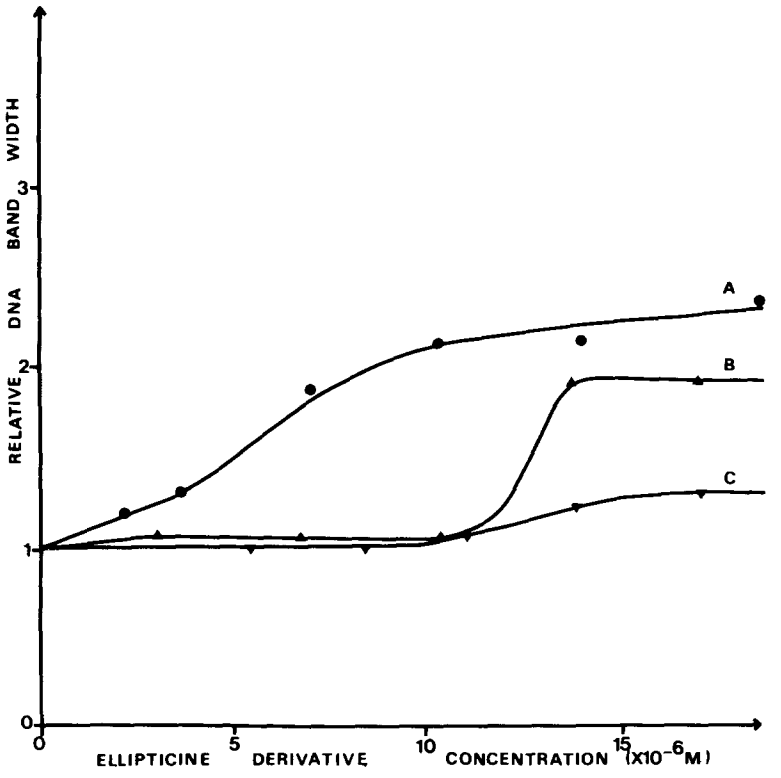


Fig 2 DNA form 1 broadening is a function of the ellipticine derivative concentration Compounds A (No 1) and B (No 6) belong to class I Compound C (No 15) belongs to class II The DNA band width at different concentrations of ellipticine is divided by the width of control DNA

ible drug–DNA interactions contribute to the better antitumoral efficiency of 9-hydroxylated derivatives of ellipticine

Acknowledgements—The technical assistance of G François and M Pottier are gratefully acknowledged We thank Dr Y Lanni for english corrections This work was supported by grants from the "Association pour la Recherche sur le Cancer" and by CNRS and INSERM

LA 147 CNRS, U 140 Inserm,
Institute Gustave Roussy
Rue Camille Desmoulins
94800 Villejuif, France

CLAUDE MALVY
SUZANNE CROS*

* *Laboratoire de Pharmacologie et Toxicologie Fondamentales*
205 route de Narbonne
31400 Toulouse, France

REFERENCES

1 J B Le Pecq, N Dat-Xuong, C Gosse and C Paoletti, *Proc natn Acad Sci U.S A* **71**, 5078 (1974)
2. P Juret, A Tanguy, A Girard, J Y Le Talaer, J S Abbatucci, N Dat-Xuong, J B Lepecq and C Paoletti, *Eur J Cancer* **14**, 205 (1978)
3 J Bernadou, B Meunier, G Meunier, C Auclair and C Paoletti, *Proc natn Acad Sci U S A* **81**, 1297 (1984)
4 S Douc-Rasy, E Multon, A Kayser and G Riou, *C r hebd Séanc Acad Sci Paris,* **296**, 899 (1983)
5 R. T Espejo, E. S Canelo and R L Sinsheimer, *Proc natn Acad. Sci U S A* **63**, 1164 (1963)
6 T Maniatis, E F Fritsch and J Sambrook, *Molecular Cloning, A Laboratory Manual* (1982)
7 C Paoletti, S Cros, N Dat-Xuong, P Lecointe and A Moisand, *Chem. biol Interact* **25**, 45 (1979)
8 S Mong, J. E Strong, J A Bush and S T Crooke, *Antimicrobials and Chemotherapeutics* **16**, 398 (1979)