Biochemical Pharmacology, Vol. 37, No. 9, pp. 1887–1888, 1988.
Printed in Great Britain.

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

DO QUINOLONES BIND TO DNA?

G. PALU', S. VALISENA, M. PERACCHI, M. PALUMBO*

Institute of Microbiology and *Department of Organic Chemistry, University of Padova, Padova - Italy

INTRODUCTION

A critical observation concerning the mechanism of action of quinolones was the recent report of their high affinity binding to DNA (1). This finding, should it be confirmed, could entail a number of consequences, among which, the safety of these drugs (2) and an entirely new definition of their chemotherapeutic potential. Moreover, it brings about a certain difficulty when trying to understand the nature of bacterial resistance to this class of antibiotics (3).

For all these reasons the possibility of quinolone binding to DNA has been further investigated.

MATERIALS AND METHODS

Norfloxacin and ofloxacin were kind gifts from Merck Sharp & Dohme and Glaxo. They were checked to be chemically pure by means of HPLC. [^{14}C] norfloxacin (S.A. 7mCi/mmol) was also from Merck Sharp & Dohme.

DNAs from several sources including calf thymus, salmon sperm, C.perfringens, M.lysodeikticus, pUC8, pBR 322, ColE1 and ssDNA from bovine lung were used in this study. Molar extinction coefficients at 260 nm varying from 6,400 to 6,900 M^{-1} cm^{-1} were used for dsDNAs while 8,400 M^{-1} cm^{-1} was used for ssDNA. Drug and DNA solutions were freshly made each time, just before use, in 10 mM Tris buffer, normally containing 50 mM NaCl, 1 mM EDTA, pH 7.5. The possibility of an interaction taking place between drugs and DNA was studied by means of fluorescence spectroscopy and equilibrium dialysis under varying conditions of ionic strength and divalent cation concentration. Different macromolecule to ligand (P/D) ratios were employed in these experiments. Spectrofluorometric titrations were performed using a Perkin-Elmer MPF 66 apparatus interfaced to a PE 7500 data processor. Radiometric measurements were carried out using a liquid scintillator spectrometer mod. LKB Wallac 1214 rackbeta.

RESULTS AND DISCUSSION

Norfloxacin and ofloxacin have a high fluorescence response. Both drugs exhibit excitation peaks at 280 and 340 nm. Their emission maxima are centered at 421 nm (norfloxacin) and 467 nm (ofloxacin).

Fluorescence spectra of the antibiotics were not modified by the addition of a large excess of DNA (P/D up to 2,000) either in their profiles or in their emission maxima, parameters which are both likely to change upon complex formation. A representative example is reported in Fig. 1.

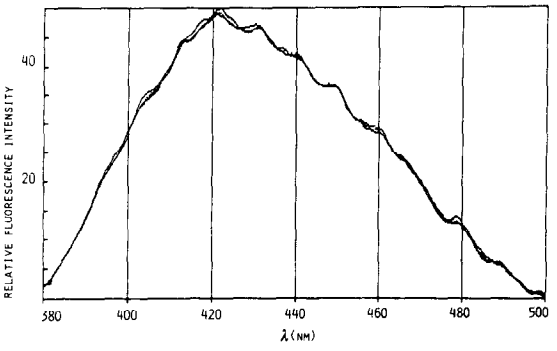

Fig. 1 Fluorescence emission spectra of Norfloxacin ($5x10^{-8}$ M) after addition of increasing amounts of pUC8 DNA (P/D ratios of 0, 50, 500 and 2,000).

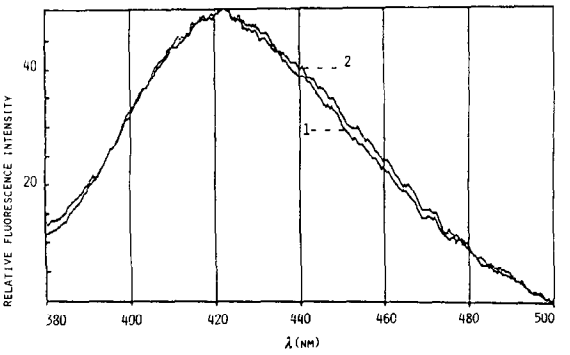

Fig. 2 Fluorescence response of Norfloxacin following equilibrium dialysis in the presence of $2x10^{-3}$ M ssDNA (P/D ratio of 5,000). 1 = compartment with DNA; 2 = external compartment.

Measurements performed under varying conditions of ionic strength and cation concentration similarly produced no evidence for an interaction taking place. However, it has to be pointed out that the spectral response of the quinolones is quite sensitive to minimal changes of divalent cation relative molarity.

To confirm the lack of interaction, equilibrium dialysis measurements were additionally performed which give a direct evaluation of DNA-bound and free drug. Fluorometric and radiometric methods were combined to have a thorough and precise evaluation of drug concentrations in the compartments of the dialysis cell. In no case were we able to detect uneven distribution of the drug in the presence of DNA. Fig. 2 illustrates the result of one of such experiments using ssDNA. Similar data were obtained when using ofloxacin as the drug and DNAs from different sources.

In conclusion, no evidence was obtained for a binding process occurring between naked DNA and quinolone drugs. Thus, we were unable to confirm recent literature data (1) by using a similar experimental approach. The assays were always performed in the presence of an exceedingly large excess of DNA so as to allow detection of one drug molecule bound per plasmid copy, i.e. to possibly unique nucleotide sequences, in the hypothesis put forward by Shen and Pernet of the binding constant being of the order of 10^6 M^{-1} (1).

We conclude that DNA is unlikely to be the direct target of these antibiotics.

Evidence is being seeked for the presence of a ternary complex involving quinolone, DNA, and gyrase.

REFERENCES

1. L.L. Shen and A.G. Pernet. Proc. Natl. Acad. Sci. U.S.A. 82, 307 (1985)

2. R. Albrecht. Progress in Drug Res. 21, 9 (1977)

3. D.C. Hooper, S.S. Wolfson, K.S. Souza, C. Tung, G.C. McHugh and M.N. Swartz. Antimicrob. Ag. Chemother. 29, 639 (1986)

ACKNOWLEDGEMENTS

We wish to thank Mr. M. Guida for technical assistance and greatly acknowledge support from CNR (grant N. 85.00865.52) and MPI.