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ANTIVIRAL ACTIVITY OF GYRASE INHIBITORS NORFLOXACIN, COUMERMYCIN A_1 AND NALIDIXIC ACID.

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INTRODUCTION

The quinolone and coumarin antibiotics are well known inhibitors of bacterial DNA gyrase (1). This enzyme is a topoisomerase II wich is capable of introducing negative supercoils into a relaxed DNA molecule (2). It has recently been reported that topoisomerases I and II are involved in the replication of SV40 (3), whose DNA, as a distinctive feature of all papovaviruses, is organized in a typical chromatin structure (minichromosome) (4). This observation has prompted us to study the effects of some representative substances of the above classes of antibiotics on the replication of SV40 and BK virus. Our aim was to test the possible activity of these drugs as antiviral agents and to gain further insight into their mechanism of action.

MATERIALS AND METHODS

Inhibition of viral DNA synthesis

Norfloxacin and coumermycin A_1 were dissolved in dimethylsulfoxide at the concentration of 20 mg/ml while nalidixic acid was dissolved in water.
Drug solutions were made fresh each time before use. BSC-1 and Vero cells, after infection with SV40 and BK virus at a multiplicity of 50-100 PFU/cell, were exposed to increasing drug concentrations for different periods of time (usually 24,48 and 72 hours). Viral DNA was extracted from infected cells with a modified Hirt's method (5). DNA samples were electrophoresed in a 1% agarose gel in TBE buffer, transferred to nytrocellulose filters and hybridized to nick-translated viral DNA probes. The inhibition of viral DNA synthesis was evaluated directly on the autoradiograms by a densitometric analysis of the bands.

DNAase I protection assay

Aliquots of nuclei from infected cells, corresponding to 10 ug of total DNA, (cell plus viral DNA), were treated with increasing amounts of pancreatic DNAase I (ranging from 0.03 to 2.5 ug/ml) after a previous exposure to the drugs under study. DNA was eventually purified and treated as reported above. The level of transition from form I to forms II and III was evaluated from the autoradiograms by quantifying relative band intensity.

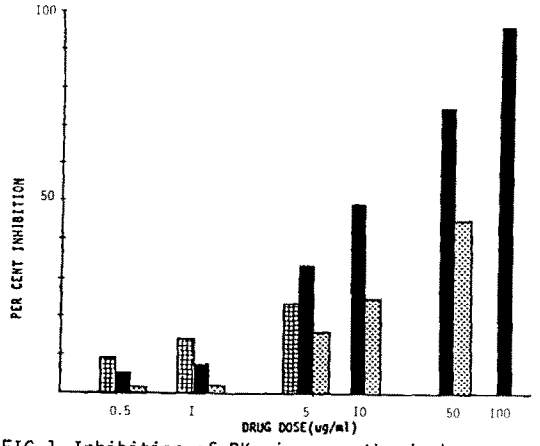

FIG.1. Inhibition of BK virus synthesis by: Coumermycin A_1, Nalidixic Acid, and Norfloxacin.

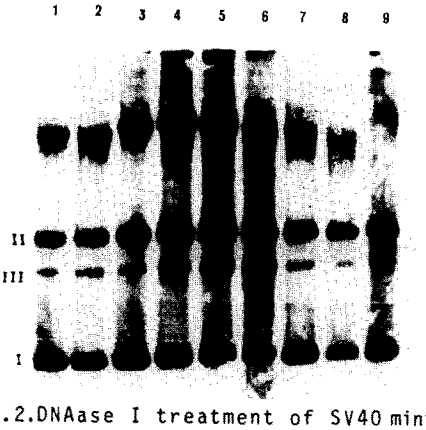

FIG.2. DNAase I treatment of SV40 minichromosomes (Enzyme was present at 0.03, 0.1, 0.3, 1 and 2.5 γ/ml, lanes 1 to 6) and protection assay using 1 γ/ml DNAase I, and 1, 0.4, 0.1 u/ml Norfl. (lanes 7 to 9).

RESULTS AND DISCUSSION

Inhibition of SV40 and BK virus DNA replication.

The results of these experiments are reported in Fig.1 and are expressed as a percentage of inhibition of viral DNA synthesis relative to untreated controls. It is evident from inspection of this figure that norfloxacin showed an antiviral activity only at relatively high drug concentrations. In fact, a 50% inhibition of viral DNA replication was observed at doses in excess of 50 ug/ml, which are closed to the cellular ED_{50} of this antibiotic. Similar results were obtained at various times after infection and using both SV40 and BK virus. Nalidixic acid was more potent, reaching a 50% reduction at approximately 10 ug/ml, in the absence of any sign of cytotoxicity. Coumermycin A_1 was also active, but, like norfloxacin, close to cytotoxic concentrations (5-10 ug/ml). In all cases, inhibition of DNA synthesis correlated with reduction of viral growth, as determined by titration of BK virus haemagglutinin.

Norfloxacin interference with DNAase I digestion of SV40 chromatin.

A typical result is shown in Fig. 2. DNAase I treatment led to the formation of increasing amounts of form II (nicked circular) and form III (linear) DNA, by the progressive introduction of single cuts whithin the SV40 chromatin (lanes 1 to 6). A significant inhibition of DNAase I digestion was produced by norfloxacin at different drug doses: 1 ug/ml (lane 7), 400 ng/ml (lane 8), and 100 ng/ml (lane 9), which represent drug to DNA molar ratios of 0.1, 0.4, and 0.01 respectively. The same effect was seen at different time periods following infection (from 28-to 45 hours). To test whether this effect was due to a direct interference of the antibiotic molecule with DNAase I itself, we repeated control experiments using purified naked SV40 DNA instead of whole nuclei. In these conditions norfloxacin did not altered the pattern of DNAase I digestion.

In conclusion, quinolone and coumarin antibiotics showed antiviral activity when assayed against papovaviruses. Norfloxacin was capable of interfering specifically with the activity of DNAase I on the regulatory region of SV40 chromatin, which is recognized by this enzyme. Since the drug does not seem to bind directly to DNA (Palù et al.: this issue) we postulate that quinolones may interfere with some enzymes (either viral or cellular), which are acting on the viral nucleic acid, perhaps through the formation of a ternary complex with DNA. Such a possibility is currently under investigation.

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