Effect of Novobiocin and Other DNA Gyrase Inhibitors on Virus Replication and DNA Synthesis in Herpes Simplex Virus Type 1-infected BHK Cells

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SUMMARY

The four known inhibitors of the bacterial DNA gyrase (nalidixic acid, oxolinic acid, novobiocin and coumermycin A) were investigated with respect to their effect on the growth of uninfected BHK cells and the yield of virus from herpes simplex virus type 1 (HSV-1)-infected BHK cells. High concentrations of nalidixic acid and oxolinic acid (about 10 mM) were needed for 50% inhibition of cellular and viral multiplication with less than fourfold preferential inhibition of virus over cell growth. Novobiocin and coumermycin were effective at lower molar concentrations and the amount needed for 50% inhibition was 10-fold higher for cell growth than for virus yield. At $5 \times 10^{-4}$ M, novobiocin inhibited DNA synthesis in uninfected cells to approx. 20% of non-treated controls, while virus DNA in infected cells was almost completely inhibited (approx. 1% of controls). Residual cellular DNA synthesis in infected cells was rather insensitive (approx. 90% of controls) to this concentration of novobiocin.

The first order of coiling of the DNA in chromatin of eukaryotic cells is accomplished by its winding around the histone octamers (Kornberg, 1977). Herpes simplex virus (HSV) DNA, which replicates in the nucleus of eukaryotic cells, does not appear to be contained in a chromatin-like structure (Francke, 1977 b; Leinbach & Summers, 1980). In prokaryotic cells, which also have no histones, DNA supercoiling is retained only partially by histone-like proteins while DNA topoisomerases are likely to be responsible for maintaining the remaining supercoils (Pettijohn & Pfenninger, 1980). The DNA gyrase of Escherichia coli (a type II DNA topoisomerase) was first identified through its sensitivity to two groups of antibiotics (Gellert et al., 1977; Higgins et al., 1978): nalidixic acid (nal) and oxolinic acid (oxo) which interact with the A subunit of DNA gyrase; and novobiocin (nov) and coumermycin (cou) which interact with the B subunit. In the study reported here, carried out to investigate the possibility that a DNA gyrase-like activity might be involved in HSV DNA replication, the effect of the four inhibitors on HSV-1-infected baby hamster kidney cells (BHK) was investigated.

The effects of nal, oxo, nov and cou on the growth rate of uninfected and the virus yield from HSV-1-infected cells are compared in Table 1. Nal and oxo were the least inhibitory, showing effects only at concentrations approaching their limit of solubility, while on a molar basis nov and cou were more effective, with cou being the most potent inhibitor. The relative effectiveness of the four antibiotics in reducing virus growth parallels their effect on cell growth, indicating that none of them is strikingly specific as an inhibitor of HSV-1 replication. Although the two parameters measured (virus yield and cell growth) may not be strictly comparable, it appears that the nov/cou group shows a difference of about 10-fold in favour of virus inhibition, while that difference is less in the case of the nal/oxo group. The following experiment was therefore performed with novobiocin.

The effect of nov on the synthesis of DNA was investigated by measuring the incorporation of $^3$H-thymidine into viral and cellular DNA of infected and uninfected cells.
Table 1. Effect of DNA gyrase inhibitors on BHK* cells

<table>
<thead>
<tr>
<th>Inhibitor†</th>
<th>Uninfected (50% reduction of growth rate)</th>
<th>HSV-1-infected§ (50% reduction of virus yield)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nalidixic Acid</td>
<td>$2.6 \times 10^{-3}$ M</td>
<td>$8.6 \times 10^{-4}$ M</td>
</tr>
<tr>
<td>Oxolinic Acid</td>
<td>$2.2 \times 10^{-3}$ M</td>
<td>$5.0 \times 10^{-4}$ M</td>
</tr>
<tr>
<td>Novobiocin</td>
<td>$3.0 \times 10^{-4}$ M</td>
<td>$2.9 \times 10^{-5}$ M</td>
</tr>
<tr>
<td>Coumermycin</td>
<td>$5.5 \times 10^{-5}$ M</td>
<td>$3.4 \times 10^{-6}$ M</td>
</tr>
</tbody>
</table>

* BHK-21 (C13) cells (McPherson & Stoker, 1962) were grown in Dulbecco's modified Eagle's medium supplemented with 10% calf serum and 10% tryptose phosphate broth (Difco). Growth rates were determined after seeding 1.5 x 10^5 cells/5 cm plastic dish (Nunc).

† Nalidixic acid and novobiocin were from Sigma, oxolinic acid from Parke, Davies and Co. (Detroit, Mich., U.S.A.) and coumermycin A from Godfrey Science and Design (Syracuse, N.Y., U.S.A.). Stock solutions were prepared fresh for each experiment at 10^{-2} M. The solvent for nalidixic and oxolinic acid was 0.1 N-KOH; for novobiocin, tris-buffered saline; and for coumermycin, dimethyl sulphoxide. At the concentrations used (1 x 10^{-6} to 5 x 10^{-3} M for each inhibitor) the solvents had no effect on the growth rate of BHK cells or on the yield of virus.

‡ Every 24 h duplicate cultures were subjected to trypsinization and the cell number determined using a Coulter counter. During the exponential phase of growth, the BHK cells had a doubling time of 15 h at 37 °C in the absence of the inhibitors. Percentage growth rate is defined as: (generation time of uninhibited control/generation time with inhibitor) x 100.

§ The Glasgow strain 17 of HSV-1 was grown and titred as described earlier (Francke, 1977 a).

Yield reduction assays were performed on confluent monolayers of BHK cells infected with 1 p.f.u./cell HSV 1. After 1 h at 37 °C the inoculum was removed and the cells washed with tris-buffered saline. Fresh medium was added and the culture incubated at 37 °C. Two h after addition of the medium, the inhibitors were added at the desired concentration. Three days later the cells were harvested into the medium and frozen at −90 °C. After thawing and disruption by sonication, the virus yields were determined by plaque assay. HSV-1 yields in uninhibited controls varied from 300 to 1000 p.f.u./infected cell.

Fig. 1. CsCl density-gradient profiles of DNA labelled in uninfected and infected BHK cells in the presence of 5 x 10^{-4} M-novobiocin. Cells were labelled with 4 μCi 3H-thymidine medium from 4 to 21 h after mock or virus infection (10 p.f.u./cell). Aliquots were analysed in CsCl density-gradients (Bone et al., 1978). DNA from (a) infected and (b) uninfected cultures is shown. In each case the profiles from the cultures without nov (O——O) are superimposed with those from the cultures with 5 x 10^{-4} M-nov (●——●). Density increase is from right to left. The denser peak in (a) represents virus DNA.

The inhibitor had very little effect on DNA synthesis at concentrations up to 1 x 10^{-4} M. At 5 x 10^{-4} M, virus DNA synthesis was extremely sensitive, while the incorporation of label into cellular DNA in infected cells was less affected (Fig. 1). It appears, therefore, that in HSV-1-infected cells the synthesis of virus DNA involves a function that is inhibited by this concentration of nov, while the residual synthesis of cellular DNA after virus infection is
independent of that function. When comparing the CsCl density-gradient profiles of the DNA labelled in the presence of $5 \times 10^{-4}$ M it is of interest to note that the absolute amount of 'nov-resistant' synthesis in uninfected cells corresponds closely to that seen for cellular DNA in infected cells in the presence or absence of nov.

For all four antibiotics tested, the concentration required to inhibit HSV-1 replication in infected cells is several orders of magnitude higher than their effective concentrations against the E. coli DNA gyrase (Higgins et al., 1978). We therefore have to conclude that if HSV depends on a gyrase-like activity, it would not share the inhibitor specificity with the E. coli enzyme. Topoisomerase II enzymes found in T4 and eukaryotic cells are insensitive to these inhibitors (Liu et al., 1979, 1980; Stetler et al., 1979). It is therefore possible that HSV DNA replication depends on a DNA topoisomerase II with a low sensitivity to nov, but the question has to be left open whether this would be a virus-induced or pre-existing cellular enzyme. To answer this, we are currently attempting to select a nov-resistant virus variant.

Collins & Johnson (1979) have used nov as a means to differentiate between different types of DNA synthesis in mammalian cells. X-ray-induced repair, for instance, was resistant to nov, while replication and u.v.-induced repair were sensitive. The results reported here on DNA synthesis in the presence of $5 \times 10^{-4}$ M-nov are of interest since they show a clear difference between viral and cellular DNA in infected cells. If inhibition by nov can be taken as a criterion for replicative DNA synthesis, the results imply that label incorporated into cellular DNA during virus infection is due to a non-replicative type of synthesis. The results also imply that processes involving incorporation of label into virus DNA, be they replication, recombination or repair, are dependent on a nov-sensitive function, and strongly suggest that intranuclear HSV DNA is maintained in a supercoiled state in spite of its lack of association with histones.

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REFERENCES


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