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Replication of *Escherichia coli* DNA *in vitro*: Inhibition by Oxolinic Acid

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Oxolinic acid, a quinolone antibacterial agent, inhibits reversibly the ATP-dependent replicative DNA synthesis in permeable cell systems as well as in cellophane disk lysates. It is about 10-fold more active than the structurally related nalidixic acid. Both drugs have no effect on the ATP-independent DNA repair, but interfere to some extent with RNA synthesis in permeable cells. They appear to interact with the same target since spontaneous nalidixic-acid-resistant mutants of *nalA* phenotype are also resistant to oxolinic acid. Full sensitivity to oxolinic acid can be conferred to lysates from resistant cells by addition of extracts from sensitive cells.

Oxolinic acid belongs to a group of quinolone antibacterial agents the best known of which is nalidixic acid. Nalidixic acid has been used extensively as a specific and reversible inhibitor of *Escherichia coli* DNA replication, although its mode of action is poorly understood [1]. Nalidixic acid inhibits replicative DNA synthesis in permeabilized cell systems [2], in bacterial lysates [3], and in soluble systems [4, 5], but has no effect on DNA repair [6]. Therefore nalidixic acid allows one to differentiate between these two types of DNA synthesis. Insensitivity to nalidixic acid previously reported for certain DNA replicating systems [7] is now considered as an indication of their non-physiological character.

However, the usefulness of nalidixic acid as a specific inhibitor of DNA replication *in vitro* is restricted by the comparatively high concentrations required for maximum inhibition. Replicative DNA synthesis is approximately 10 times less sensitive *in vitro* than *in vivo* and it is sometimes difficult to rule out secondary effects of high drug concentrations. Furthermore the inhibition is only partial (55–70% depending on the system) and the residual synthesis cannot be reduced by higher concentrations of the inhibitor. For these reasons it would be advantageous to have a structurally related quinolone compound available which is a more potent inhibitor of DNA replication *in vitro*.

A comparison of antibacterial activities has shown oxolinic acid to be at least 10-fold more active against enterobacteriacae than nalidixic acid [8]. This increased activity could be an indication for a stronger interaction of oxolinic acid with the nalidixic-acid-sensitive component of the DNA replication apparatus. We therefore compared the effects of oxolinic acid and nalidixic acid on DNA replication *in vitro* employing nucleotide-permeable cells and cellophane disk lysates. The results presented in this paper show that there is a good correlation between the antibacterial activities of these drugs and their capacity to block replicative DNA synthesis. It was found that oxolinic acid is a 10-fold more potent inhibitor of

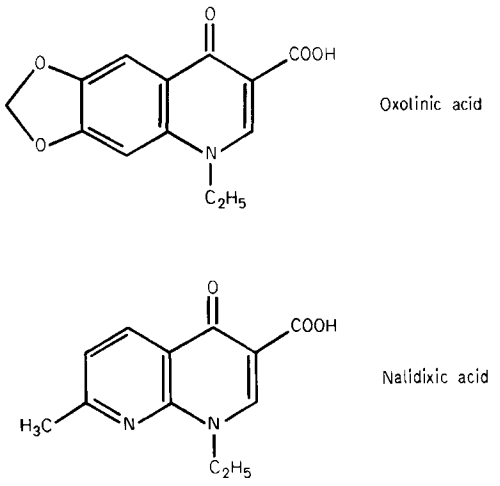

Fig. 1. *Structural formulae of oxolinic acid (1-ethyl-6,7-methylenedioxy-4-quinolone-3-carboxylic acid) and nalidixic acid (1-ethyl-7-methyl-1,8-naphthyridine-4-one-3-carboxylic acid)*

Abbreviations. Hepes, *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonate; EGTA, glycol-bis(2-aminoethyl ether)-*N,N'*-tetraacetic acid.

Trivial Names. Oxolinic acid, 1-ethyl-6,7-methylenedioxy-4-quinolone 3-carboxylic acid; nalidixic acid, 1-ethyl-7-methyl-1,8-naphthyridine-4-one 3-carboxylic acid.

DNA replication *in vitro* than nalidixic acid although the mode of action of these two agents appears to be identical.

MATERIALS AND METHODS

Bacterial Strains

E. coli 159 (F^+ thy^- hcr^-) was obtained from Dr D. Pratt (Davis, Calif.). *E. coli* BT 1000 (thy^- $endoI^-$ polAl) was from Dr F. Bonhoeffer (Tübingen). Spontaneous Nal^r mutants (*i.e.* resistant to nalidixic acid) were isolated by their ability to grow in the presence of 500 µg/ml nalidixic acid. Mapping of Nal^r mutants indicates that there are two genetic loci, *nalA* and *nalB* [9]. *nalA* mutants are resistant to more than 40 µg/ml of the drug, whereas *nalB* mutants are resistant to 4 µg/ml but not to 10 µg/ml. It has been shown that *nalB* is a transport mutant whereas *nalA* is not [10]. In merodiploids the $nalA^s$ allele is dominant over the $nalA^r$.

Chemicals

Oxolinic acid was obtained from Gödecke AG (Freiburg), nalidixic acid and hydroxy-nalidixic acid were obtained from Sterling-Winthrop Laboratories. The sources of all other reagents have been described previously [11].

Preparation Systems Used in vitro

Cells were grown in 1.6 l M-3 medium (antibiotic medium 3 from Difco) at 37 °C to an $A_{600} = 0.5$ and harvested at room temperature.

Toluenized Cells. Cells were resuspended in 60 ml 20 mM Hepes pH 8.0 containing 1% toluene and permeabilized for 5 min at room temperature by vortex treatment. Cells were washed free of toluene and resuspended in buffer at 5×10^{10} cells/ml. Aliquots were quick frozen and stored in liquid nitrogen.

Plasmolysed Cells. Cells were resuspended at room temperature in 5 ml 20 mM Hepes pH 8.0, 5 mM EGTA, 2 M sucrose. Aliquots were quick frozen in liquid nitrogen and stored at −20 °C.

Cellophane Disk Lysates. Cells were resuspended in 20 mM Hepes pH 8.0, 20% (w/w) sucrose, quick frozen and stored at −20 °C. Lysates were prepared by the procedure of Schaller *et al.* [3]. Preparation of DNA-free extracts was essentially as described by Wickner *et al.* [12].

Assay of DNA Synthesis

Standard incubation mixtures (25 µl) contained 20 mM Hepes pH 8.0, 0.1 M KCl, 10 mM magnesium acetate, 2 mM ATP, 0.1 mM NAD, 0.02 mM each of dATP, dCTP, and [^{3}H]dTTP (250 counts min^{-1} $pmol^{-1}$). Incubations were carried out for 30 min at 30 °C.

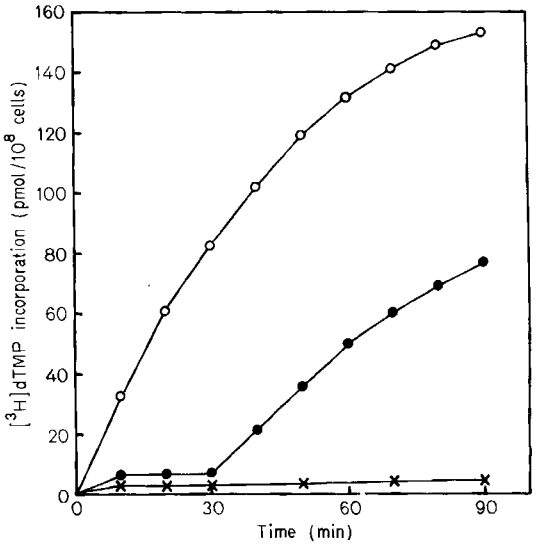

Fig. 2. *Effect of oxolinic acid on DNA synthesis in toluenized cells.* 2×10^9 toluenized cells of *E. coli* 159 were incubated in 1-ml standard incubation mixtures (see Methods). At the times indicated 25-µl aliquots were removed and assayed for acid-insoluble radioactivity. After 30 min the incubation mixtures were cooled in an ice bath. The cells were pelleted, washed once with cold 0.02 M Hepes pH 8.0, and resuspended in fresh reaction mixtures. Incubation was then continued for 60 min at 30 °C. (O—O) Control; (●—●) oxolinic acid (50 µg/ml) present during the first 30 min of incubation; (×—×) control without ATP

RESULTS

Effect on DNA Synthesis in Nucleotide-Permeable Cells

Toluene-treated *E. coli* cells incorporate deoxynucleoside 5′-triphosphates into DNA in a semiconservative replication process [13,14]. DNA replication in toluenized cells represents a continuation of replication *in vivo* and proceeds only at the region of the genome about to be replicated before the treatment [15]. Replicative synthesis is nearly completely dependent upon the addition of ATP, which is not required for the repair synthesis carried out by DNA polymerase I. This allows a convenient measurement of nonreplicative synthesis by omission of ATP. Repair synthesis can be markedly stimulated by addition of exogenous nucleases.

The experiment described in Fig. 2 shows the effect of oxolinic acid on the incorporation of [^{3}H]dTMP in toluenized *E. coli* cells. It is evident that oxolinic acid (50 µg/ml) reduces the amount of replicative DNA synthesis almost to the background level of ATP-independent DNA repair. The inhibition of DNA replication is fully reversible since if the inhibitor is

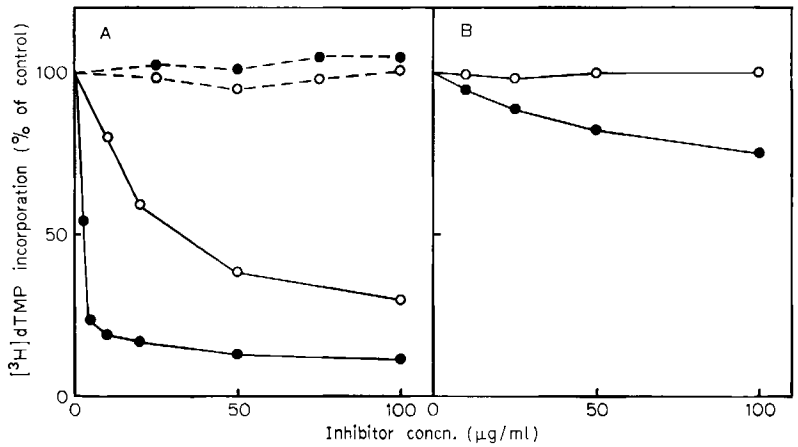

Fig. 3. *Selective inhibition of ATP-dependent DNA synthesis.* Standard incubation mixtures (50 μl) contained 10^8 toluenized cells of either (A) *E. coli* 159 or (B) *E. coli* 159 (Nal^r). Oxolinic acid (●——●) or nalidixic acid (○——○) were added at the concentrations indicated. Repair synthesis was assayed in incubation mixtures containing 0.5 μg/ml pancreatic DNAase and no ATP with either oxolinic acid (●-- ●) or nalidixic acid (○——○) present. Incubation time: 30 min at 30 °C. DNA synthesis is expressed as percentage of incorporation in the absence of inhibitors. 100% corresponds to an incorporation of (A) 110 pmol or (B) 135 pmol [^{3}H]dTMP (ATP-dependent synthesis), and 35 pmol [^{3}H]dTMP (repair synthesis) respectively

removed after a 30-min incubation the rate of incorporation is practically indistinguishable from the untreated control.

Fig. 3A compares the inhibitory activities of oxolinic acid and nalidixic acid. It can be seen that oxolinic acid inhibits replicative DNA synthesis by 75% at a drug concentration of 5 μg/ml, whereas a 10-fold higher concentration of nalidixic acid is required to achieve the same effect. The maximum degree of inhibition (88%) that can be obtained with oxolinic acid under standard assay conditions also compares favourably with nalidixic acid (70%). On the other hand, both drugs have no effect on the ATP-independent DNA repair triggered by pancreatic DNAase. These conclusions were confirmed by density labelling experiments as described previously [15].

It appears likely that because of their structural similarity oxolinic acid and nalidixic acid interact with the same component of the replication apparatus. To test this, we compared the effects of oxolinic acid and nalidixic acid on DNA replication in a permeabilized cell system prepared from a spontaneous mutant of *E. coli* 159 that is resistant to high concentrations of nalidixic acid (Nal^r). If both drugs interact with the same target the sensitivities of DNA replication to oxolinic acid and nalidixic acid should both be abolished by one single mutation. It can be seen (Fig. 3B) that in the Nal^r cell system the inhibitory effect of oxolinic acid is also drastically reduced. Therefore, oxolinic acid and nalidixic acid appear to block semi-conservative DNA replication by binding to the same receptor.

Interference with RNA Synthesis

It has been reported recently that nalidixic acid causes a substantial inhibition of the transcription of some bacterial genes [17] independent of its effect on DNA replication. Puga and Tessman [18] have previously observed an inhibitory action of nalidixic acid on phage S-13 transcription. These findings indicate that nalidixic acid, besides blocking DNA replication, also affects the expression of certain genes. Therefore we investigated whether oxolinic acid and nalidixic acid can also interfere with RNA synthesis in permeabilized cells. For these studies plasmolysed cells, *i.e.* cells made permeable by treatment with 2 M sucrose were employed. It has been shown previously [19, 20] that this treatment renders the cells permeable to small molecules while leaving them able to carry out physiological RNA and protein synthesis.

As can be seen in Fig. 4A, oxolinic acid and nalidixic acid markedly reduce the amount of [^{3}H]-UMP incorporation. Both drugs give a maximum inhibition of approximately 50% albeit at concentrations 10–20-fold higher than those required for a similar inhibition of DNA replication. It should be noted that for better comparison the incorporation mixture was identical with the one used for assaying DNA replication except for replacing the deoxynucleoside triphosphates with ribonucleoside triphosphates.

The observed inhibition could obviously represent a secondary effect only observed at high drug concentrations. Therefore a control experiment was per-

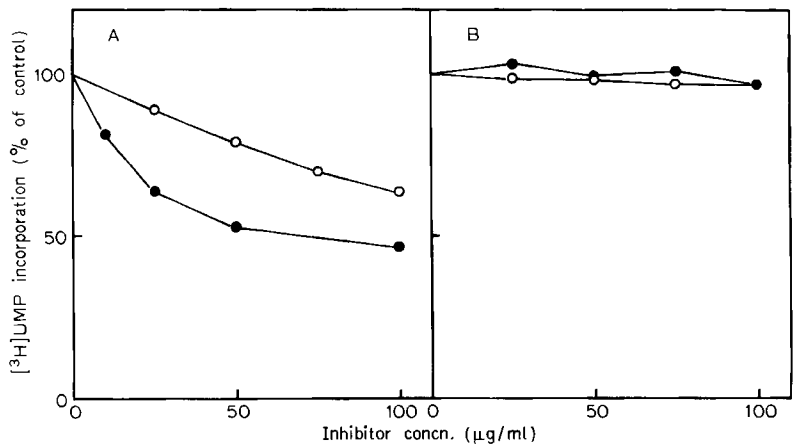

Fig. 4. *Effect of oxolinic acid and nalidixic acid on RNA synthesis in plasmolysed cells.* [^{3}H]UMP incorporation was assayed in incubation mixtures (50 μl) containing 20 mM Hepes pH 8.0, 0.1 M KCl, 10 mM magnesium acetate, 2 mM ATP, 0.1 mM each of CTP, GTP, and [^{3}H]UTP (100 counts min^{-1} pmol^{-1}) and 10^8 plasmolysed cells. Incubations were performed for 30 min at 30 °C. Oxolinic acid (●——●) or nalidixic acid (○——○) were added at the concentrations indicated. RNA synthesis is expressed as percentage of incorporation of an untreated control. 100% corresponds to an incorporation of (A) 310 pmol or (B) 330 pmol [^{3}H]UMP respectively. (A) *E. coli* 159, (B) *E. coli* 159 (Nal^r)

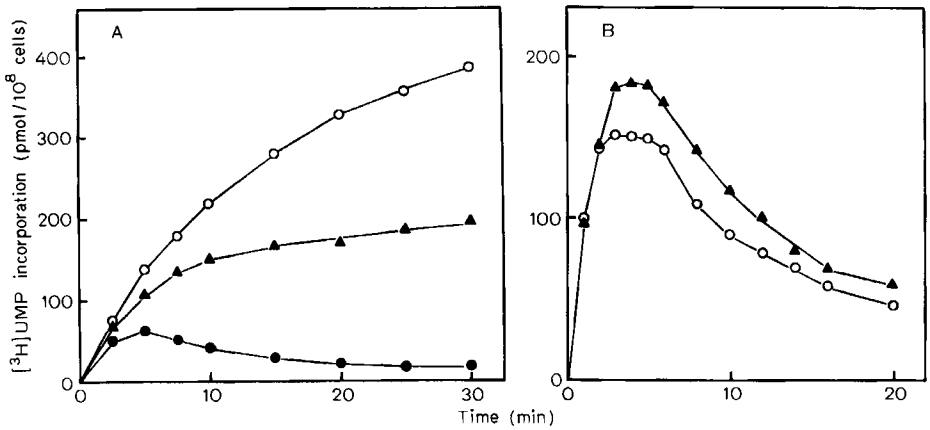

Fig. 5. *Kinetics of [^{3}H]UMP incorporation.* (A) 2×10^9 plasmolysed cells of *E. coli* 159 were incubated in 1-ml incubation mixtures at 30 °C as described in Fig. 4. At the times indicated 50-μl aliquots were removed and assayed for acid-insoluble radioactivity. (○ —○) Control; (▲——▲) oxolinic acid (100 μg/ml) present; (●——●) rifampicin (5 μg/ml) present. (B) Cells were pre-incubated for 3 min at 30 °C in 1-ml incubation mixtures as in (A) but omitting the radioactive label. 25 μCi [^{3}H]UTP and rifampicin (final concntration 5 μg/ml) were then added together with or without oxolinic acid as indicated. At 1-min intervals 50-μl aliquots were removed and assayed for acid-precipitable radioactivity. (○——○) Control; (▲——▲) oxolinic acid (200 μg/ml) added

formed employing plasmolysed *E. coli* 159 (Nal^r) cells. No inhibitory effect of either oxolinic acid or nalidixic acid on [^{3}H]UMP incorporation was observed in Nal^r cells (Fig. 4B). Therefore we conclude that the inhibition of DNA replication by these agents must share a common step with the inhibition of transcription.

The inhibitory effect of oxolinic acid on RNA synthesis was further analysed in the experiment described in Fig. 5. It can be seen (Fig. 5A) that plasmolysed cells incorporate [^{3}H]UMP during a 30-min incubation at a gradually decreasing rate. This incorporation is blocked by low concentrations of rifampicin and represents the synthesis of messenger-type RNA [20]. In the presence of oxolinic acid (100 μg/ml) the incorporation reaches a plateau already after 10 min. This could be the result from different modes of drug action: oxolinic acid might interfere directly with RNA synthesis by blocking the initiation or elongation of RNA chains, or indirectly by enhancing the rate of mRNA degradation. To decide between these possibilities the following experiment was performed (Fig. 5B). The cells were preincubated in the absence of radioactive label for 3 min, [^{3}H]UTP was then added together with rifampicin and oxolinic acid (200 μg/ml). It can be seen that oxolinic acid has no inhibitory effect on the amount of label incorporated under these condi-

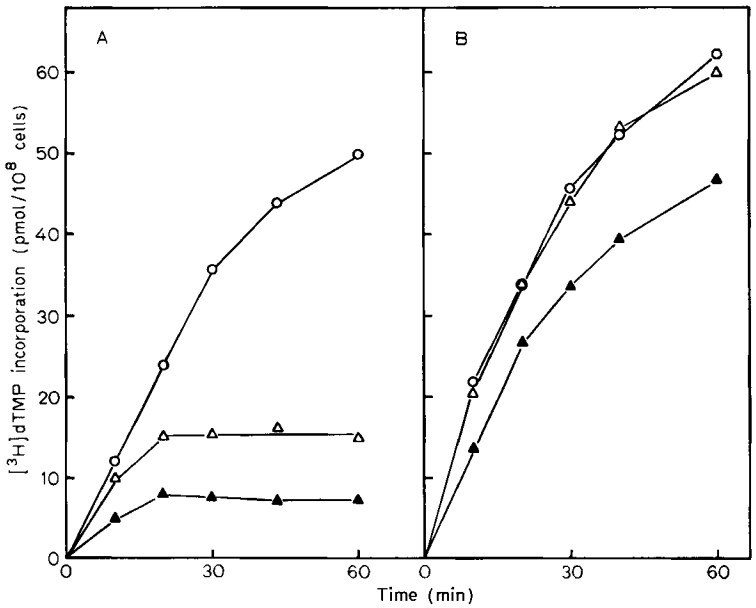

Fig. 6. *Kinetics of DNA synthesis in cellophane disk lysates.* 1 µl of a cell suspension (5×10^{10} cells/ml) of (A) *E. coli* BT 1000 (Nal^s) or (B) *E. coli* BT 1000 (Nal^r) was spread with 1 µl of lysozyme solution (1 mg/ml lysozyme, 1% Brij, 10 mM EGTA, 20% sucrose, 20 mM Hepes pH 8.0) on a cellophane disk of 12-mm diameter lying on a cold agar plate A (2% agar, 5 mM EGTA, 10% sucrose, 20 mM Hepes pH 8.0). After 20 min at 4 °C the disks were transferred onto another agar plate B (2% agar, 5 mM magnesium acetate, 20 mM Hepes pH 8.0) and dried by cold air. The disks were incubated on 25-µl drops of incubation mixture in a Petri dish which was covered and floated in a 30 °C water bath. The incubation was stopped and the acid-insoluble radioactivity determined as described by Schaller *et al.* [3] but omitting the heating step. (○——○) Control; (▲——▲) oxolinic acid (50 µg/ml) present; (△——△) nalidixic acid (50 µg/ml present

tions, nor is there any evidence for an accelerated degradation of the labeled RNA in the presence of the drug. Identical results were obtained for nalidixic acid (data not shown). This argues that both drugs interfere specifically with the rifampicin-sensitive initiation step of RNA synthesis.

Inhibition of DNA Replication in Cellophane Disk Lysates

Schaller *et al.* [3] have described a DNA replication system *in vitro* which in contrast to the nucleotide-permeable cell can be complemented by the addition of exogenous macromolecules and still retains many of the characteristics of normal DNA replication including sensitivity to nalidixic acid. This method involves the gentle lysis of cells on a cellophane disk held on an agar surface. In order to test the inhibitory activity of oxolinic acid in this system cellophane disk lysates were prepared from quick frozen cells of *E. coli* BT 1000 stored at −20 °C. Fig. 6A shows that the rate of ATP-dependent DNA synthesis is linear for at least the first 20 min of incubation at 30 °C and amounts to approximately 1.25 pmol of [^{3}H]dTMP per min per 10^8 lysed cells. Similar to the results with permeable cells, the incorporation is strongly inhibited by oxolinic acid (50 µg/ml) and to a lesser extent by nalidixic acid. However, there appears to be a delay of drug action which is not observed in the cellular systems *in vitro*. The cellophane disk lysate from *E. coli* BT 1000 (Nal^r) cells is completely resistant to nalidixic acid (50 µg/ml) but exhibits some residual sensitivity to oxolinic acid (Fig. 6B). However, 20-fold higher concentrations of oxolinic acid are required for the same degree of inhibition as compared to the Nal^s lysates.

Bourguignon *et al.* [10] have shown that soluble extracts from Nal^s cells confer nalidixic acid sensitivity on the DNA synthesis in lysates from Nal^r cells. It was therefore of interest to test whether addition of Nal^s extracts also confers oxolinic acid sensitivity to Nal^r lysates and whether this might provide a more sensitive assay for the drug receptor present in the soluble extract. Highly concentrated DNA-free extracts of both the Nal^s and the Nal^r strain were prepared according to the procedure of Wickner *et al.* [12]. 1 µl of extract (15−20 µg protein) is roughly equivalent to the lysate from 5×10^7 cells. 1−4 µl of extract were applied onto the disks and air-dried in the cold. 5×10^7 cells were then spread onto the disk together with lysozyme solution and lysed as described in Fig. 7. The lysed cells were then assayed for sensitivity to oxolinic acid employing an inhibitor concentration of 25 µl/ml. This drug concentration reduces the DNA synthesis in the Nal^s lysate by 75% whereas less than 10% inhibition is observed in the Nal^r lysate. Upon addition of increasing amounts of a Nal^r extract to the Nal^s lysate only a small increase in resistance to oxolinic acid is found (Fig. 7A). By contrast, adding a 4-fold excess of Nal^s

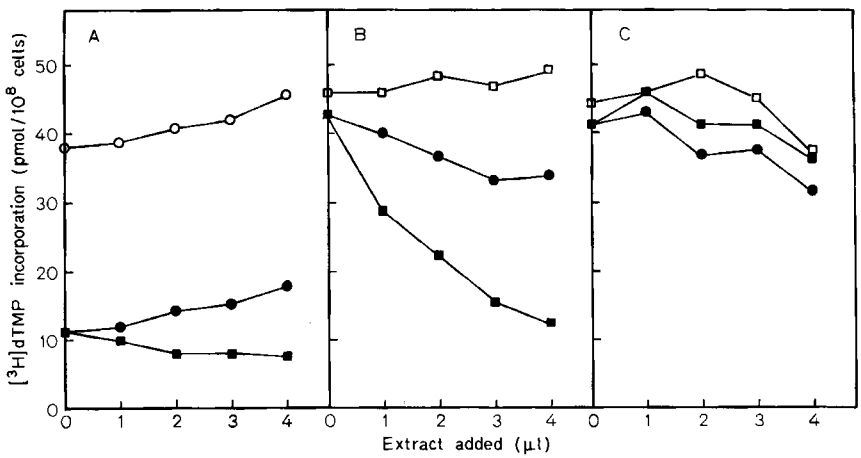

Fig. 7. *Effect of added cell-free extracts on oxolinic acid sensitivity of lysates.* Cell-free extracts were prepared from (A) *E. coli* BT 1000 (Nal^s) or (B, C) *E. coli* BT 1000 (Nal^r) by the lysis procedure of Wickner *et al.* [12]. 1–4 µl of extract were applied per cellophane disk and dried on an agar plate by cold air. 1 µl of cell suspension was then added per disk and lysed as described in Fig. 6. Incubations were carried out for 30 min at 30 °C in the presence or absence of oxolinic acid (25 µg/ml). (A) Lysates were prepared from *E. coli* BT 1000 (Nal^s). (○——○) Nal^r extract added; (●——●) Nal^r extract added plus oxolinic acid; (■——■) Nal^s extract added plus oxolinic acid. (B) Lysates were prepared from *E. coli* BT 1000 (Nal^r). (□——□) Nal^s extract added; (■——■) Nal^s extract added plus oxolinic acid; (●——●) Nal^r extract added plus oxolinic acid. (C) Lysates were prepared from *E. coli* BT 1000 (Nal^r). Extracts were added to incubation droplets. Symbols as in (B)

extract to a Nal^r lysate increases the oxolinic acid sensitivity drastically from 10% to 75% (Fig. 7B). This is nearly identical with the degree of inhibition observed in the Nal^s lysate at this drug concentration. The control experiments show that addition of the Nal^s extract to the lysate has no inhibitory effect if the DNA synthesis is measured in the absence of the drug. However, an increase in oxolinic acid sensitivity (up to 30%) is also observed upon adding an excess of the homologous Nal^r extract. This probably results from the leakiness of the Nal^r mutation which is also indicated by the limited oxolinic acid resistance of this strain. Next we tested whether it is possible to confer oxolinic acid sensitivity to cellophane disk lysates from resistant cells by adding Nal^s extract to the incubation droplet instead of mixing it with the lysate on top of the disk. As can be seen in Fig. 7C, the results of such an experiment are clearly negative and no drug sensitivity was observed to be conferred under these conditions.

DISCUSSION

As might be expected from their structural similarity, the effects of oxolinic acid on DNA synthesis *in vitro* closely resemble those of nalidixic acid. Both agents function as reversible inhibitors of the ATP-dependent replicative DNA synthesis in permeabilized cell systems as well as in cellophane disk lysates. They have no effect on the ATP-independent DNA repair carried out by DNA polymerase I. Sensitivity to oxolinic acid is drastically reduced if the system used *in vitro* are prepared from spontaneous Nal^r mutants, presumably mutated in the *nalA* locus. As has been previously reported for nalidixic acid [10], sensitivity to oxolinic acid is dominant and can be conferred to resistant lysates by addition of sensitive extracts.

However, oxolinic acid and nalidixic acid differ markedly in their inhibitory activity. Oxolinic acid is about 10-fold more active in inhibiting DNA replication in all systems tested *in vitro*. It is also superior to nalidixic acid as far as the maximum obtainable inhibition is concerned. Furthermore, the one-step Nal^r mutants that we have isolated so far (resistant to 500 µg/ml nalidixic acid) have still retained some sensitivity to oxolinic acid and do not form colonies at 100 µg/ml oxolinic acid. At present we do not know whether this is simply an indication of a stronger interaction of oxolinic acid with the same target or whether additional molecular activities are involved. At any rate, there exists a correlation of the antibacterial activities of these drugs and their capacities to block DNA replication *in vitro*.

Both drugs are not strictly specific inhibitors of DNA replication. They also interfere with transcription in permeable cells, although the inhibition of RNA synthesis is incomplete and occurs at concentrations at least 10-fold higher than those required to block DNA replication. This is in agreement with data obtained *in vivo* [17] demonstrating that nalidixic acid causes a preferential inhibition of the transcription of certain bacterial operons. Oxolinic acid and nalidixic acid seem to interfere with the initiation but not with the propagation of RNA chains. There is no evidence for an accelerated degradation of

mRNA in the presence of these drugs. Similar results were obtained by Puga and Tessman [18] studying the inhibition of phage S-13 transcription by nalidixic acid *in vivo*. These authors demonstrated that nalidixic acid does not lead to a premature termination of RNA chains nor does it enhance the degradation of phage mRNA. It should be pointed out that no inhibition of RNA synthesis by oxolinic acid or nalidixic acid is observed in a plasmolysed cell system prepared from a spontaneous Nal^r mutant. This suggests that a common step affects both replication and transcription. The molecular mechanism by which the quinolone antibacterial agents interfere with these processes remains to be elucidated.

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REFERENCES

1. Goss, W. A. & Cook, T. M. (1975) in *Antibiotics* (Corcoran, J. W. & Hahn, F. E., eds) vol. 3, pp. 174–196, Springer-Verlag, Berlin.
2. Pedrini, A. M., Geroldi, D., Siccardi, A. & Falaschi, A. (1972) *Eur. J. Biochem. 25*, 359–365.
3. Schaller, H., Otto, B., Nüsslein, V., Huf, J., Herrmann, R. & Bonhoeffer, F. (1972) *J. Mol. Biol. 63*, 183–200.
4. Sakakibara, Y. & Tomizawa, J. (1974) *Proc. Natl Acad. Sci. U.S.A. 71*, 802–806.
5. Kornberg, T., Lockwood, A. & Worcel, A. (1974) *Proc. Natl Acad. Sci. U.S.A. 71*, 3189–3193.
6. Simon, T. J., Masker, W. E. & Hanawalt, P. C. (1974) *Biochim. Biophys. Acta, 349*, 271–274.
7. Okazaki, R., Sugimoto, K., Okazaki, T., Imae, Y. & Sugino, A. (1970) *Nature (Lond.) 228*, 223–226.
8. Kaminsky, D. & von Strandtmann, M. (1969) *Annu. Rep. Med. Chem. 5*, 87–100.
9. Hane, M. W. & Wood, T. H. (1969) *J. Bacteriol. 99*, 238–241.
10. Bourguignon, G. J., Levitt, M. & Sternglanz, R. (1973) *Antimicrob. Agents Chemother. 4*, 479–486.
11. Staudenbauer, W. L. (1974) *Eur. J. Biochem. 47*, 353–363.
12. Wickner, W., Brutlag, D., Schekman, R. & Kornberg, A. (1972) *Proc. Natl Acad. Sci. U.S.A. 69*, 965–969.
13. Moses, R. E. & Richardson, C. C. (1970) *Proc. Natl Acad. Sci. U.S.A. 67*, 674–681.
14. Pisetsky, D., Berkower, I., Wickner, R. & Hurwitz, J. (1972) *J. Mol. Biol. 71*, 557–571.
15. Burger, R. M. (1971) *Proc. Natl Acad. Sci. U.S.A. 68*, 2124–2126.
16. Staudenbauer, W. L. (1975) *J. Mol. Biol. 96*, 201–205.
17. Shuman, H. & Schwartz, M. (1975) *Biochem. Biophys. Res. Commun. 64*, 204–209.
18. Puga, A. & Tessman, I. (1973) *J. Mol. Biol. 75*, 99–108.
19. Gros, F., Gallant, J., Weisberg, R. & Cashel, M. (1967) *J. Mol. Biol. 25*, 555–557.
20. Ben-Hamida, F. & Gros, F. (1971) *Biochimie (Paris) 53*, 71–80.

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