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International Journal of Antimicrobial Agents 18 (2001) 579–582

INTERNATIONAL JOURNAL OF
Antimicrobial Agents

www.ischemo.org

Short communication

In vitro activity of the chelating agents nitroxoline and oxine against *Mycobacterium bovis* BCG

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Received 13 June 2001; accepted 12 July 2001

Abstract

The chelating antibiotic nitroxoline (5-nitro-8-hydroxyquinoline) showed a bacteriostatic effect at a concentration of 10 μM for *Mycobacterium bovis* BCG. At higher concentrations the compound showed moderate cidal activity against growing bacilli. In contrast, its non-nitrated derivative oxine (8-hydroxyquinoline, MIC = 2 μM) reduced the viability of a growing culture rapidly, 5000-fold at a concentration where the nitroxoline was merely bacteriostatic. Both compounds showed appreciable cidal activity against bacilli in their hypoxic dormant state.

Keywords: *Mycobacterium*; Nitroxoline; Oxine

1. Introduction

Tuberculosis remains a leading cause of illness and death. One-third of world's population is latently infected with *Mycobacterium tuberculosis*. Latently infected people are at a risk of developing the active disease. Eight million cases of active tuberculosis, partially due to the reactivation of the latent state, and three million deaths from the disease occur each year [1]. A major problem in the chemotherapy of tuberculosis is the persistence of infection despite prolonged therapy: current regimes require six months of treatment. This may be due to sub-populations of the bacillus shifting down to a non-replicating or dormant state; growth-arrested bacteria are less susceptible to conventional anti-tuberculosis drugs than growing bacilli [2]. The difficulty of tuberculosis control is compounded by the increasing incidence of infection caused by the genetically drug-resistant strains. The problem of genetic and possibly the above-mentioned physiological drug resistance due to quiescent organisms demonstrate the requirement for drugs with new mechanisms of action.

Nitroxoline (5-nitro-8-hydroxyquinoline, quinoline = benzopyridine) is an antibiotic that does not belong to any known anti-microbial class. The drug is used in the treatment of acute or recurrent urinary tract infections as it shows activity against uropathogenic *Escherichia coli* strains (MIC = 8 mg/l) [3]. The pharmacokinetics of nitroxoline in plasma and urine are well established [4]. Bourlioux and co-workers demonstrated a correlation between the chelating property and the anti-bacterial activity of the compound [5–9]. The hydroxy group of the compound acts as a weak acid ($pK_a = 6.3$) and divalent metal cations such as Mg^{2+} and Mn^{2+} are coordinated by the corresponding $-O^-$ and the nitrogen within the pyridine moiety of the quinoline [3]. Although it appears that its chelating activity plays a role, the mechanism by which the nitroxiline-cation complexes exert cidal activity as well as the identity of the actual cations involved remains to be elucidated.

In addition to the unusual chelating-based mechanism of action of the drug the compound contains a nitro-group. Nitro-heterocycles (nitroimidazoles, nitroimidazopyrans) were the first anti-dormancy leads identified [10,11] and recently, we demonstrated that nitrofurans exhibit cidal activity against growing and dormant tubercle bacilli [12]. The cidal activity of the nitro-heterocyclic compounds is presumably due to the reduction of the nitro-group to a reactive intermediate

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0924-8579/01/$ - $20
PII: S0924-8579(01)00437-X

that then causes damage and subsequent death. The novel chelating-based mechanism of action of nitroxoline together with the fact that the compound is a nitro-heterocycle prompted us to question whether the drug has cidal activity against growing and/or dormant tubercle bacilli and whether the nitro-group plays a role in its activities.

2. Material and methods

2.1. Antimicrobial agents

Nitroxoline and oxine were from Sigma. Stock solutions were prepared in dimethylformamide.

2.2. Strain and cultivation

All experiments were carried out using *Mycobacterium bovis* BCG ATCC 35734. Liquid culture experiments were conducted at 37 °C in screw cap tubes with 17 ml Dubos Tween-albumin broth (pH 6.6; BD Biosience) exactly as described in Ref. [12]. To grow experimental exponential phase bacilli, pre-cultures were diluted to 5×10^6 cfu/ml. Drugs (170 μl) were added and caps were loosely screwed down allowing exchange of air. Cultures were aerated by incubation on a shaker-incubator at 250 rpm. For MIC determination cultures were exposed to serial 2-fold dilutions of the agents. The effect of the drug was monitored by measuring the optical density (A_{600}) of the cultures after 24 and 48 h of exposure. The lowest drug concentration that caused inhibition of growth was recorded as MIC. To grow dormant cultures pre-cultures were diluted to 5×10^5 cfu/ml. Magnetic stirrers were added, the caps (with rubber septa) were tightly screwed down to seal the tubes and the cultures were incubated on stirring platforms at 170 rpm. After 20 days of growth (10^8 cfu/ml) drugs were injected using a needle into the hypoxic stationary phase cultures. Oxygen depletion was monitored with the redox indicator methylene blue as described in Ref. [12]. Colony-forming unit counts were determined by plating appropriate dilutions of the culture onto Dubos oleic-albumin agar as described in Ref. [12]. Typically, cultures were diluted at least 10^3-fold before plating. Thus, considering the MICs of nitroxoline (10 μM) and oxine (2 μM) and the maximum drug concentration used (250 μM in dormant cultures), the dilutions for plating contained less that $\cong 10\%$ of the MIC. However, to demonstrate directly that the drug carry over effects were negligible, colony counts were compared in which cultures had been diluted in a medium containing either no drug, or 200 μM oxine or nitroxoline and plated immediately. Colony counts were found to be similar and thus not affected by the presence of the drugs.

3. Results

3.1. Cidal activity of nitroxoline and oxine for growing tubercle bacilli

The MIC of nitroxoline was 10 μM (1.9 mg/l). Fig. 1A shows that nitroxoline at MIC was bacteriostatic. Moderate bactericidal activity was found at higher concentrations: $5 \times$ MIC (50 μM) resulted in a 100-fold reduction of viability after three days of exposure. To determine whether the nitro-group of nitroxoline plays a role in the bactericidal activity of the compound, the experiments were carried out the same way with its non-nitrated derivative oxine (8-hydroxyquinoline). The MIC for oxine was found to be 2 μM (0.3 mg/l). Oxine at 10 μM, i.e. a concentration at which nitroxoline was merely bacteriostatic, resulted in a rapid 5000-fold decrease in viable counts within one day (Fig. 1B). Thus, the cidal activity of oxine was significantly higher than that of nitroxoline. This shows that the nitro-group does not play a role in the cidal effect of nitroxoline against the growing tubercle bacilli. On the contrary, the nitro-group appears to attenuate the potency of the compound.

3.2. Cidal activity of nitroxoline and oxine for dormant tubercle bacilli

To grow dormant BCG we employed the dormancy culture model that was developed by Wayne and coworkers [13–15]. Wayne's model is based on the observation that the obligate aerobe tubercle bacillus encounters hypoxic environments once it resides within their host [2]. To mimic this in vivo condition bacteria are grown in sealed tubes. Under these oxygen-limited conditions the bacilli self-generate a temporal oxygen gradient and the culture enters stationary phase when oxygen is depleted. The bacilli in the hypoxic stationary phase are in a synchronised state of low metabolic activity, in which the cells maintain viability for extended periods without division [13,14]. Both nitroxoline (Fig. 1C) and oxine (Fig. 1D) showed moderate cidal activity against dormant bacteria on increasing the concentration of the compounds: 250 μM caused a 20- to 200-fold reduction of the viability of the dormant culture over a period of three days. The finding that both, nitroxoline and oxine showed a similar effect on dormant bacilli suggests that the nitro-group does not play a role in the cidal activity on dormant cells.

4. Discussion

We report the anti-microbial activity of nitroxoline and its non-nitrated derivative oxine on growing and dormant tubercle bacilli. Both compounds showed a

similar (moderate) activity against dormant organisms, suggesting that the nitro-group in nitroxoline does not play a role in the toxicity of the compound. Nitroxoline showed appreciable cidal activity against the growing bacilli. However, oxine was found to be markedly more potent; the compound reduced the viability of the growing culture rapidly by 5000-fold at a concentration (10 μM, 1.5 mg/l) at which nitroxoline showed only a bacteriostatic effect.

The mechanisms underlying the strong cidal activity of oxine against growing bacilli and why its cidal activity is reduced for dormant cells is not clear. Oxine is used in chemistry as a chelating agent in the determination of trace metal ions (e.g. Co^{2+}, Cu^{2+} and Ni^{2+} [16]), and halogenated oxines have been used as luminal amoebicides [17]. A few reports in the literature offer some interesting observations regarding the biological activity of oxine such as the RNA synthesis in yeasts is rapidly inhibited by the compound. Work on *E. coli* RNA polymerase then showed that this might be due to the chelating of the dissociable cations Mn^{2+} and Mg^{2+} and possibly the tightly bound Zn^{2+} [18]. This might argue for a rather unspecific mechanism of action via titration of essential cations. On the other hand, copper complexes of oxine, but not the free ligand, were shown to inhibit amino acyl tRNA synthetase activity [19]. Further, it was shown that iron bound to this chelator causes DNA strand breakage in cultured human lung cells, possibly due to the action of metal-bound oxyl radical formed from the complex [20]. Thus, it is conceivable that multiple mechanisms involving different cations are responsible for the anti-mycobacterial activity of oxine. Inhibition of transcription and translation as well as DNA damage via the formation of various metal–oxin complexes would be consistent with the observed reduction of oxin's cidal activity for dormant bacilli; mycobacteria in their dormant state show reduced gene expression and do not replicate. Consequently, they show reduced sensitivity for inhibitors of these processes such as rifampicin and ciprofloxacin [13]. Whether the above-mentioned mechanisms are specific to oxine or whether they could also play a role in the cidal activity of nitroxoline, to our knowledge, has not been determined. Genetic approaches are now in progress to define the targets and mechanism of action of these interesting anti-microbial compounds in mycobacteria.

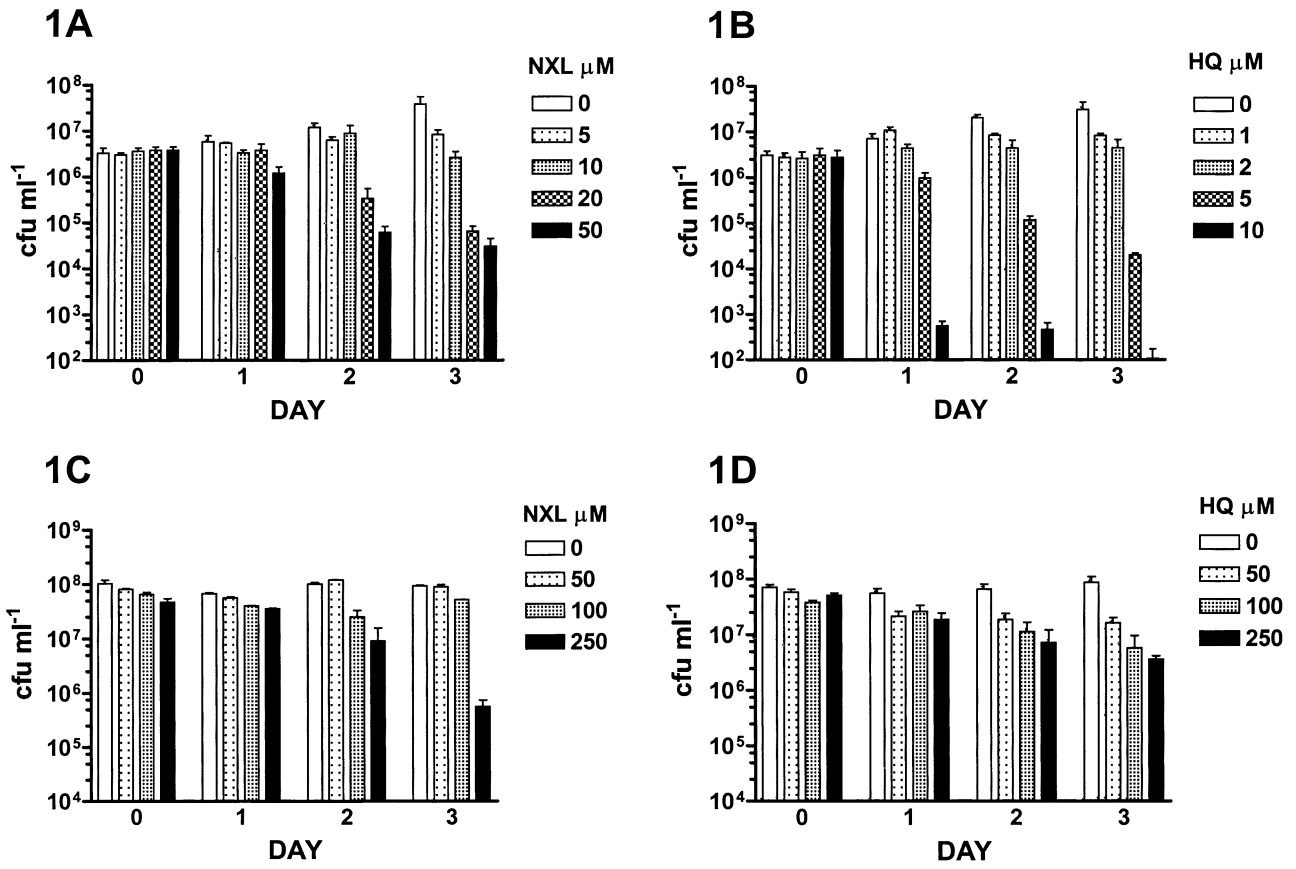

Fig. 1. Effect of nitroxoline (NXL) and oxine (HQ) on the viability of (A, B) growing and (C, D) dormant BCG cultures. Drugs were added at day 0 and cfu were determined by plating and colony count. The experiments were carried out three times and each experiment was performed with duplicate cultures. Mean values and standard deviations are shown. Significant clumping or pH change was not observed in any culture.

Acknowledgements

This study was supported by the Institute of Molecular and Cell Biology (IMCB).

References

[1] Manabe YC, Bishai WR. Latent Mycobacterium tuberculosis-persistence, patience, and winning by waiting. Nat Med 2000;6:1327–9.
[2] Dick T. Dormant tubercle bacilli: the key to more effective TB chemotherapy? J Antimicrob Chemother 2001;47:117–8.
[3] Pelletier C, Prognon P, Bourlioux P. Roles of divalent cations and pH in mechanism of action of nitroxoline against *Escherichia coli* strains. Antimicrob Agents Chemother 1995;39:707–13.
[4] Bergogne-Bérézin E, Berthelot G, Muller-Serieys C. Present status of nitroxoline. Pathol Biol 1987;35:873–8.
[5] Karam D, Amgar A, Bourlioux P. Inhibition of bacterial adhesion of uropathogenic *Escherichia coli* strains by the urine of patients treated with nitroxoline. Pathol Biol 1988;36:452–5.
[6] Bourlioux P, Botto H, Karam D, Amgar A, Camey M. Inhibition of bacterial adherence by nitroxoline on cellular adhesion and on urinary catheter surfaces. Pathol Biol 1989;37:451–4.
[7] Oliviero L, Perdiz M, Bourlioux P. Direct effect of nitroxoline in the inhibition of bacterial adherence to urinary catheters. Pathol Biol 1990;38:455–8.
[8] Pelletier C, Prognon P, Latrache Villart HL, Bourlioux P. Mircobiological consequences of chelation of bivalent metal cations by nitroxoline. Pathol Biol 1994;42:406–11.
[9] Latrache H, Bourlioux P, Karroua M, Zahir H, Hakkou A. Effects of subinhibitory concentrations of nitroxoline on the surface properties of *Escherichia coli*. Folia Microbiol 2000;45:485–90.
[10] Wayne LG, Sramek HA. Metronidazole is bactericidal to dormant cells of *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 1994;38:2054–8.
[11] Stover CK, Warrener P, Vandevanter DR, et al. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. Nature 2000;405:962–6.
[12] Murugasu-Oei B, Dick T. Bactericidal activity of nitrofurans against growing and dormant *Mycobacterium bovis* BCG. J Antimicrob Chemother 2000;46:917–9.
[13] Wayne LG, Hayes LG. An in vitro model for sequential study of shift down of *Mycobacterium tuberculosis* through two stages of non-replicating persistence. Infect Immun 1996;64:2062–9.
[14] Lim A, Eleuterio M, Hutter B, Murugasu-Oei B, Dick T. Oxygen depletion induced dormancy in *Mycobacterium bovis* BCG. J Bacteriol 1999;181:2252–6.
[15] Boon C, Li R, Qi R, Dick T. Proteins of *Mycobacterium bovis* BCG induced in the Wayne dormancy model. J Bacteriol 2001;183:2672–6.
[16] Kim YS, Shin JH, Choi YS, Lee W, Lee YI. Solvent sublation using 8-hydroxyquinoline as a ligand for the determination of trace elements in water samples. Microchem J 2001;68:99–107.
[17] Tracy JW, Webster LT Jr. Drugs used in the chemotherapy of protozoal infections. In: Hardman JG, Limbird LE, editors. Goodman and Gilmans: the pharmacological basis of therapeutics, 9th ed. New York: McGraw-Hill, 1996:987–1008.
[18] Fraser RS, Creanor J. The mechanism of inhibition of ribonucleic acid synthesis by 8-hydroxyquinoline and the antibiotic lomofungin. J Bacteriol 1975;147:401–10.
[19] Rhode W, Cordell B, Webster R, Levinson W. Inhibition of amino acyl tRNA synthetase activity by copper complexes of two metal binding ligands. *N*-Methyl isatin beta-thiosemicarbazone and 8-hydroxyquinoline. Biochem Biophys Acta 1977;477:102–11.
[20] Leanderson P, Tagesson C. Iron bound to the lipophilic chelator, 8-hydroxyquinoline, causes DNA strand breakage in cultured lung cells. Carcinogenesis 1996;17:545–50.