Printed in U.S.A.

## In Vitro Activity of Chlorhydroxyquinoline Against Mycoplasma Species

## R. F. COSGROVE\* AND SANDRA BAINES

International Development Laboratory, E. R. Squibb & Sons, Moreton, Merseyside L46 1QW, England

## **Received for publication 16 November 1977**

The in vitro activities of 5-chloro-8-hydroxyquinoline (CHQ) against single strains of 12 different species of mycoplasma and the impacts of repeated exposure of these strains to CHQ on their susceptibility to this agent have been studied. On initial exposure, the minimal inhibitory concentrations for these strains ranged from 0.24 to  $1.92 \mu g$  of CHQ per ml of test medium; activities remained unchanged during 10 serial transfers in CHQ-containing medium.

Previous reports (1-5) have shown that 5chloro-8-hydroxyquinoline (CHQ) has significant activity in vitro against a wide variety of bacteria, fungi, and protozoa. This report summarizes the results of in vitro studies on the activity of CHQ against both pathogenic and saprophytic species of mycoplasma and the impact of repeated exposure to this agent on such activity.

The species and strains of mycoplasma used in this study and their origins are listed in Table 1. The organisms were grown and tested for their susceptibility to CHQ in modified Chanock medium (20 ml of inactivated horse serum, 10 ml of yeast extract, 1 ml of 10% glucose solution, 1 ml of 1% nicotinamide adenine dinucleotide solution, 1 ml of 10% arginine solution, 1 ml of 5% thallous acetate solution, 100,000 U of benzylpenicillin, 2 ml of 0.1% phenol red, 1.47 g of PPLO broth [Difco Laboratories], and 70 ml of distilled water).

CHQ (Halquinol, E. R. Squibb & Sons Ltd.) was dissolved in dimethylformamide at a concentration of 8,000  $\mu$ g/ml. Doubling dilutions were made in dimethylformamide, and 0.05 ml of each dilution was added to 6-ml portions of sterile medium in bijou bottles (0.25 ounce, ca. 7.5 ml). A 0.5-ml amount of a 24-h actively growing culture of mycoplasma was added to each bijou. The final concentrations of CHQ in the medium ranged from 61.5 to 0.12  $\mu$ g/ml. Control cultures, with 0.05 ml of dimethylformamide, were included with each minimal inhibitory concentration (MIC) determination. The cultures were incubated at 37°C for 48 h, and the MIC was determined. Portions (0.5 ml) were

Organism	Source <sup>a</sup>	MIC (µg of CHQ per ml of test medium		
		On initial test	After 5 serial transfers	After 10 serial transfers
Acholeplasma laidlawii G23/6	Α	0.96	1.92	1.92
A. laidlawii B	Α	0.96	0.96	0.96
Iowa 695 cloned	Α	1.92	1. <b>92</b>	3.85
Mycoplasma synoviae (fresh-field isolate)	Α	0.96	0.96	0.96
Type 8 FB serotype	Α	0.96	0.96	0.96
WRI 431/10 serotype	Α	0.96	0.96	1.92
M. gallisepticum serotype A	Α	0.96	1.92	1.92
M. gallisepticum A 514	Α	0.96	1.92	1.92
M. bovigenitalium NCTC 10122	В	0.24	0.12	0.12
M. agalactiae var. bovis NCTC 10131	В	0.96	1.92	1.92
M. hyopneumoniae	С	0.24	0.48	0.48
M. hyorhinis	С	0.24	0.12	0.12

 TABLE 1. MIC data for the activity of CHQ against mycoplasma species

<sup>a</sup> A, University of Liverpool Veterinary Field Station (Leahurst); B, National Collection of Type Cultures; and C, Royal Veterinary College, London.

then removed from the bijou containing the highest concentration of CHQ that still allowed normal or nearly normal growth and transferred to another series of bijous containing a range of CHQ concentrations similar to those above. Successive transfers were made every 48 h until 10 transfers were completed.

The data in Table 1 show that the MICs of CHQ for the various strains ranged from 0.24 to 1.92  $\mu$ g/ml of test medium. The data also show that the MICs for the respective strains did not change significantly during 10 serial transfers in CHQ-containing medium.

All of the strains examined, whether they were pathogenic or saprophytic strains, fresh-field isolates, or serotypes, showed a sensitivity to CHQ that compared favorably with MICs of 3 to 4  $\mu$ g/ml previously (4) reported for a range of bacteria, molds, and yeasts. Thus, it may be concluded that CHQ does have significant antimycoplasmal activity. The gifts of mycoplasma cultures from the Avian Medicine Department of Liverpool University's Veterinary Field Station (Leahurst) and from the Royal College of Veterinary Medicine, London University, are gratefully acknowledged. We would also like to thank Judith Power of Leahurst for her help and advice on the cultivation of mycoplasma species.

## LITEPATURE CITED

- Ellenrieder, V. M., and K. H. Sensch. 1972. The influence of chlorinated oxyquinoline derivatives on anaerobic micro-organisms. Arzneim. Forsch. 22:908-909.
- Fiedler, H., and U. Kaben. 1966. Antimycotic and antibacterial action of 8 quinolinols and nicotinic acid esters. Pharmazie 21:233-238.
- Forster, T. C., and G. Duggan. 1974. In vitro experiments to determine the resistance of strains of *E. coli* and *Salmonellae* to halquinol and tetracycline, p. D28-1-D28-2. In Proceedings of the 3rd International Congress International Pig Veterinary Society, Lyon.
- Heseltine, W. W., and P. J. Campbell. 1960. Laboratory studies of chlorhydroxyquinoline. J. Trop. Med. Hyg. 63:1-3.
- Lamy, L. 1964. A comparative experimental study of the activity of certain hydroxyquinoline derivatives on multiplication of *Entamoeba histolytica* cultures and associated bacteria. Ann. Inst. Pasteur Paris 107:98-108.