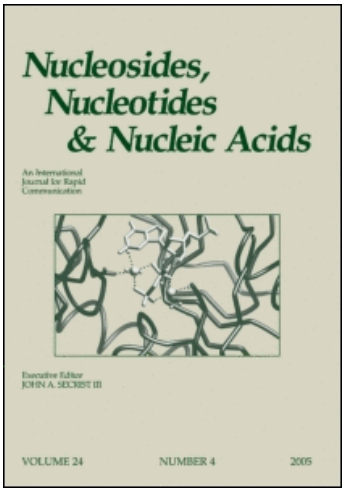

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Effect of Fluoropyrimidines on the Growth of *Ureaplasma urealyticum*

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ABSTRACT

In the present study, we investigated the effect of fluoropyrimidines on the growth of *Ureaplasma urealyticum*. Addition of fluoropyrimidines strongly inhibited bacterial growth. Growth inhibition by these analogues could be reversed by addition of either thymidine or deoxyuridine, suggesting inhibition of thymidylate biosynthesis as the mechanism in operation.

Key Words: *Ureaplasma*; Nucleoside analogues; Growth inhibition; Fluoropyrimidines.

INTRODUCTION

Mycoplasmas, a class of Mollicutes, are found in both the animal and plant kingdoms as free-living saprophytes and parasites. *Ureaplasma urealyticum* (*U. urealyticum*), a human pathogen, colonizes the oral cavity, respiratory tract and urogenital tract. This organism is involved in neonatal infection and plays a role in

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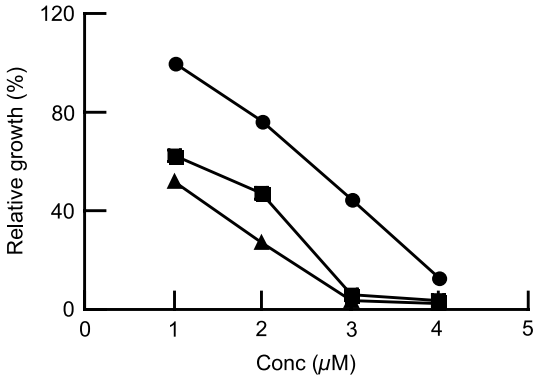

Figure 1. The inhibition of 5-FdUrd and reversal by pyrimidine deoxynucleosides in the growth of *U. urealyticum*. 5FdUrd:dUrd (1:1,●); 5-FdUrd:dThd (1:2,■) and 5-FdUrd alone (▲).

genital disease such as urethritis and reproduction failure in both men and women.[1] Fluoropyrimidines are known to inhibit thymidylate synthase (TS) activity and thereby interfere with DNA synthesis. The effects of fluoropyrimidines on the growth of *U. urealyticum* are here described.

MATERIALS AND METHODS

Ureaplasma cell culture was performed as described.[2] Cell growth was estimated by measuring the colour change units (CCU) of the growth media. To determine the CCU, 20 μl of Ureaplasma cells was inoculated into 80 μl growth medium in 96 well plates with or without nucleoside and diluted 10^{-2} to 10^{-10} fold. The plates were sealed and incubated as described.[3]

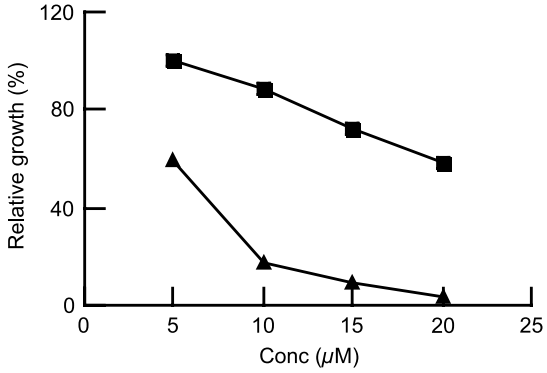

Figure 2. The relative growth of *U. urealyticum* culture in the absence and presence of dTHU. 5-FdCyd:dTHU (1:1,■) and 5-FdCyd alone (▲).

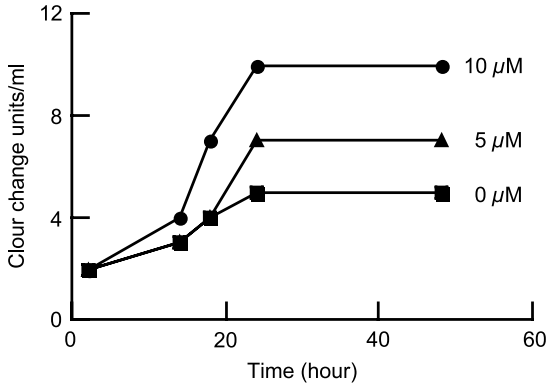

Figure 3. Effect of dUrd on the growth of *U. urealyticum*. dUrd concentrations are marked besides the lines.

RESULTS

To understand the mechanism of inhibition by 5-FdUrd, we examined the inhibitory effect of 5-F-dUrd on *U. urealyticum* growth in the presence or absence of deoxyuridine (dUrd) and deoxythymidine (dThd) (Fig. 1). In the absence of dUrd 5-FdUrd strongly inhibited *U. urealyticum* growth, with an inoculum of 10^5 cfu/ml of *U. urealyticum* and 3 μM 5-FdUrd there was no survival of the bacterium. However, when dUrd was added to the growth medium in 1:1 ratio to 5-FdUrd, the inhibitory effect of 5-FdUrd was drastically reduced (Fig. 1). The bactericidal effect of 5-F-dUrd was completely blocked by 10-fold excess dUrd. Addition of dThd to the culture medium had similar effect. Therefore, the growth inhibition by 5-FdUrd was probably due to inhibition of TS-like enzyme activity and the rescue by the addition of dUrd suggested that this TS-like enzyme is a key enzyme in pyrimidine nucleotide biosynthesis in *U. urealyticum*. The addition of dThd, on the other hand, provided an alternative pathway for dTTP synthesis.

Addition of the cytidine deaminase inhibitor deoxytetrahydrouridine (dTHU) to *U. urealyticum* cultures drastically reduced the inhibition by 5-fluorodeoxycytidine (5-F-dCyd) on *U. urealyticum* grwoth (Fig. 2). This finding indicated that inhibition by 5-F-dCyd was depending on the deamination of 5-FdCyd to 5-FdUrd and it was 5-FdUrd which exhibited the inhibitory activity.

U. urealyticum cell growth was stimulated by the addition of dUrd into the growth medium (Fig. 3). Cells that grown in the presence of 10 μM dUrd showed twice the CCU at 26 h compared to cells grown without dUrd. These results indicated that *U. urealyticum* growth is limited by the availability of pyrimidines in the media.

DISCUSSION

Infections caused by mycoplasmas have gained recognition as medical problems in the past decades; moreover, resistance of some mycoplamas to current antibiotics has

been noted. As far as the antibacterial mechanism of pyrimidines is concerned, we showed here that fluoropyrimidine nucleoside analogues strongly inhibited *Ureaplasma* growth. The mechanism of inhibition is most likely by blocking the synthesis of thymidylate. 5-fluoronucleosides may serve as lead compounds for future development of new mycoplasma agents.

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