The Activity of PTK 0796 (BAY 73-6944) Against Tetracycline Resistance

Paratek Pharmaceuticals, Inc., Boston, MA - The University of Alberta, Edmonton, Alberta, Canada

Abstract 2611
Poster F-752
The Activity of PTK 0796 Against Tetracycline Resistance

S. Weir1, A. Macone1, J. Donatelli1, C. Triebel1, D.E. Taylor2, S.K. Tanaka1, and *S.B. Levy1

1Paratek Pharmaceuticals, Inc., Boston, MA, - 2The University of Alberta, Edmonton, Alberta, Canada

Abstract 2611

ABSTRACT

Background: PTK 0796 (Bay 73-6944), a novel multichrome-resistant inhibitor of tetracycline, exhibits ultra activity against gram positive clinical isolates resistant to approved tetracyclines (1,2). Studies were conducted to evaluate the potential emergence of resistance and the mechanism by which PTK 0796 overcomes resistance determinants.

Methods: MIC testing was performed according to NCCLS guidelines. In vivo, the influence of efflux was determined in Tet(K), Tet(L), Tet(M) were identified using multiplex PCR. Single-step mutant selection and passage studies were used to establish the likelihood of emergence of resistance. The influence of efflux was determined in Tet(O).

Results: PTK 0796 was active against tetracycline resistant and efflux mediated tetracycline. MICs were not significantly affected by the presence of Tet(O) (MIC range of 0.12-32 µg/ml), presence of Tet(K) or Tet(M) (0.12-2 µg/ml), or deletion of susceptible strain (PTK 0796 1µg/ml). Tet(M) exhibited no tendency to promote resistance in vivo whereas with Tet(K) promoting resistance to single step selection in a serial passage. PTK 0796 class for Tet(K) is 0.12-4 µg/ml and inhibits protein synthesis in the presence of Tet(O).

Conclusions: PTK 0796 overcomes resistance mechanisms in gram positive bacteria as determined by its potent activity against resistance to currently marketed drugs. This activity was the result of PTK 0796 having high affinity for efflux pumps and genomic targets for ribosome binding that the resistant protein. Regardless of the state of the resistance protein, the resistance to PTK 0796 was not observed.

INTRODUCTION

Tetracycline resistance remains a clinically significant determinant in the utility of tetracyclines, doxycycline, minocycline, and other clinically available tetracyclines. There are two major mechanisms of resistance: efflux and ribosome protection. Both mechanisms have been described in gram positives and gram negatives. Both resistance proteins generally carry a common gene products and efflux in gram negatives. The major common genes of resistance proteins are tetracycline and the tet(A) determinant. The tet(A) determinant was the first to be identified in a plate with the ribosome protection protein, Tet(O).

METHODS

As in vitro MICS

MICs were determined against standard clinical isolates using NCCLS methods.

Macromolecular Synthesis

Macromolecular synthesis analysis was used to establish the effectiveness of Tet(K), tetracycline efflux protein against PTK 0796. Macromolecular synthesis of tetracyclines were evaluated with 32P-labeled tetracyclines using autoradiography (1,2, and 3).

% Inhibition of Protein Synthesis

% Activity

PTK 0796 inhibition of protein synthesis was assessed by comparing the results of PTK 0796 on total cell protein synthesis in the presence and absence of tetracycline. (Figure 1) The ability of PTK 0796 to overcome tetracycline efflux was determined by comparing the potency of PTK 0796 on total cell protein synthesis in the presence and absence of tetracycline. (Figure 1)

% Activity

In vitro translation experiments were performed using human translation buffer conditions in the presence and absence of tetracycline. (Figure 2) The ability to select PTK 0796 resistant mutants was determined in single and multiple passage experiments. (Table 2)

RESULTS

The ability to select PTK 0796 resistant mutants was determined by comparing the potency of PTK 0796 on total cell protein synthesis in the presence and absence of tetracycline. (Table 2)

CONCLUSIONS

Mutants resistant to PTK 0796 were found either as single step mutants or multiple step mutants after up to 10 passages. Mutants were not observed whether the initial strain was susceptible or resistant to tetracycline (efflux or ribosome protection).