ABSTRACT

Minimum inhibitory concentration (MIC) determinations: MIC values for the EBO-resistant mutants of M. avium ATCC 700898 were determined in calcofluor-stained Mueller Hinton broth according to Clinical and Laboratory Standards Institute document M24-A3. MIC values were determined as essentially described by CLSI M4-A7 with 7H11 Middlebrook agar and 5% FADC. Antibiotic synergy testing: The effect of combining EBO with clarithromycin (CLR), rifabutin (RBT), EMB, amikacin (AMK) or bedaquiline (BDQ) were determined in 7 strains of NTM. Synergy, additive effects, indifference or antagonism was characterized by broth microdilution method (BMD). MICs of selected EBO mutants were determined against AMK, BDQ, CLR, RBT, EMB, and clofazimine (CFZ) and the mutants were further characterized by genomic DNA analysis. Resistant colonies were confirmed by plaque splitting on agar plates containing ortho-free MIC used to select resistance. Control plates containing no drug were divided by the MIC of drug B alone. A combination of drugs is considered synergistic if the product of the MIC of drug A and drug B divided by the MICs of drug A and B divided by the MICs of drug A and B is ≤ 0.5. Results: Spontaneous resistance frequency determination: The RF of M. avium ATCC 700898 at 2x, 4x and 8x the MIC (mg/mL) of EBO was determined. EBO resistance frequency was essentially the same when selected on 2-8x agar MIC (Table 1). The RF of EBO ranged from 1.58x10-7 to 8.48x10-9 when selected on 2-8x agar MIC. The spontaneous resistance frequency against EBO resistant strains did not change significantly. The activity of EBO was not antagonized, and was mainly indifferent to the addition of CLR, RBT, AMK, or BDQ for all the NTM strains tested against EBO-resistant strains did not change significantly. The activity of EBO was not antagonized, and was mainly indifferent to the addition of CLR, RBT, AMK, or BDQ for all the NTM strains tested. The MIC for EBO increased 32-256-fold for the resistant mutants; however, the MICs for the other drugs were not impacted by EBO resistance suggesting that cross-resistance did not occur.

INTRODUCTION

There are an estimated 200,000 patients with non-tuberculosis mycobacterial (NTM) lung disease in the United States with many remaining undiagnosed. The number of patients is expected to increase by an estimated 8% per year. Among the approximately 10,000 patients diagnosed with NTM lung disease in the United States, approximately 44,000 have lung disease caused by Mycobacterium avium Complex (MAC). There are 210,000 patients diagnosed with nontuberculous mycobacteria (NTM). The standard of care therapy for MAC disease is urgent.

In the checkerboard studies, no evidence of antagonism was observed with any strain. EBO combined with CLR, RBT, AMK or EMB. MICs of selected EBO mutants were determined against AMK, BDQ, CLR, RBT, EMB, and clofazimine (CFZ) and the mutants were further characterized by genomic DNA analysis. Resistant colonies were confirmed by plaque splitting on agar plates containing ortho-free MIC used to select resistance. Control plates containing no drug were divided by the MIC of drug B alone. A combination of drugs is considered synergistic if the product of the MIC of drug A and drug B divided by the MICs of drug A and B divided by the MICs of drug A and B is ≤ 0.5. Results: Spontaneous resistance frequency determination: The RF of M. avium ATCC 700898 at 2x, 4x and 8x the MIC (mg/mL) of EBO was determined. EBO resistance frequency was essentially the same when selected on 2-8x agar MIC (Table 1). The RF of EBO ranged from 1.58x10-7 to 8.48x10-9 when selected on 2-8x agar MIC. The spontaneous resistance frequency against EBO resistant strains did not change significantly. The activity of EBO was not antagonized, and was mainly indifferent to the addition of CLR, RBT, AMK, or BDQ for all the NTM strains tested against EBO-resistant strains did not change significantly. The activity of EBO was not antagonized, and was mainly indifferent to the addition of CLR, RBT, AMK, or BDQ for all the NTM strains tested. The MIC for EBO increased 32-256-fold for the resistant mutants; however, the MICs for the other drugs were not impacted by EBO resistance suggesting that cross-resistance did not occur.

RESULTS

The in vitro activity of EBO was tested in the presence of key components of the standard of care drugs for the treatment of MAC pulmonary disease, clarithromycin, ethambutol, amikacin and rifabutin. MICs of selected EBO mutants were determined against AMK, BDQ, CLR, RBT, EMB, and clofazimine (CFZ) and the mutants were further characterized by genomic DNA analysis. Resistant colonies were confirmed by plaque splitting on agar plates containing ortho-free MIC used to select resistance. Control plates containing no drug were divided by the MIC of drug B alone. A combination of drugs is considered synergistic if the product of the MIC of drug A and drug B divided by the MICs of drug A and B divided by the MICs of drug A and B is ≤ 0.5. Results: Spontaneous resistance frequency determination: The RF of M. avium ATCC 700898 at 2x, 4x and 8x the MIC (mg/mL) of EBO was determined. EBO resistance frequency was essentially the same when selected on 2-8x agar MIC (Table 1). The RF of EBO ranged from 1.58x10-7 to 8.48x10-9 when selected on 2-8x agar MIC. The spontaneous resistance frequency against EBO resistant strains did not change significantly. The activity of EBO was not antagonized, and was mainly indifferent to the addition of CLR, RBT, AMK, or BDQ for all the NTM strains tested against EBO-resistant strains did not change significantly. The activity of EBO was not antagonized, and was mainly indifferent to the addition of CLR, RBT, AMK, or BDQ for all the NTM strains tested. The MIC for EBO increased 32-256-fold for the resistant mutants; however, the MICs for the other drugs were not impacted by EBO resistance suggesting that cross-resistance did not occur.

REFERENCES AND ACKNOWLEDGMENTS


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