In Vitro Activities of the New Antitubercular Agents PA-824 and BTZ043 against Nocardia brasiliensis

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The in vitro activity of PA-824 and BTZ043 against 30 Nocardia brasiliensis isolates was tested. The MIC50 and MIC90 values for PA-824 were both >64 µg/ml. The same values for BTZ043 were 0.125 and 0.250 µg/ml. Given the MIC values for benzothiazinone (BTZ) compounds, we consider them good candidates to be tested in vivo against N. brasiliensis.

Nocardia brasiliensis is a natural inhabitant of the soil that in some cases gains entry to human skin by trauma with splinters or wood material contaminated with this bacterium (17). Once in the subcutaneous tissues, the bacteria proliferate, producing local inflammation, abscesses, and fistulae, and may affect subjacent organs, depending on the topographic localization of the lesions. The production of chronic inflammation and scarring of tissue makes it difficult for antimicrobials to penetrate and act against the bacteria. Several antimicrobials, including sulfonamides, aminoglycosides, beta-lactams, etc., have been used in the treatment of actinomycetoma (2, 5, 17). However, in some cases cure is not obtained, making it important to evaluate in vitro and in vivo the activity of new antimicrobials.

Given the close phylogenetic relationship among actinobacteria, it is possible that some antimicrobial agents are active against nocardiae. Among the most recently developed antitubercular compounds, PA-824 ((S)-2-nitro-6-{4-(trifluoromethoxy)benzyl}oxy)-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine) has shown the best and most promising results (4, 12). In vitro, those compounds present MIC values for Mycobacterium tuberculosis isolates similar to those of isoniazid (MIC of PA-824, 0.015 to 0.25 µg/ml; MIC of isoniazid, 0.03 to 0.06 µg/ml) (12). PA-824 acts as a prodrug activated through a bioreduction process within the M. tuberculosis cell, and it is efficient against both latent and replicating M. tuberculosis. Transcriptional analysis has revealed a mixed potential mechanism of action that operates both by affecting cell wall synthesis and by chemical poisoning. The latter is achieved by increasing the intracellular amount of toxic nitric oxide (NO) (7, 10, 11). The development of M. tuberculosis mutants and its whole-genome resequencing showed the importance of a gene named ddn (Rv3547) in PA-824 resistance. This gene encodes a 151-amino-acid protein, a deazaflavin-dependent nitroreductase (Ddn); orthologous genes have been found in other actinobacteria (7).

1,3-Benzothiazin-4-one (benzothiazinone [BTZ]) compounds have been recently described that have excellent activity against actinobacteria, including Corynebacterium, Mycobacterium, Rhodococcus, and Nocardia (6). They are particularly active against Mycobacterium tuberculosis in vitro and in vivo, with BTZ043 showing a MIC of 1 ng/ml for the control strain M. tuberculosis H37Rv. This value is far below that of other active drugs, including rifampin and isoniazid. The biochemical target, the decaprenyl-phosphoribose-2’-epimerase (encoded by gene dprE1), is commonly distributed among actinobacteria (6, 13).

In the present work, we analyze the susceptibility of 30 N. brasiliensis isolates from human mycetoma to these compounds by a broth microdilution method.

We studied 30 isolates from the collection of the Laboratorio Interdisciplinario de Investigación Dermatológica (LIID) of the Servicio de Dermatología, Hospital Universitario, Universidad Autónoma de Nuevo León (UANL), including N. brasiliensis HUJEG-1 utilized previously in other in vitro and in vivo assays (1, 9, 15). All the isolates came from human cases of actinomycetoma and were identified as N. brasiliensis by biochemical methods and by nucleotide sequence analysis of a fragment of the 16S rRNA gene as described before (14).

PA-824 was kindly donated by the Global Alliance for TB Drug Development; BTZ043 was provided by one of the authors of the present study.

The broth microdilution method based on the CLSI M2-A2 document that we used has been described before (3). As external controls, we used Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 29213. PA-824 was tested at concentrations of 64 to 0.25 µg/ml. In the case of BTZ043, the lowest concentration used was 0.0015 µg/ml. M. tuberculosis H37Rv was used also as a control.

The dprE1 gene from N. brasiliensis was obtained by comparing the dprE1 (locus Rv3790) gene sequence of M. tuberculosis H37Rv to the entire genome sequence of N. brasiliensis HUJEG-1 obtained by our group (16) by using the BLAST program available at the NCBI Internet site. To establish the presence of a putative ddn (Rv3547) ortholog in N. brasiliensis, we utilized the M. tuberculosis H37Rv nucleotide sequence published in GenBank and compared it to the complete chromosomal sequence of N. brasiliensis HUJEG-1 by the use of the BLAST program.

The MIC50 and MIC90 values of PA-824 for the N. brasiliensis isolates were >64 µg/ml in both cases. N. carnea ATCC 6847 and N. transvalensis ATCC 6865 showed the same MIC value. As a resistant control, we tested M. smegmatis LR222, for which the

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drug MIC was >64 μg/ml. The MIC value for the susceptible control, *M. tuberculosis* H37Rv, was 0.125 μg/ml.

The BTZ043 MIC<sub>50</sub> and MIC<sub>90</sub> values were 0.125 and 0.25 μg/ml, respectively. The MIC for *N. carnea* ATCC 6847 was 0.003 μg/ml, for *N. transvalensis* ATCC 6865 was 0.003 μg/ml, for *N. brasiliensis* NCTC10300 was 0.03 μg/ml, and for *N. brasiliensis* HUJEG-1 was 0.125 μg/ml. The MIC value for *M. tuberculosis* H37Rv was 0.000976 μg/ml. The MIC values of both PA-824 and BTZ-043 were >64 μg/ml for *Escherichia coli* ATCC 25922 and *S. aureus* ATCC 29213.

Comparing the *M. tuberculosis dprE1* gene sequence to the complete sequence of *N. brasiliensis*, we found a sequence with 74% homology encoding a 493-amino-acid protein (accession number ZP_09840341.1) similar to the FAD binding 4 superfam-

**FIG 1** Alignment of the BTZ resistance-determining region of *N. brasiliensis* HUJEG-1 with the corresponding DPR protein sequences of *N. farcinica* and *M. tuberculosis*, both BTZ-sensitive organisms. In red we show the Cys387 amino acid related to resistance to this antimicrobial.

**REFERENCES**


