Correlation between Pyrazinamide Activity and pncA Mutations in Mycobacterium tuberculosis Isolates in Taiwan

Tsi-Shu Huang,1,2 Susan Shin-Jung Lee,3 Hui-Zin Tu,1 Wen-Kuei Huang,3 Yao-Shen Chen,3 Chung-Kai Huang,3 Shue-Ren Wann,3 Hsi-Hsun Lin,3 and Yung-Ching Liu1,3*

Section of Microbiology and Infectious Diseases, Kaohsiung Veterans General Hospital,1 Department of Medical Technology, Foo-Yin Institute of Technology,2 and National Yang-Ming Medical College,3 Taipei, Taiwan

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A total of 76 clinical Mycobacterium tuberculosis isolates from Taiwan were tested for pyrazinamidase activity, pyrazinamide susceptibility, and pncA mutations. Frequency of resistance to PZA rose with increases in resistance to first-line drugs. Of 17 pyrazinamide-resistant strains, 7 (3 of which had not been previously described) possessed mutations in the pncA gene.

Pyrazinamide (PZA) has become increasingly important because of its ability to enhance the efficacy of isoniazid and rifampin and to allow shorter courses of therapy (2, 8, 20, 21). The unique feature of PZA is believed to be its ability to kill a population of semidormant tubercle bacilli that reside in acidic inflammatory environments (6). Unfortunately, susceptibility testing for PZA is still not sufficiently standardized to help guide therapy (7, 17, 19). Accordingly, there is a major need for more rapid and reliable tests. One approach is to detect mutations in the pncA gene. It has been reported that these mutations correlate well with a MIC of PZA of $>100 \mu g/ml$, with the frequency of pncA mutation among resistant strains (depending on the geographic area) ranging from 66.7 to 96.8% (1, 3–5, 8–12, 13–14, 18).

This study was designed to determine the frequency of PZA-resistant strains with pncA mutations among PZA-resistant and -susceptible M. tuberculosis strains isolated in Taiwan. In vitro susceptibility to PZA was correlated with PZase activity and the composition of the entire pncA nucleotide sequence. The frequency of PZA resistance among strains with various patterns of resistance to first-line drugs (isoniazid, rifampin, streptomycin, and ethambutol) was also investigated.

A total of 76 M. tuberculosis strains with variant drug susceptibility patterns isolated from 1994 to 2000 from clinical specimens collected in Kaohsiung Veterans General Hospital, Taiwan, were randomly selected. They included 27 strains susceptible to the four first-line antimycobacterial drugs, 28 multidrug-resistant (MDR) strains, and 21 strains with variable drug resistance patterns.

PZA MICs were tested by a BACTEC MGIT 960 PZA system because it requires no radioactive materials and has been shown to be as reliable as the BACTEC 460TB system (16). The susceptibility tests were performed at 100 and 300 \( \mu g/ml \) according to the manufacturer’s instructions. The critical concentration of PZA for determination of resistance recommended by the manufacturer is 100 \( \mu g/ml \). All the PZA-resistant strains were retested.

The PZase activity was assayed using the Wayne method (22). Several loopfuls of colonies were in two tubes of medium for each strain; one tube was examined after 7 days of incubation, and the other was examined after 14 days of incubation. All the strains that initially lacked PZase activity were retested.

DNAs were extracted using a Qiagen MiniElute PCR purification kit (Qiagen, Valencia, Calif.) according to the manufacturer’s instructions and stored at 4°C. A 720-bp region that included the entire open reading frame of pncA and 82 bp of an upstream putative regulatory sequence was amplified by PCR with the forward (P1) and reverse (P6) primers (GenBank accession number U59967; published by Scorpio) (18). A GeneAmp system 9600 thermocycler (Perkin-Elmer Corp., Foster City, Calif.) was used for target amplification with the following parameters: 5 min at 4°C followed by 30 cycles of 60 s at 94°C, 30 s at 63°C, and 60 s 72°C and termination with a final extension step at 72°C for 10 min. The PCR products were purified with the Qiagen MiniElute PCR purification kit according to the manufacturer’s instructions. The purified PCR products were sequenced in an ABI PRISM 310 genetic analyzer (Applied Biosystems, Inc., Foster City, Calif.). DNA sequencing reactions were performed with the Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems, Inc.). Sequence data were compared with a published sequence for pncA (GenBank accession number U59967). Differentiation of M. tuberculosis from other members of M. tuberculosis complex was done using specific deletion profiles (15).

A total of 59 of the 76 isolates were PZA susceptible; they all had identical wild-type pncA sequences. Among the 17 PZA-resistant isolates, 7 had a pncA nucleotide sequence change and lacked PZase activity (Table 1) (isolates 1 to 7). Three of these mutations (a deletion of nucleotides 352 to 358, a G insert at nucleotide 397, and a Phe 94→Ser mutation) have not been previously reported. The Phe 94→Ser mutation was located in one of the clusters of hydrophobic residues which are close to Lys 96 and which point towards the active-site region (11). Of the resistant strains, 10 carried wild-type pncA. One strain (isolate 8) had a wild-type pncA sequence without PZase activity. The strain...
was MDR and formed small colonies on a 7H11 agar plate. A negative reverse transcription-PCR result indicated that there could be a mutation of a pncA-regulatory gene and that this mutation could affect expression of pncA, thereby causing PZA resistance. One strain (isolate 9) had reduced PZase activity, giving negative PZase results at 7 days but giving positive results at 14 days; the other eight strains (isolates 10 to 17) had normal PZase activity. There might be mechanisms of PZA resistance at 14 days; the other eight strains (isolates 10 to 17) had normal PZase activity and expression, such as mutation could affect expression of pncA gene mutations in pyrazinamide-resistant strains of Mycobacterium tuberculosis. A strong (98.7%) correlation between the loss of PZase activity and that only 7 out of 17 PZA-resistant strains possessed a mutation in the pncA gene for 76 clinical M. tuberculosis isolates. The emergence of resistance to the first-line antituberculosis drugs has led to an increased use of MDR strains increased. The frequency of PZA-susceptible strains according to resistance level in BACTEC for 21 Mycobacterium tuberculosis clinical isolates from China. Epidemiol. Infect. 124:227–232.


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We found a strong (98.7%) correlation between the loss of PZase activity and the presence of a pncA genotype. We were surprised to find that only 7 out of 17 PZA-resistant strains possessed a mutation in the pncA sequence and low (88.2%) correlation between the loss of PZase activity and PZA sus-
ceptibility. This finding is in contrast to prior reports (1, 3–5, 8–12, 13–14, 18). This method is therefore not sufficiently sen-
tive to be used as a surrogate marker for PZA resistance in Taiwan. Nevertheless, in view of the problems of standardiza-
tion with the in vitro susceptibility tests we suggest that strains with borderline or poorly reproducible susceptibility should be examined for PZase activity and that rapid automated DNA sequencing should be used.

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