Tuberculosis 90 (2010) 152–157

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

DRUG DISCOVERY AND RESISTANCE

A mutation in *Mycobacterium tuberculosis rpoB* gene confers rifampin resistance in three HIV-TB cases

Sangita Malhotra [a], Victoria J. Cook [a,b], Joyce N. Wolfe [c], Patrick Tang [b,d], Kevin Elwood [a,b], Meenu K. Sharma [c,e,*]

[a] Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada
[b] British Columbia Center for Disease Control, Vancouver, British Columbia, Canada
[c] National Reference Centre for Mycobacteriology, National Microbiology Laboratory, Public Health Agency of Canada, 1015 Arlington St., Winnipeg, Manitoba, Canada
[d] Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada
[e] Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada

ARTICLE INFO

Article history:
Received 8 September 2009
Received in revised form
18 December 2009
Accepted 2 January 2010

Keywords:
rpoB
Mycobacterium tuberculosis
Rifampin resistant tuberculosis

SUMMARY

Rifampin is a key component of standard short-course first-line therapy against *Mycobacterium tuberculosis* (MTB). Rifampin monoresistant MTB, previously a rare phenomenon, is now being reported at increasing rates worldwide. We report a mutation in the *rpoB* region leading to low level rifampin monoresistance in a cluster of HIV-positive patients. All rifampin monoresistant isolates identified from 2004 to 2006 underwent susceptibility confirmation, sequencing of *rpoB* and genotyping. Three patients were found to have a previously undocumented 3 base pair insertion at codon 525 in the *rpoB* region. The earliest initial case was infected with fully susceptible MTB. Disease relapse occurred 7 months later with a genotypically identical MTB isolate, showing acquired rifampin monoresistance. MTB isolates from 2 subsequent patients showed primary rifampin monoresistance with an identical genotype to the index case. Patients with rifampin monoresistant MTB tend to have poorer outcomes than those with fully susceptible strains. Risk factors for the development of rifampin monoresistance include co-morbid HIV infection and previously treated tuberculosis. HIV infection has been associated with malabsorption of anti-tuberculous medications leading to sub-therapeutic levels of administered drugs. These factors may have played a role in the development of this previously undocumented mutation.

1. Introduction

Drug resistance in *Mycobacterium tuberculosis* (MTB) disease has become an increasing concern in the fight against this global epidemic. Monoresistance to rifampin, a key component of standard short-course, first-line therapy against MTB, is uncommonly reported compared to monoresistance to isoniazid (INH) or multidrug resistance (MDR).[1–4] In fact, rifampin resistance in MTB is often a marker of MDR tuberculosis (TB) as it is more easily acquired in the setting of INH monoresistance.[5] However, rates of both acquired and primary rifampin monoresistant MTB are increasing worldwide.[5,6] Risk factors for rifampin monoresistant MTB include HIV co-infection, previously treated TB or inappropriately treated TB.[5–7] Patients with monoresistance to rifampin tend to have poor treatment outcomes requiring longer hospital stays and treatment courses.[7–9] Rifampin, a semi-synthetic derivative of rifamycin B, acts against MTB by inhibiting mycobacterial transcription by targeting DNA dependant RNA polymerase.[3,5] Resistance in MTB can develop after a single step mutation and is defined as resistance to a rifampin critical concentration of 2 μg/mL by BACTEC 460 methodology (Becton Dickinson Sparks, MD).[10,11] More than 95% of rifampin monoresistant MTB strains have a mutation in a 27 codon (81 base pair) region of the gene that encodes the beta subunit of RNA polymerase (*rpoB*).[9,12]

We describe a previously undocumented mutation involving the *rpoB* region in a rifampin monoresistant cluster of three HIV related TB cases and review the factors that may have led to the development of this novel mutation.

2. Case report

All rifampin monoresistant cases were identified based on susceptibility results from Tuberculosis Control at the British

* Corresponding author at: National Reference Centre for Mycobacteriology, National Microbiology Laboratory, Public Health Agency of Canada, 1015 Arlington St., Winnipeg, Manitoba, Canada. Tel.: +1 204 789 6036; fax: +1 204 789 2036.
E-mail address: meenu_sharma@phac-aspc.gc.ca (M.K. Sharma).

1472-9792/$ – see front matter
doi:10.1016/j.tube.2010.01.001

Columbia Centre for Disease Control (BCCDC), Vancouver, Canada. Patient 1 (index case) was a 39-year-old man known to be HIV-positive for 14 years (Table 1). His medical history was significant for co-infection with hepatitis C, psoriasis, cerebral toxoplasmosis and injection drug use (IDU). A tuberculin skin test (TST) in 2000 showed greater than 20 mm induration. Treatment for latent TB was offered but declined.

The patient presented to hospital in December 2002 with fever, chills and weight loss. Chest radiography showed a right middle lobe infiltrate and mediastinal lymphadenopathy. He was not receiving highly active antiretroviral therapy (HAART) and his CD4 count was 30 cells/mm^3. Sputum samples were positive for acid fast bacilli (AFB) and culture positive for a fully susceptible MTB isolate. The patient was started on daily directly observed therapy (DOT) with isoniazid (INH) (300 mg), pyrazinamide (PZA) (1500 mg), ethambutol (EMB) (1500 mg) and rifampin (600 mg) for 6 weeks followed by daily DOT with INH (300 mg) and rifampin (600 mg) during the continuation phase. He completed 9 months of DOT ($\geq$80% of dosages) by September 2003 with clinical and microbiological resolution of TB. There was no history of gastrointestinal disease during the duration of his therapy.

In April 2004, seven months after completion of anti-tuberculous therapy, the patient had a recurrence of disease. His CD4 count at that time was less than 10 cells/mm^3 and he had declined HAART. Sputum smears were positive for AFB, cultures grew MTB and susceptibility testing showed monoresistance to rifampin (MIC 2–4 μg/mL). The isolate was sent to the National Reference Centre for Mycobacteriology (NRCM), Winnipeg, Canada, for confirmation of susceptibility results, sequencing of *rpoB*, and MTB genotyping using IS*6110*-based restriction fragment-length polymorphism (RFLP) and mycobacterial interspersed repetitive units-variable number tandem repeats (MIRU-VNTR) techniques.

He was initially restarted on a standard quadruple regimen by DOT (Table 1). When rifampin resistance was observed, rifabutin was discontinued and streptomycin (1 g three times weekly) and moxifloxacin (MOX) (400 mg daily) were added to the regimen. Due to persistently positive sputum cultures, serum drug levels were assessed at the National Jewish Medical and Research Centre (Denver, CO) revealing a potentially sub-therapeutic serum INH level of 1.56 μg/mL (target level is 3–6 μg/mL). As a result, the INH dose was increased to 600 mg daily. The patient completed 12 months of DOT with clinical and microbiological resolution. Compliance was estimated to be approximately 80–90%.

In November 2005, seven months after completing the second course of anti-tuberculous therapy, the patient presented to hospital with fever, cough and axillary lymphadenopathy. The patient was not receiving HAART and his CD4 count remained less than 10 cells/mm^3. A multi-loculated abdominal fluid collection was noted on abdominal ultrasound. Sputum, axillary node fine needle aspiration and drainage from the abdominal collection were positive for AFB by smear and positive for rifampin monoresistant MTB by culture. Daily treatment included INH (600 mg), EMB

Table 1
Summary of rifampin monoresistant *M. tuberculosis* case cluster.

	Patient 1 39-year-old male	Patient 2 48-year-old female	Patient 3 48-year-old male
Co-morbid Conditions and Past Medical History	■ HIV-positive (14 years), CD4 < 30 (2002), CD4 < 10 (2004) ■ Hepatitis C positive ■ Psoriasis ■ Cerebral toxoplasmosis ■ IDU	■ HIV-positive, CD4 20 ■ Hepatitis C positive ■ IDU	■ HIV-positive (24 years), CD4 90 (4%) ■ PCP ■ Chronic diarrhea (microsporidia, *E.histolytica)* ■ Diverticulosis
Medications	■ Sulfadiazine, pyrimethamine, hydroxychloroquine, prednisone	■ ddI, 3TC, efavirenz, sulfamethazole/trimethoprim (double strength), fluconazole	■ Abacavir, 3TC, atazanavir/ritonavir, sulfamethazole/trimethoprim (double strength)
Tuberculosis History	■ 2000 TST >20 mm ■ 12/2002 Pulmonary TB ■ 04/2004 Pulmonary TB ■ 11/2005 Disseminated TB	■ 2001 TST negative ■ 01/2006 Pulmonary TB	■ 1993 TST negative ■ 04/2006 Pulmonary TB
Microbiology	■ 12/2002 – sputum: MTB (fully susceptible) ■ 01/2003 – 2 culture and AFB smear negative sputums ■ 04/2004 – sputum: MTB rifampin monoresistant ■ 11/2005 – abdominal fluid collection, sputum, axillary drainage: MTB rifampin monoresistant ■ 07/2006 – AFB smear negative	■ 01/2006 lung biopsy (post mortem): MTB rifampin monoresistant	■ 04/2006 – BAL: MTB rifampin monoresistant
Treatment	■ 12/2002 – 09/2003 completed 9 months of standard therapy by DOT (80–90% compliance) ■ 04/2004 – started INH, PZA, EMB and rifabutin ■ 05/2004 –rifabutin discontinued ■ 06/2004 – moxifloxacin and streptomycin added ■ 07/2004 – INH increased to 450 mg OD ■ 10/2004 – noted to have sub-therapeutic INH levels; dose of INH increased to 600 mg OD ■ 04/2005 – completed 12 months of treatment (80–90% compliance) ■ 11/2005 – restarted INH, EMB, PYR, moxifloxacin, streptomycin	■ no anti-tuberculous treatment received	■ 04/2006 – on standard treatment (INH, rifabutin, PZA, EMB) ■ 05/2006 – rifabutin discontinued, moxifloxacin added
Epidemiology	■ Admitted at same time as Patient 2 ■ Attended same respite facility as Patient 3 ■ Resided in Downtown Eastside (DES) area of Vancouver, Canada	■ Admitted at same time as Patient 1 ■ Resided in DES	■ Attended same respite facility as Patient 1 ■ Resided in DES