Annals of Clinical Microbiology and Antimicrobials

Research

Prevalence of genotypic HIV-1 drug resistance in Thailand, 2002

Ekachai Jenwitheesuk*[1,2], Chotip Watitpun[3], Asda Vibhagool[4] and Wasun Chantratita[3]

Address: [1]Department of Microbiology, University of Washington School of Medicine, Seattle, WA, USA, [2]Department of Clinical Microbiology, Faculty of Medical Technology, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, [3]Department of Pathology Ramathibodi Hospital, Mahidol University, Bangkok, Thailand and [4]Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Email: Ekachai Jenwitheesuk* - ekachai@compbio.washington.edu; Chotip Watitpun - c_watitpun@yahoo.com; Asda Vibhagool - raavb@mahidol.ac.th; Wasun Chantratita - rawct@mahidol.ac.th

* Corresponding author

Published: 6 March 2003

Annals of Clinical Microbiology and Antimicrobials 2003, **2**:4

Received: 28 December 2002
Accepted: 6 March 2003

This article is available from: http://www.ann-clinmicrob.com/content/2/1/4

Abstract

Background: The prices of reverse transcriptase (RT) inhibitors in Thailand have been reduced since December 1, 2001. It is expected that reduction in the price of these inhibitors may influence the drug resistance mutation pattern of HIV-1 among infected people. This study reports the frequency of HIV-1 genetic mutation associated with drug resistance in antiretroviral-treated patients from Thailand.

Methods: Genotypic resistance testing was performed on samples collected in 2002 from 88 HIV-1 infected individuals. Automated DNA sequencing was used to genotype the HIV-1 polymerase gene isolated from patients' plasma.

Results: Resistance to protease inhibitors, nucleoside and non-nucleoside reverse transcriptase inhibitors were found in 10 (12%), 42 (48%) and 19 (21%) patients, respectively. The most common drug resistance mutations in the protease gene were at codon 82 (8%), 90 (7%) and 54 (6%), whereas resistant mutations at codon 215 (45%), 67 (40%), 41 (38%) and 184 (27%) were commonly found in the RT gene. This finding indicates that genotypic resistance to nucleoside reverse transcriptase inhibitors was prevalent in 2002. The frequency of resistant mutations corresponding to non-nucleoside reverse transcriptase inhibitors was three times higher-, while resistant mutation corresponding to protease inhibitors was two times lower than those frequencies determined in 2001.

Conclusion: This study shows that the frequencies of RT inhibitor resistance mutations have been increased after the reduction in the price of RT inhibitors since December 2001. We believe that this was an important factor that influenced the mutation patterns of HIV-1 protease and RT genes in Thailand.

Background

During the last decade, the prevalence of human immunodeficiency virus type 1 (HIV-1) drug resistance has increased in developed countries as a result of widespread antiretroviral therapy [1–9]. Genotypic evidence of resistance for any drug was found in fewer than 2% of cases in one study from 1989 [4], increased to 10%-16% in cohorts recruited after 1995 [2,3], and attained between

20% and 26% in studies performed since 1997 [5–9]. Overall, several studies show rates of primary genotypic drug resistance between 10% and 18% for nucleoside reverse transcriptase inhibitors (NRTIs), of none to 13% for non-nucleoside reverse transcriptase inhibitors (NNRTIs), and of 3% to 7% for protease inhibitors (PIs) [1–9].

In July 2002, S Sirivichayakul, et al. [10] reported genotypic resistant mutations of HIV-1 reverse transcriptase (RT) in HIV-1 infected Thai patients who had been treated with double nucleoside reverse transcriptase inhibitors (NRTIs) for more than eight years. They found 54 (55.7%) out of 97 patients failed to achieve viral suppression. The genotypic analysis of HIV-1 RT isolated from these 54 patients showed that 61% and 18% of them had RT gene mutations related to azidovudine (AZT) and lamivudine (3TC) resistance, respectively. Mutations in the RT gene related to other NRTI and multi-NRTI resistance were found in low levels (1% to 4%). Interestingly, these patients' sequences also included NNRTI mutation (G190A) without evidence of exposure to this drug class. In addition, no PI resistant mutations were reported. However, the genotypic data showed in this report represents the resistance information of only one ARV class (NRTIs). YK Cho, et al. (2002) [11] reported the prevalence of HIV-1 drug resistance in South Korea where the AIDS epidemic started at almost the same time that it started in Thailand. They found that the viruses from 80% of NRTI-experienced patients had mutations related to AZT resistance while mutations related to 3TC and didanosine (ddI) resistance were found in 11% and 5% of the patients, respectively. In addition, no Q151M and NNRTI resistance-related mutations were found.

There are approximately 1 million people infected with HIV-1 in Thailand with 30,000 new infections every year and 4,200 among children [12]. Only a few patients can afford ARV drugs due to the high monthly price of effective treatment regimens. In addition, it is estimated that only 5% of HIV-infected people can get access to ARV double therapy and a very small number can get treatment with three drugs [13].

On December 1, 2001, the Thai Government Pharmaceutical Organization (GPO) reduced the monthly cost of their ARV medications from 5,000 Baht (US$112) to 2,500 Baht (US$ 55). This has helped many infected people (especially, low-income people) to improve their immune status and control the virus load. However, it is expected that the increased availability of the drugs may change the drug resistance mutation pattern of HIV-1 among infected people in Thailand. To date, the frequency of ARV drug resistance in Thailand has not been well characterized. So far, only one report concerning the frequencies of both PI and RT inhibitor resistance mutations in Thai patients has been found [14]. Therefore, this study, which aims to investigate the prevalence and patterns of HIV-1 genotypic resistance among antiretroviral-treated Thai patients in 2002, may provide valuable information that could guide the modification of regimen and treatment strategies.

Methods

Study subjects

To investigate the prevalence of primary and secondary drug-resistant viral genotypes circulating in Thailand, mutations critical to the HIV-1 protease and reverse transcriptase sequences were examined in plasma samples collected from patients living in four large cities. Eighty-eight samples (from 88 patients) were obtained from four hospital centers, which are located in four regions of Thailand: Central (Bangkok; 56 samples), Northern (Chiang Mai; 12 samples), Northeastern (Khon Kaen; 10 samples), and Eastern (Chonburi; 10 samples). All plasma samples were collected during 2002 from individuals living permanently in Thailand, who have been treated extensively by ARV drug regimens and have failed to respond to treatment (current plasma viral load of over 500 HIV-1 RNA copies/ml). They were treated to all three classes of ARV drugs (NRTIs, NNRTIs, and PIs) and were receiving a combination of at least 2 classes of ARV drugs while the plasma samples were collected. Samples from individuals on drug holidays for longer than 2 weeks before the time of the study were excluded because of the possibility of the reversion of circulating mutant genotypes [15,16].

Genotyping

Viral RNA was isolated from plasma samples, using the QIAamp viral extraction kit (Qiagen, Inc., Chatsworth, CA, USA). The TRUGENE HIV-1 Genotyping Assay was used in conjunction with the Open Gene automated DNA sequencing system (Visible Genetics Inc., Toronto, Canada) to sequence the protease and reverse transcriptase (RT) regions of the HIV-1 cDNA. Testing involved simultaneous clip sequencing of protease and codons 35–244 of the RT from the amplified cDNA in both the 3' and 5' directions. Sequences were aligned and compared with a lymphoadenopathy-associated virus type 1 (HIV-B-LAV1) consensus sequence using Visible Genetics Gene Librarian software. We focused on mutation at positions in the polymerase gene known to be associated with the commonly used drugs against HIV-1. Interpretation of the genotype in terms of drug resistance was based on an algorithm established by the Stanford HIV RT and Protease Sequence Database http://hivdb.stanford.edu/hiv/[17]. The frequencies of resistant mutations were also compared with the frequencies among Thai patients reported by our group in 2001 [18]. DNA sequences generated in this study have been submitted to GenBank under the accession numbers AF457212-AF457387.

Table 1: Frequency of mutations associated with resistance to protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) in Thai patients in 2002.

Mutation(s)	Frequency (%)
PIs	12
NRTIs	48
NNRTIs	21
All three drug families	12

Table 2: Distribution of resistant mutations in the reverse transcriptase and protease genes among the HIV-1 infected patients in 2001 (83 patients) and 2002 (88 patients).

Mutations	Patients, no. (%)	
	2001	2002
Mutations to NRTIs		
M41L	28	38
E44D	0	9
A62V	2	3
K65R	2	3
D67N	38	40
T69D	5	6
K70R	25	23
L74V	2	6
V75M/T	0	15
F77L	0	4
F116Y	0	4
V118I	0	22
Q151M	3	3
V179D	2	3
M184V/I	46	27
H208Y	0	6
L210W	19	24
T215Y/F	36	45
K219Q	23	16
Mutations to NNRTIs		
A98G	2	5
L100I	0	3
K101E	4	5
K103N	1	12
V108I	0	6
Y181C	4	8
G190A/S	7	12
Mutations to PIs		
L10I/V	64	18
K20R	11	12
M36I	88	70
M46I	8	3
G48V	10	3
F53L	6	4
I54V	11	6
L63P	36	20
A71T/V	10	4
V82A/F	13	8
L90M	19	7

Results and Discussion

Sequence analysis of protease and RT regions

The details of patients' current ARV regimen are not shown in this report, since only 30% of this data was well recorded. Thus, in this report, we show only the frequencies of resistant mutations of HIV-1 isolated from ARV-treated Thai patients, and compare them with the frequencies that were determined in 2001.

Genotypic resistance to protease inhibitor was noticed in 10 (12%) patients, whereas nucleoside reverse transcriptase inhibitor and non-nucleoside reverse transcriptase inhibitor key mutations were recognized in 42 (48%) and 19 (21%) patients, respectively (Table 1). The most common resistant mutations among 88 subjects were found at codons 82 (8%), 90 (7%) and 54 (6%) within the protease gene and at codons 215 (45%), 67 (40%), 41 (38%) and 184 (27%) within the sequenced region of the RT gene. However, in contrast to the frequency in 2001, the mutation at codon 75 of the RT gene was found in up to 15% of the patients. Resistance to zidovudine was mostly found (45%). The multi-nucleoside-resistant genotype (Q151M) was found in three patients while the insertion at codon 69 of RT gene was not found in this study. Resistance to all three drug families (multi-drug resistance) was recognized in 10 (12%) patients. Resistance to NNRTIs in 2002 was much higher (21%) than those found in 2001 (8%). As expected, changes at positions 103, 181 and 190 were the most frequently found (Table 2). The results of our study also show that, in 2002, the prevalence of mutations conferring PI resistance in Thai patients was much lower (12%) than the prevalence in 2001 (25%), whereas the mutations conferring NRTI resistance stabilized at approximately 48%.

High prevalence of mutations conferring RT inhibitor resistance noted in our study could be related to the reduction in the price of RT inhibitors in Thailand since December 1, 2001. Moreover, in March 2002, the first batch of 120,000 tablets of GPO-VIR (a combination of nevirapine 200 mg, lamivudine 150 mg and stavudine 30 mg), produced by GPO, was made available at a cost of 20 Baht (US$ 0.46) a tablet. Reduced prices of the RT inhibitors has led not only to an improvement in affordability for patients but also increased the chance of RT inhibitor resistant variants to become predominant in RT inhibitor experienced patients. In addition, inadequate adherence and the absence of monitoring the efficacy of the treatment can rapidly lead to treatment failure as well as promote widespread of drug-resistant HIV strains. We believe that the reduction of RT inhibitor cost was an important factor that influenced the mutation patterns of HIV-1 protease and RT in 2002.

Bias concerning the sampling issues could explain the lower rate of resistant mutations in protease and the higher rate of mutations conferring NNRTI resistance in this study. However, the frequencies of resistant mutations reported in 2001 and 2002 were from the same group and the criteria for selecting patients and the geographic regions within Thailand from where the patients were recruited did not change. Therefore, no sampling bias is apparent.

Conclusions

The frequencies of HIV-1 drug resistance mutations among treated patients have changed after the reduction in the price of RT inhibitor in Thailand since December 2001. The mutations related to RT inhibitor resistance were found with high frequency, while the frequency of mutations related to PI resistance was low. The patterns of drug resistance mutations constantly change due to the wide use of ARV drug therapy. Thus, one should be concerned that the widespread of HIV-1 drug resistance strains might seriously limit treatment options in the near future.

Authors' contributions

AV recruited patients, provided clinical data, and patients' histories. CW performed HIV-1 RNA extraction, RT-PCR and sequencing. EJ interpreted and evaluated the results and drafted the manuscript. WC helped with evaluation of the results produced, and provided intellectual guidance and mentorship. All authors read and approved the final manuscript.

Written consent was obtained from the patient for the publication of this study.

Acknowledgements

Authors would like to thank Shing-Chung Ngan, Aaron Chang, Mike Inouye, Jason McDermott and Ram Samudrala for the critical reading of the manuscript, comments and suggestions.

References

1. Dietrich U, Ruppach H, Gehring S, Knechten H, Knickmann M, Jager H, Wolf E, Husak R, Orfanos CE, Brede HD, Rubsamen-Waigmann H and von Briesen H **Large proportion of non-B HIV-1 subtypes and presence of zidovudine resistance mutations among German seroconvertors.** *AIDS* 1997, **11:**1532-1533
2. Yerly S, Kaiser L, Race E, Bru JP, Clavel F and Perrin L **Transmission of antiretroviral-resistant HIV-1 variants.** *Lancet* 1999, **354:**729-733
3. Boden D, Hurley A, Zhang L, Cao Y, Guo Y, Jones E, Tsay J, Ip J, Farthing C, Limoli K, Parkin N and Markowitz M **HIV-1 drug resistance in newly infected individuals.** *JAMA* 1999, **282:**1135-1141
4. Little SJ, Daar ES, D'Aquila RT, Keiser PH, Connick E, Whitcomb JM, Hellmann NS, Petropoulos CJ, Sutton L, Pitt JA, Rosenberg ES, Koup RA, Walker BD and Richman DD **Reduced antiretroviral drug susceptibility among patients with primary HIV infection.** *JAMA* 1999, **282:**1142-1149
5. Brodine SK, Shaffer RA, Starkey MJ, Tasker SA, Gilcrest JL, Louder MK, Barile A, VanCott TC, Vahey MT, McCutchan FE, Birx DL, Richman DD and Mascola JR **Drug resistance patterns, genetic sub-**

types, clinical features, and risk factors in military personnel with HIV-1 seroconversion. *Ann Intern Med* 1999, **131:**502-506
6. Salomon H, Wainberg MA, Brenner B, Quan Y, Rouleau D, Cote P, LeBlanc R, Lefebvre E, Spira B, Tsoukas C, Sekaly RP, Conway B, Mayers D and Routy JP **Prevalence of HIV-1 resistant to antiretroviral drugs in 81 individuals newly infected by sexual contact or injecting drug use.** *AIDS* 2000, **14:**F17-F23
7. Wegner SA, Brodine SK, Mascola JR, Tasker SA, Shaffer RA, Starkey MJ, Barile A, Martin GJ, Aronson N, Emmons WW, Stephan K, Bloor S, Vingerhoets J, Hertogs K and Larder B **Prevalence of genotypic and phenotypic resistance to antiretroviral drugs in a cohort of therapy-naive HIV-1 infected US military personnel.** *AIDS* 2000, **14:**1009-1015
8. Little S **Transmission and prevalence of HIV resistance among treatment-naive subjects.** *Antiviral Ther* 2000, **5:**33-40
9. Perez-Olmeda M, Del Romero J, Rubio A, Ruiz L, Rodriguez C, Leal M, Clotet B and Soriano V **Primary HIV-1 drug resistance in Spain before and after the introduction of protease inhibitors.** *J Med Virol* 2001, **63:**85-87
10. Sirivichayakul S, Ruxrungtham K, Sa-nguansilp C and Phanuphak P **High prevalence of multi-drug resistance mutations in Thai patients treated for prolonged period with double nucleosides [abstract TuPeB4605].** *In Proceeding of XIV International AIDS conference: 2002 July 7–12; Barcelona, Spain*
11. Cho YK, Sung H, Ahn SH, Bae IG, Woo JH, Won YH, Kim DG and Kang MW **Frequency of mutations conferring resistance to nucleoside reverse transcriptase inhibitors in human immunodeficiency virus type-1 infected patients in Korea.** *J Clin Microbiol* 2002, **40:**1319-1325
12. UNAIDS/WHO **epidemiological fact sheet on HIV/AIDS and sexually transmitted infections in Thailand, update**
13. Kraisintu K **Generic production of HIV/AIDS-related drugs in Thailand.** *In: Improving Access to Care in developing countries: lessons from practice, research, resources and partnerships. UNAIDS November – December 2001*
14. Chantratita W, Jenwitheesuk E, Watitpun C, Pongthanapisith V, Vibhagool A, Leechawengwong M, Sookpranee M and Apairatana A **Prevalence of HIV-1 polymerase gene mutations in pretreated patients in Thailand.** *Southeast Asian J Trop Med Public Health* 2002, **33:**80-84
15. Devereux H, Youle M, Johnson M and Loveday C **Rapid decline in detectability of HIV-1 drug resistance mutations after stopping therapy.** *AIDS* 1999, **13:**F123-F127
16. Verhofstede C, Wanzeele FV, Van der Gucht B, De Cabooter N and Plum J **Interpretation of RTI or a switch from RTI to PI resulted in a fast reapperance of virus strains with a RT-sensitive genotype.** *AIDS* 1999, **13:**2541-2546
17. Shafer RW, Jung DR, Betts BJ, Xi Y and Gonzales MJ **Human immunodeficiency virus reverse transcriptase and protease sequence database.** *Nucleic Acids Research* 2000, **28:**346-348
18. Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Rapp BA and Wheeler DL **GenBank.** *Nucleic Acids Res* 2002, **30:**17-20