The influence of cytochrome P450 and drug transporter protein polymorphism on the virological /immunological response in HIV infected patients

Yoonseon Park

Department of Medicine

The Graduate School, Yonsei University

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Directed by Professor June Myung Kim

The Doctoral Dissertation
submitted to the Department of Medicine
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the
degree of Doctor of Philosophy

Yoonseon Park

December 2008

This certifies that the Doctoral Dissertation of Yoonseon Park is approved.

Thesis Supervisor: June Myung Kim

Dong Soo Kim

Hyon Suk Kim

Chung Mo Nam

Kyungsoo Park

The Graduate School

Yonsei University

December 2008

ACKNOWLEDGEMENTS

I would like to thank professor June Myung Kim who gave me the opportunity to participate in such a great project and always provided me with good and thorough comments with deep sincerity. I am deeply grateful to him for giving me a good run in infectious diseases part. He has my role model whom I have learned from and who shaped my perspective career as a doctor.

I am in debt to professor Dong Soo Kim, Hyun Suk Kim and Jung Mo Nam who gave me many great advices during this study. In addition, I am especially thanks to Kyungsoo Park who was one of pharmacokinetics expert and Ji Hyun Lee who was an excellent helper about the pharmacogenetics.

I want to express my thanks to professor Min Soo Park who has provided valuable insight as well as perspective to my study.

I express my greatest love to my wise husband, parents and parents-in-law who supported me with deep love and faith, and especially, Minseong and Minje.

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The use of highly active antiretroviral therapy (HAART) has proven efficacy in controlling viral replication in HIV (human immunodeficiency virus) infected patients. Efavirenz, recommended NNRTI (nonnucleoside

reverse transcriptase inhibitor) by DHHS (department of health and human service) guideline, is known to be metabolized by CYP2B6 mainly and by CYP2D6 partially and is a substrate for P-glycoprotein, which is encoded by MDR1. The aims of this study were to evaluate the influence of CYP2B6, CYP2D6 and MDR1 protein polymorphism on the virological /immunological response and neuropsychiatric adverse effects of efavirenz using a population genetics approach.

Total 82 Korean HIV infected patients were divided into two groups, those who had good immunologic or virologic responses with efavirenz based regimens and or not. And, it was considered to have neuropsychiatric adverse effects of efavirenz or not. Eleven single nucleotide polymorphisms in the CYP2B6, CYP2D6 and MDR1 genes were analyzed.

Patients with SNP C1236T tended to have better immunologic response at week 24 and much $CD4^+$ T cells increment. There were no correlation

between SNPs of CYP450, MDR1 and virologic response to HAART. Among patient who experienced neuropsychiatric adverse effects (n = 24) and those who did not (n=58), MDR1 A893S (r.2677G>T) variant showed a strong association with the neuropsychiatric adverse effects caused by efavirenz (p = 0.007). These data strongly suggest that A893S polymorphism in the human MDR1 gene is associated with altered drug responses of efavirenz. The adequate trough concentration of efavirenz group more likely had TT genotype than inadequate group in G2677T.

It was anticipated that MDR1 polymorphism may play an important role as a genetic marker for predicting responsiveness of efavirenz prior to and after initiation of therapy by these association results in HIV infected patients.

Key words: cytochrome P450, MDR1 protein, mutation, HIV, HAART (highly active antiretroviral therapy), neuropsychiatric adverse effects

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I. INTRODUCTION

The use of highly active antiretroviral therapy (HAART) has proven efficacy in controlling viral replication in HIV-infected patients. HAART

mostly consisted of two nucleoside reverse transcriptase inhibitors (NRTIs) plus at least one protease inhibitor (PI) or nonnucleoside reverse transcriptase inhibitor (NNRTI). For PIs and NNRTIs, several studies have demonstrated a relationship between their plasma drug concentrations and efficacy and/or toxicity. Especially for PIs, an important inter-patient variability in plasma drug concentrations was observed that results in wide drug exposure from fixed dosing regimens. Therefore, pharmacokinetics of antiretroviral drugs is increasingly used in clinical care to determine the best dosage regimen adapted to each individual to reduce the risk of virologic failure from low plasma drug concentrations, to limit the toxicity linked to high plasma drug concentrations, and in some circumstances to assess adherence[1].

The cytochrome P450 enzymatic complex (CYP 450) is the main metabolizer involved in the liver clearance of NNRTIs and PIs. Several polymorphisms at different CYP 450 isoenzymes are known to influence

the metabolism of drugs in the liver, causing increased or decreased clearance. In addition, the activity of the P-glycoprotein (P-gp) cell membrane efflux pump influences the intracellular disposition of some antiretrovirals (particularly PI) and their tissue distribution. Polymorphisms at the genes encoding these protein transporters influence their expression and activity, what modulates the disposition of the transported drugs[2].

P-gp is expressed on a variety of cells including human lymphocytes, the target cells of HIV and of antiretroviral substances [3]. Though there is still controversy about the biological relevance of these SNPs, there appears to be an association between specific genotypes and mRNA expression, P-gp expression and/or P-gp function[4]. Fellay *et al*. found lower nelfinavir and efavirenz plasma levels associated with the TT genotype of the SNP C3435T in exon 26 and a greater rise of the $CD4^+$ T cell count 6 months after initiation of antiretroviral therapy in patients with

this genotype[5]. It was hypothesized that this benefit associated with the T allele could result from an enhanced HIV protease inhibitor penetration into $CD4^+$ T cells. In addition to altering intracellular drug concentration, overexpression of P-gp in vitro has been demonstrated to reduce the susceptibility of human $CD4^+$ T cells to infection with HIV by affecting viral fusion and possibly viral release[6]. However, the clinical relevance of such a role for P-gp has yet to be determined.

In contrast to PIs, NNRTIs such as efavirenz had been not known substrates of the P-gp. Therefore, it has been speculated that the P-gp may modulate the clinical course of HIV infection independent from its role in drug transport. However, these observations from in vitro studies have not been confirmed in vivo when the disease progression before treatment was assessed in HIV infected individuals with different MDR1 genotypes [7].

Because of the unresolved issues surrounding the potential effects of MDR1 polymorphisms and P-gp function in HIV infection, I investigated

whether there was an association between the MDR1 polymorphisms (C3435T, G2677T/A, C1236T), CYP2B6 and the immunological/virologic response in HIV infected individuals after initiation of antiretroviral therapy.

SNPs, G516T, A785G of CYP2B6 have recently been shown to affect efavirenz pharmacokinetics and response in HIV patients[8]. SNPs of MDR1(C1236T, G2677T/A, C3435T, -1469G/A, -935A/G, -709C/G, -693T/C) associated reduced protein expression and altered enzyme activity, but these findings are inconsistent[9].

HIV patients may have symptoms, dizziness, headache or nightmare as neuropsychiatric adverse effects with efavirenz containing regimen. Efavirenz posses a narrow therapeutic range, as plasma drug levels above 4,000 ng/mL have been associated with more central nervous system (CNS) toxicity while the rate of virologic failure seems to be increased if the drug concentration falls below 1,000 ng/mL[10].

II. MATERIALS AND METHODS

Patients

Of the HIV 1 infected patients at the Department of Infectious Diseases of Severance hospital, Yonsei University Health System in Korea, 237 patients started antiretroviral therapy containing efavirenz between 2001 and 2008. HIV load and $CD4^+$ T cell count were determined four weeks after initiation of therapy and approximately every three months thereafter. In patients who received NNRTI or PI drugs as part of their therapy, plasma levels were monitored. The treatment was based on current international treatment guidelines[11], taking into account individual circumstances of each patient (e.g. known intolerabilities, adverse-effects of previous therapies, concomitant medication).

Patient enrolled in this study meet the following entry criteria: they were diagnosed as HIV infection or AIDS and treated more than 4 weeks

with HAART (NNRTI based or PI based regimen, NNRTI; non nucleoside reverse transcriptase inhibitors, PI; protease inhibitors)

The exclusion criteria were as follows: they were less than 18 years of age; they were in, pregnancy or breast feeding state; they had elevated serum alanine aminotransferase(ALT, >5 times upper limit of normal) or decreased renal function (creatinine >300 μmol/L), anemia (Hb <10.0 g/dL); they had acute bacterial/viral infection (other than HIV) or chronic hepatitis B or hepatitis C; they were prescribed cytotoxic chemotherapy, rifampicin, verapamil, macrolide antibiotics or non-steroidal anti-inflammatory drugs within the previous 2 weeks.

The study was in accordance with the Helsinki Declaration and was approved by the local ethics committee in Severance hospital. Patients gave informed consent for the study.

Definitions

Antiretroviral treatment failure was defined as a suboptimal response to therapy. Treatment failure was often associated with virologic failure, immunologic failure, and/or clinical progression. Virologic failure on treatment was defined as a confirmed HIV RNA level >400 copies/mL after 24 weeks, >50 copies/mL after 48 weeks, or a repeated detectable HIV RNA level after prior suppression of viremia. Adequate virologic response at 24 weeks had at least a 1 log 10 copies/mL of HIV RNA. Immunologic failure can be defined as a failure to achieve and maintain an adequate $CD4^+$ T cell response despite virologic suppression and an inability to increase $CD4^+$ T cell counts above pre-therapy levels by the threshold (> 100 cells/mm) over 24 weeks or 48 weeks.

Clinical Progression was defined as the occurrence or recurrence of HIV-related events (after at least 3 months on an antiretroviral regimen), excluding immune reconstitution syndromes.

Sample collection

Blood samples (5 mL) were collected prior to (trough level), and 2–18 h after an oral dose of the prescribed drugs taken with a light standardized breakfast. The samples were centrifuged at 3,000 rpm (1850 *g*) for 10 min at 4°C (Beckmann Centrifuge) and the plasma was separated and transferred into polypropylene test tubes. The processed plasma was stored at −80°C up to the time of analysis.

Protein expression and transporter activity of MDR1

Protein expression of G2677A/T variation was confirmed by immunoblotting assay. And, Transport activity of MDR1 was evaluated by FACS using rhodamine 123, $DiOC_2$ and Calcein acetoxymethylester(calcein-AM)[12, 13]. Expression of MDR1 prevented those dye to be loaded with the fluorescent marker.

Genotype analyses

DNA was extracted with DNase MiniKit (Qiagen, Hilden, Germany) from viable peripheral-blood mononuclear cells. Genotype screening of each sample was performed by the SNaPshot or SNaPIT method (Applied Biosystems) according to protocols supplied by the manufacturer, using the primers listed in Table 1. MDR1 polymorphisms at 7 sites with over 1% allele frequency in the database of the Korea Pharmacogenomics Research Network (http://www.pharmacogenomics.or.kr/) were chosen. Positions of the genetic variations in the promoter and intragenic regions are given in relation to the translation initiation codon.

Pharmacokinetic analyses

Plasma for determination of efavirenz by LC-MS/MS (API 4000^{TM} system, Applied biosystem) was collected at more than week 4 of treatment (per oral q 24 hours). Sampling times were unspecified. Time of

prior dose was obtained by patient report, and samples drawn greater than 96 h post-dose or in which drug was not detected were excluded. Population pharmacokinetic modeling was performed assuming a one-compartment open model with first-order absorption. The model was parameterized in terms of drug clearance, volume of distribution and absorption rate, and assumed complete absorption of the dose.

Pharmacokinetic data

The PK model was a one-compartment model with first-order absorption and exclusive hepatic elimination according to the classical well-stirred model[14]. The subject-specific parameters of this model for drug are the rate constant of absorption (K_a), volume of distribution (V), and clearance (CL), where CL is equal to $Q\ CL_{int}/(Q + CL_{int})$, in which Q is as defined below and CL_{int} is intrinsic (hepatic) clearance. Covariates evaluated for inclusion in the PK model for drug were co-administered other drug, sex, race, age, weight.

Statistical analyses

Fisher's exact test was used to make pair-wise comparisons between plasma HIV-1 RNA outcome and MDR1 and CYP 450 polymorphisms. Regression models on both a linear scale and a log scale were used. The Kruschal–Wallis test was used to determine if the clinical parameters including plasma HIV-1 RNA, $CD4^+$ T lymphocyte counts parameters are similar among the different genotypes. SPSS version 15.0 (SPSS GmbH, Munich, Germany) was used for statistical analysis. A *p*-value of 0.05 was considered to be significant.

Table 1. Primer sequence & PCR condition(SNaPshot)

Gene	SNPname	rs number	Strand	Primer sequence	
				Forward Primer	CGTGACGTGCTGGTACA
CYP2B6	516G>T	rs3745274	Reverse	Reverse Primer	CTCCATGTCCCTGATTCTT
				Genotyping Primer	AGATGATGTTGGCGGTAATGGA
				Forward Primer	CTTTCTTGCAGCTGTTTG
CYP2B6	785A>G	rs2279343	Reverse	Reverse Primer	CCTCTGTCTTTCATTCTGTC
				Genotyping Primer	GGTAGGTGTCGATGAGGTCC
				Forward Primer	CATTTGGTAGTGAGGCAGGT
CYP2D6	100C>T	rs1065852	Forward	Reverse Primer	TGGTCGAAGCAGTATGGTG
				Genotyping Primer	GCGCAACGCTGGGCTGCACGCTAC
				Forward Primer	CAGGAAACAGCTATGACCCTGCTAACTGAGCACAGGAT
CYP2D6	2988G>A	rs28371725	Forward	Reverse Primer	TGTAAAACGACGGCCAGTGTCCGGCCCTGACACTCCT
				Genotyping Primer	AAACAGTGCAGGGGCCGAGGGAG
				Forward Primer	GGAGCAAAGAAATGGAATACAATA
MDR1	-1459G>A	-	Reverse	Reverse Primer	TTCTCCCGTGAAGACCAAGTTC
				Genotyping Primer	GTTTTGCTTTGTTGCTTTAT

				Forward Primer	CAGGAAACAGCTATGACCGCATGCTGAAGAAAGACCA
MDR1	-935A>G	rs2188524	Reverse	Reverse Primer	TGTAAAACGACGGCCAGTCCTTCTCCCGTGAAGACC
				Genotyping Primer	CGCGCATCAGCTGAATCA
				Forward Primer	CAGGAAACAGCTATGACCGCATGCTGAAGAAAGACCA
MDR1	-709C>G	DL1000839	Forward	Reverse Primer	TGTAAAACGACGGCCAGTCCTTCTCCCGTGAAGACC
				Genotyping Primer	CGCTCTCTTTGCCACAGGAAG
				Forward Primer	CAGGAAACAGCTATGACCGCATGCTGAAGAAAGACCA
MDR1	-693T>C	rs3213619	Reverse	Reverse Primer	TGTAAAACGACGGCCAGTCCTTCTCCCGTGAAGACC
				Genotyping Primer	GAGCTTGGAAGAGCCGCT
				Forward Primer	CAGGAAACAGCTATGACCTATTCGAAGAGTGGGCACAA
MDR1	1236C>T	rs1128503	Reverse	Reverse Primer	TGTAAAACGACGGCCAGTTCCATCAACACTGACCTGGA
				Genotyping Primer	GCCCACTCTGCACCTTCAGGTTCAG
				Forward Primer	CAGGAAACAGCTATGACCTCAGCATTCTGAAGTCATGGA
MDR1	2677G>T/A	rs2032582	Reverse	Reverse Primer	TGTAAAACGACGGCCAGTTCCAAGAACTGGCTTTGCT
				Genotyping Primer	TATTTAGTTTGACTCACCTTCCCAG
				Forward Primer	TGTTTGACTGCAGCATTGC
MDR1	3435C>T	rs1045642	Reverse	Reverse Primer	TTTATTTGAAGAGAGACTTACATTAGGC
				Genotyping Primer	TGTTGGCCTCCTTTGCTGCCCTCAC

III. RESULTS

Baseline characteristics of 82 patients were shown as table 2 at the time of HIV infection diagnosis.

Table 2. Baseline characteristics at the diagnosis of HIV infection

Variables	At the time of HIV diagnosis(N=82)
Age, mean ± SD(years)	38.01 ± 11.2
Sex, M:F	9.25:1
HIV-1 risk factor	
Homosexual	42
Heterosexual	28
Transfusion	2
Unknown	10
CDC class C	60.5%
$CD4^+$ T cell counts(cells/mm^3), median , SD	210.00, 153.51
HIV-1 RNA level(log_{10} copies/mL), median, SD	4.81, 0.91

At the initiation of antiretroviral therapy, 17 patients had viral loads of <10,000 copies/ml, 15 patients between >10,000 and <100,000 copies/ml, and 50 patients >100,000 copies/ ml. 38 patients had a $CD4^+$ T cell count of <200 cells/μl, 25 patients between >200 and <350 cells/μl, and 19 patients >350 cells/μl.

The 82 HIV patients were divided into two groups according to their responses to HAART. 46 out of 82 patients achieved good immunologic responses at 24 weeks and 49 patients including former 46 did same responses at 48 weeks with increment of 100-150 cells/mm^3. Also, 56 patients had good virologic responses at 24 weeks and 52 patients kept their responses at 48 weeks with undetectable HIV RNA load or decline in serum HIV RNA by > 1 $\log_{10}$ copies/mL. The median values of the log viral load decline and the $CD4^+$ T cell increase from baseline, determined at week 24 and 48 after initiation of therapy were 3.96, 4.34, 101.00 and 166.00, respectively.

Genotype analysis of the MDR1 gene at position 3435 in exon 26 revealed 34 patients with the CC genotype, 33 with the CT genotype and 14 with the TT genotype. Analysis of the 2677 polymorphism in exon 21 demonstrated that 23 patients had the GG-, 35 the GT-, 9 the TT-, 7 the AG-, and 7 the AT genotype; the AA genotype was not found in this group. And those analyses of 1236 polymorphism in exon showed 9 with CC type, 40 with CT type and 33 with TT type. Detailed another genotype results are presented in table 3A and 3B.

Table 3A Genotype data of CYP450 and immunologic response to HAART

Gene		Frequency	Immunologic response after 24 weeks		*p* value			Immunologic response after 48 weeks		*p* value		
			responder	nonresponder	Co-dominant	dominant	recessive	Responder	Nonresponder	Co-dominant	dominant	recessive
CYP2B6 516G>T	GG	61	38	23				37	19			
(Q172H)	GT	19	7	12	0.146	0.054	0.860	10	7	0.498	0.818	0.296
	TT	2	1	1				2	0			
	Total	82	46	36				49	26			
CYP2B6 785A>G	AA	51	31	20				30	18			
(K262R)	AG	28	14	14	0.470	0.273	0.418	16	8	0.410	0.492	0.198
	GG	3	1	2				3	0			
	Total	82	46	36				49	26			
CYP2D6 100C>T	CC	38	22	16				25	8			
(P34S)	CT	25	13	12	0.899	0.690	1.000	13	10	0.212	0.078	0.342
	TT	18	10	8				10	8			
	Total	81	45	36				48	26			
CYP2D6 2988G>A	AA	1	1	0				0	1			
	GA	1	1	0	0.440	-	-	1	0	0.302	-	-
	GG	79	43	36				47	25			
	Total	81	45	36				48	26			

All data driven by Pearson chi square test, except one cell containing less than 5 samples by Fisher exact test.

Table 3B Genotype data of MDR1 and immunologic response to HAART

Gene		Frequency	Immunologic response after 24 weeks		*p* value			Immunologic response after 48 weeks		*p* value		
			Responder	Nonresponder	Co-dominant	dominant	recessive	Responder	Nonresponder	Co-dominant	dominant	recessive
MDR1 -1459G>A	AA	7	4	3				5	2			
	GA	41	26	15	0.443	0.992	-	29	11	0.280	0.739	-
	GG	31	15	16				14	12			
	Total	79	45	34				48	25			
MDR1 -935A>G	AA	65	37	28	0.768	-	-	38	20	0.951	-	-
	GA	17	9	8				11	6			
	Total	82	46	36				49	26			
MDR1 -709C>G	CC	78	44	34	0.801	-	-	47	24	0.508	-	-
	CG	4	2	2				2	2			
	Total	82	46	36				49	26			
MDR1 -693T>C	CT	8	5	3	0.701	-	-	7	1	0.163	-	-
	TT	74	41	33				42	25			
	Total	82	46	36				49	26			
MDR1 1236C>T	CC	9	1	8				4	2			
	CT	40	27	13	0.008	0.004	0.816	26	11	0.636	0.943	0.350
	TT	33	18	15				19	13			
	Total	82	46	36				49	26			
MDR1 2677G>T/A	AT	7	4	3				4	3			
	AG	7	3	4				5	2			
	GG	23	14	9	0.841	0.877	0.477	17	4	0.324	0.054	0.171
	GT	35	18	17				18	12			
	TT	9	6	3				4	5			
	Total	81	45	36				48	26			
MDR1 3435C>T	CC	34	17	17				21	10			
	CT	33	18	15	0.181	0.294	0.070	18	12	0.766	0.660	0.716
	TT	14	11	3				9	4			
	Total	81	46	35				48	26			

All data driven by Pearson chi square test, except one cell containing less than 5 samples by Fisher exact test.

Table 4A Genotype data of CYP450 and virologic response to HAART

Gene		Frequency	virologic response after 24 weeks		*p* value			virologic response after 48 weeks		*p* value		
			responder	nonresponder	Co-dominant	dominant	recessive	Responder	Nonresponder	Co-dominant	dominant	recessive
CYP2B6 516G>T	GG	59	41	18				39	14			
(Q172H)	GT	17	13	4	0.834	0.619	0.656	11	5	0.638	0.910	0.386
	TT	2	2	0				2	0			
	Total	78	56	22				52	19			
CYP2B6 785A>G	AA	49	32	17				31	14			
(K262R)	AG	27	22	5	0.454	0.188	0.656	19	4	0.465	0.276	0.793
	GG	2	2	0				2	1			
	Total	78	56	22				52	19			
CYP2D6 100C>T	CC	36	23	13				21	12			
(P34S)	CT	24	20	4	0.433	0.223	0.865	19	3	0.178	0.101	0.963
	TT	17	12	5				11	4			
	Total	77	55	22				51	19			
CYP2D6 2988G>A	AA	1	0	1				0	1			
	GA	1	1	0	0.555	-	-	1	0	0.210	-	-
	GG	76	55	21				51	18			
	Total	78	56	22				52	19			

All data driven by Pearson chi square test, except one cell containing less than 5 samples by Fisher exact test.

Table 4B Genotype data of MDR1 and virologic response to HAART

Gene		Frequency	virologic response after 24 weeks		*p* value			virologic response after 48 weeks		*p* value		
			Responder	Nonresponder	Co-dominant	dominant	recessive	Responder	Nonresponder	Co-dominant	dominant	recessive
MDR1 -1459G>A	AA	6	5	1				5	1			
	GA	40	32	8	0.373	0.818	-	28	10	0.874	0.635	-
	GG	30	19	11				19	6			
	Total	76	56	20				52	17			
MDR1 -935A>G	AA	61	18	43	0.773	-	-	40	16	0.505	-	-
	GA	17	4	13				12	3			
	Total	78	22	56				52	19			
MDR1 -709C>G	CC	74	53	21	0.963	-	-	49	18	0.935	-	-
	CG	5	3	2				3	1			
	Total	78	56	22				52	19			
MDR1 -693T>C	CT	8	7	1	0.545	-	-	7	1	0.333	-	-
	TT	70	49	21				45	18			
	Total	78	56	22				52	19			
MDR1 1236C>T	CC	9	6	3				6	1			
	CT	37	28	9	0.803	0.876	0.625	26	8	0.497	0.432	0.285
	TT	32	22	10				20	10			
	Total	78	56	22				52	19			
MDR1 2677G>T/A	AT	7	4	3				4	3			
	AG	7	7	0				5	1			
	GG	22	16	6	0.386	0.390	0.140	18	3	0.275	0.075	0.115
	GT	33	25	8				21	8			
	TT	9	4	5				4	4			
	Total	78	56	22				52	19			
MDR1 3435C>T	CC	32	23	9				23	5			
	CT	31	22	9	0.833	0.701	0.895	20	10	0.362	0.154	0.596
	TT	14	10	4				8	4			
	Total	77	55	22				51	19			

All data driven by Pearson chi square test, except one cell containing less than 5 samples by Fisher exact test.

Table 5A Genotype data of CYP450 and adverse effects of Efavirenz

Gene		Frequency	Experience of EFV AEs		*p* value			Experience of EFV AEs		*p* value		
			No	Yse	Co-dominant	dominant	recessive	No*	Yes*	Co-dominant	dominant	recessive
CYP2B6 516G>T	GG	60	33	27				41	20			
(Q172H)	GT	19	10	9	0.976	0.836	0.901	16	3	0.294	0.233	0.514
	TT	2	1	1				1	1			
	Total	81	44	37				58	24			
CYP2B6 785A>G	AA	50	27	23				34	17			
(K262R)	AG	28	15	13	0.908	0.941	0.662	22	6	0.532	0.299	0.875
	GG	3	2	1				2	1			
	Total	81	44	37				58	24			
CYP2D6 100C>T	CC	37	21	16				28	10			
(P34S)	CT	25	11	14	0.323	0.770	0.258	16	9	0.569	0.696	0.510
	TT	18	12	6				14	4			
	Total	80	44	36				58	23			
CYP2D6 2988G>A	AA	1	0	1				1	0			
	GA	1	1	0	0.360	-	-	1	0	0.666	-	-
	GG	78	43	35				56	23			
	Total	80	44	36				58	23			

All data driven by Pearson chi square test, except one cell containing less than 5 samples by Fisher exact test.

*Experience of neuropsychiatric adverse effects more than one episode designated “Yes” and vice versa

Table 5B Genotype data of MDR1 and adverse effects of efavirenz

Gene		Frequency	Experience of AEs		*p* value			Experience of CNS AEs		*p* value		
			No	Yes	Co-dominant	dominant	recessive	No*	Yes*	Co-dominant	dominant	recessive
MDR1 -1459G>A	AA	7	3	4				6	1			
	GA	41	23	18	0.790	0.494	-	29	12	0.703	0.402	-
	GG	30	17	13				22	9			
	Total	78	43	35				57	22			
MDR1 -935A>G	AA	64	38	26	0.076	-	-	47	18	0.540	-	-
	GA	17	6	11				11	6			
	Total	81	44	37				58	24			
MDR1 -709C>G	CC	77	42	35	0.859	-	-	57	21	0.039	-	-
	CG	4	2	2				1	3			
	Total	81	44	37				58	24			
MDR1 -693T>C	CT	8	6	2	0.216	-	-	7	1	0.273	-	-
	TT	73	38	35				51	23			
	Total	81	44	37				58	24			
MDR1 1236C>T	CC	9	6	4				8	1			
	CT	39	24	15	0.393	0.937	0.184	30	10	0.181	0.204	0.098
	TT	33	15	18				20	13			
	Total	81	44	37				58	24			
MDR1 2677G>T/A	AT	7	5	2				5	2			
	AG	7	3	4				5	2			
	GG	23	15	8	0.194	0.486	0.036	21	2	0.026	0.021	0.007
	GT	34	19	15				24	11			
	TT	9	2	7				3	6			
	Total	80	44	36				58	23			
MDR1 3435C>T	CC	34	19	15				26	8			
	CT	32	16	16	0.857	0.742	0.779	23	10	0.409	0.306	0.233
	TT	14	8	6				8	6			
	Total	80	43	37				57	24			

All data driven by Pearson chi square test, except one cell containing less than 5 samples by Fisher exact test.

*Experience of neuropsychiatric adverse effects more than one episode designated “Yes” and vice versa

As to the $CD4^+$ T cell response at 24 weeks on the HAART, there were significant differences between patient groups with different genotypes at positions 1236 like the table 3B. But, $CD4^+$ T cell response at 48 weeks or virologic response at week 24 and 48 had no correlation with CYP450 or MDR1 polymorphisms (table 3A, 3B, 4A and 4B).

There was a trend of a more pronounced mean $CD4^+$ T cell increase in 1236C>T at week 24(124.41 cells/mm^3) and this trend did persist at week 48(184.50 cells/mm^3).

The frequencies of CYP450 and MDR1 variants as shown in Table 5A, 5B were not correlate with neuropsychiatric adverse effects(AEs) of efavirenz except 2677G>T/A. The group having neuropsychiatric adverse effects more likely had TT genotype than the other group. The genotype *p* value of this site was 0.007.

In exon 21, G 2677T/A genotyping revealed GG in 23.4 %, GT in 43.2 %, TT in 0.11%, GA in 8.6% and TA in 8.6% . Because GA and AA genotypes are included in the GG genotype and the TA genotype is included in the GT genotype, a significant difference was observed in neuropsychiatric adverse effects occurrence (GG: GT: TT= 4/30:13/42:6/9, *p* value 0.007) by Pearson chi-square method. Figure 1 showed neuropsychiatric adverse effects according to genotypes.

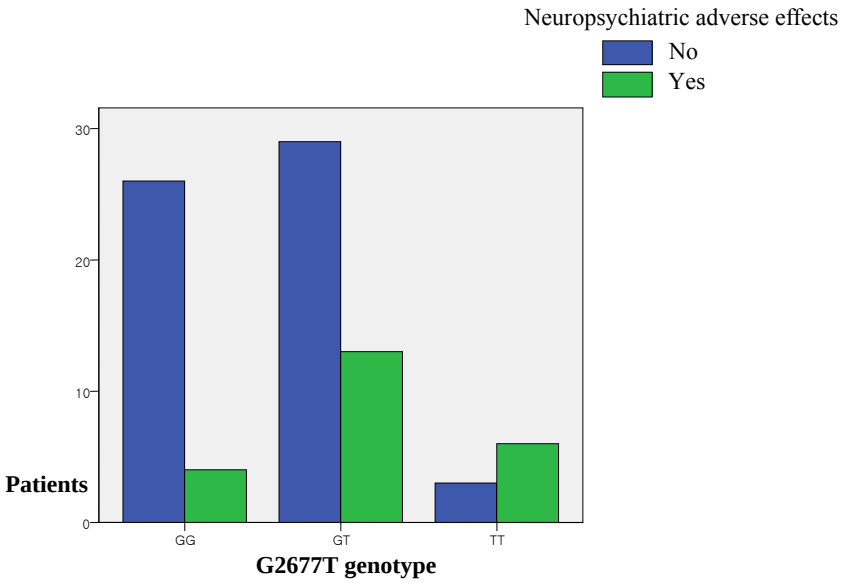

Fig 1. Neuropsychiatric adverse effects in relation to 2677 G> T . Experience of neuropsychiatric adverse effects more than one episode defined as “Yes” and vice versa.

Trough plasma concentration (median, range) of efavirenz in all patients was 614.41ng/mL(70.4–6,117.0). Each points of concentration are shown in figure 2. There were no difference of the changes in $CD4^+$ T lymphocyte counts between 49% of adequate concentration group and inadequate group from baseline to week 24 and 48. The adequate concentration group more likely had TT genotype than inadequate group in G2677T/A (p = 0.045) by chi-square test. It was also revealed by Kruskal-Wallis test(p = 0.034) that TT genotype concentration was found to be more adequate than those of the other groups.

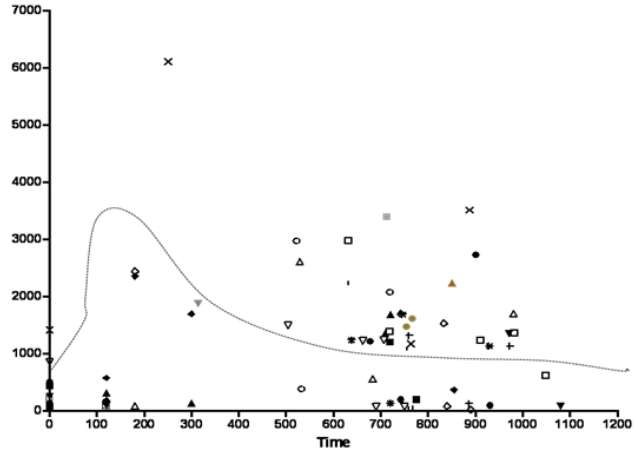

Fig 2. Steady state concentration of efavirenz

Protein expression level and MDR1 activity

Because these gene sites were known to be non-synomous SNPs, evaluation of protein expression level and MDR1 activity was needed. The cells transfected with MDR1 and with *neo* displayed p-glycoprotein expression. A mock control demonstrated no evidence of background signal. Representative Western blots are shown as figure 3. After anti-MDR1 antibody and Goat antimouse antibody, treated with neomycin phosphotransferase, there were no differences between MDR1 protein expression levels as different genotypes.

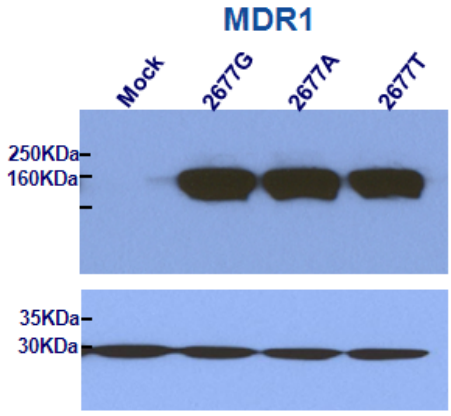

Fig. 3 Protein expression of G2677A/T variation.

To measure the EFV transport activity by 2677G>A/T, the dye as like rhodamine 123, DiOC2 or Calcein-AM, which is the substrate of MDR1, was accumulated after an amount of efflux time. Figure 3 showed results of FACS assay. The DiOC2 dye could inhibit the efflux of EFV and, according to increased EFV concentration, fluorescent activity was decreased.

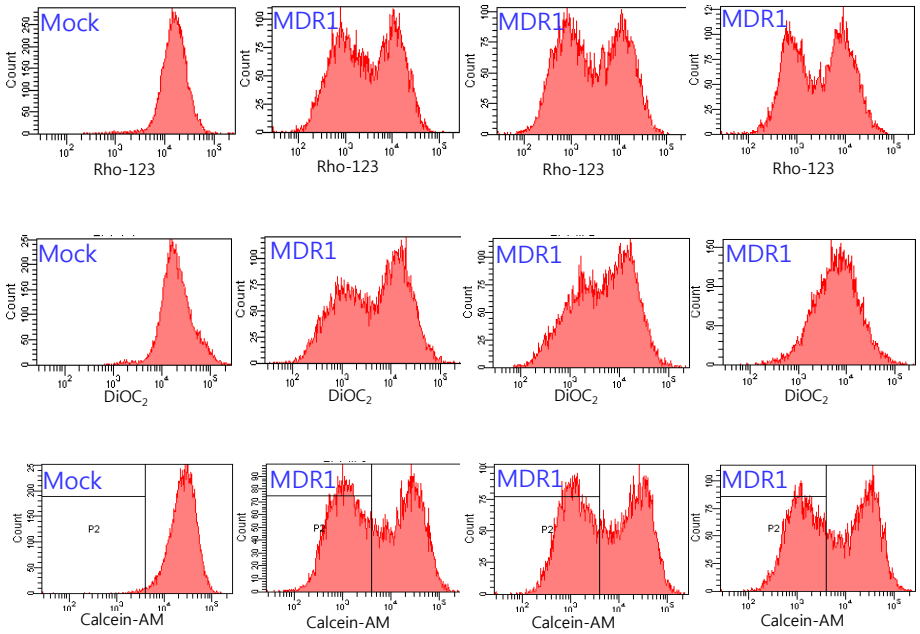

Fig. 4 Dye selection for Inhibition by EFV

IV. DISCUSSION

Highly active antiretroviral therapy (HAART), a combination of at least three antiretroviral drugs, has dramatically improved the prognosis of HIV/AIDS. However, viral replication under therapy can lead to the selection of drug resistant viruses and subsequent virologic failure. While poor adherence is likely to be the main cause of treatment failure, individual pharmacokinetic variability can also play an important role[15].

Recent pharmacogenetic studies of antiretroviral drugs reported the influence of several genetic polymorphisms on antiretroviral drug exposure, toxicity and response to treatment[2]. Efavirenz is known to be metabolized by cytochrome P 450(CYP) 2B6 and CYP2C19, respectively, with some involvement by CYP3A. Variations in the hepatic metabolism of EFV are one of the main causes of important inter-individual variations in the plasma concentration of the drug[16]. And, racial heterogeneity for CYP2D6 alleles has been reported, and efavirenz is an in vitro inhibitor for 2D6. Over 1% allele frequency in the database of the Korea Pharmacogenomics Research Network (http://www.pharmacogenomics.or.kr/), were chosen and genotyped, but there were no significant SNPs in CYP450 in this study.

In an analysis of the virological and immunological response of HAART with respect to the MDR1 C1236T, G2677T/A and C3435T polymorphisms, virus load and $CD4^+$ T cell count were assessed longitunally after initiation of

antiretroviral therapy.

These data are similar to a report of Fellay *et al*. that showed a significantly greater mean $CD4^+$ T cell increased in patients with the MDR1 3435TT genotype during an observation period of 24 weeks[5]. I saw a trend towards a greater mean $CD4^+$ T-cell rise in patients with the 1236, 2677, 3435 genotype as well. But, a possible explanation for this results that it did not persist at week 48 is the adaptation of protein, MDR1.

The 3435 TT genotype may have a reduced translation efficiency or lead to post-translational differences[17]. It has been shown, that a linkage disequilibirum exists between the exon 26 C3435T and the exon 21 G2677T/A polymorphism[4].

In a retrospective study of 455 treatment naïve patients initiating antiretroviral therapy with 40 months of follow-up [18], there was a trend to earlier virological failure in the 3435CC group ($p = 0.07$) with no effect of the C3435T polymorphism in the MDR-1 gene on immunological failure. However, the difference in the virological response was not observed during the first 10 months. Further follow-up of our patient group is ongoing in order to detect long-term effects that may not have been apparent during the observation period analysed in this report.

Because one study insisted that efavirenz is not a substrate for the multidrug transporter P-glycoprotein, the confirmation of MDR protein

expression and activity was needed. Use of dye like calcein-AM, rhodamine and DiOC2 could help efavirenz prove a substrate of MDR1 in figure 2.

Subjects with the 2677A allele have been evaluated mainly as subjects with the 2677G or 2677T allele. 2677A or 2677T results showed relation with Neuropsychiatric adverse effects. Because the 2677A allele was recently found to be functional in vivo and was detected at higher frequency in Koreans than other Asians[19], the genotyping of this variant in HIV patients is meaningfull. Thus the activity of the variant 2677A allele needs to be evaluated by haplotype analysis to avoid misinterpretations.

At present, 28 single nucleotide polymorphisms (SNPs) have been found at 27 positions, and 11 SNPs alter the amino acid sequences of MDR1(p-glycoprotein)[20]. Several groups have reported that a synonymous polymorphism, C3435T in exon 26, is associated with lower *MDR1* expression[21, 22], and higher plasma levels of *MDR1* substrates[21, 23-25] but other studies have contradicted these results[5, 26-28]. On the other hand, there are also studies that have not found an association between reported SNP and *MDR1* expression in the placenta[29] and duodenum[30] or disposition of P-gp substrates[31, 32]. Another frequent SNP with an amino acid exchange, G2677T/A (Ala893Ser/Thr) in exon 21[33] is located in the second trans-membrane spanning domain, and there is also disagreement regarding the effects of this polymorphism (G2677T/A) on *MDR1* transport activities[24, 27, 29, 30]. The reasons for these discrepancies concerning *MDR1* polymorphisms and

P-gp expression or function are unclear.

The G2677T/A polymorphisms are unique, with 3 allelic variants at the same gene locus. Moreover, ethnic differences in the allelic frequencies of G2677T/A have also been observed. Of the 3 allelic variants, the allelic frequency of the variant A allele ranged from 3.3% to 36% in Asians[23, 34-37] compared with 1.9% to 10% in white subjects[31, 33, 37] and 0.5% in black subjects[37].

Generally, therapeutic drug monitoring is recommended for drugs with a narrow therapeutic range, whereas pharmacogenetics is aimed at personalizing prescription, which in a perfect world would render therapeutic drug monitoring unnecessary. Both approaches would possibly lead to dose reduction (and drug saving) if many individuals are exposed to supratherapeutic dosing in a given population. These conditions are partially fulfilled for efavirenz. There is a certain ground for dose adjustment by using drug levels, although there is no consensus on the association of pharmacokinetics and efavirenz neuropsychiatric toxicity[38]. There is some evidence to suggest that individuals with efavirenz levels below 1,000 ng/mL are at risk for drug resistance/treatment failure and those with levels above 4,000 ng/mL are at risk for neurotoxicity [10]. Our data showed no definite association with drug level, because of small study population.

Several studies on efavirenz have reported higher plasma exposure and early adverse effects with the homozygous variant of the hepatic cytochrome

P450 enzyme CYP2B6 G516T polymorphism, which are more frequently found in African–American subjects. However, despite its association with efavirenz exposure this polymorphism was not associated with time to virologic or toxicity-related failure. Genetic analysis has also proven to be a valuable predictor of antiretroviral drug hypersensitivity reactions; genetic screening of patients prior to initiation of specific antiretrovirals has proven to reduce the incidence of drug hypersensitivity in certain settings. The reasons for antiretroviral treatment failure are multi-factorial but as the individualization of HAART increases understanding the influence of specific genotypes on treatment success and toxicity could further optimize these life-saving treatments.

Recently, Gatanaga et al. performed a priori dose reduction to 400 mg in four efavirenz-naive individuals homozygous as results of CYP2B6. Despite the preemptive dose reduction, only one individual remained with the 400 mg dose, two individuals required a further decrease to 200 mg/day, and efavirenz[39]. Efavirenz is uniformly prescribed at 600 mg once daily. The possibility that a lower efavirenz dose in many such individuals may reduce adverse effects without compromising efficacy should be studied. Additional large studies with longer follow up are needed to determine whether a sustained threefold difference in plasma efavirenz exposure affects long-term efficacy or toxicity, including the risk of developing reverse transcriptase efavirenz-associated resistance mutations.

In the meantime, clinical decision-making regarding the choice of ART grows more complex as our available therapies expand. It is important, then, that current genotype associations are confirmed in larger populations in the world and that target genes of interest continue to be identified and associations established between these genes and response to ART to help guide our decisions when prescribing antiretrovirals. As these data accumulate, we may be able to prospectively increase the chance of treatment success while avoiding toxicity as personalized HIV therapy evolves.

V. CONCLUSION

During a follow-up of 48 weeks, I found some evidence for an association between the MDR1 G2677T/A and C3435T polymorphisms and the virological and immunological response to therapy or drug adverse effects in HIV infected patients. The individual response to antiretroviral therapy is a complex phenomenon, which is influenced by a large number of biological variables. Further studies on the role of polymorphisms of the MDR1 and other transporters and enzymes involved in drug metabolism are necessary in order to elucidate the role of pharmacogenetic effects in HIV therapy.

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< 국문 요약>

한국인 HIV 감염자에서 약물 대사 효소와 약물 수송단백의 변이에 따른 HAART 의 치료 효과 분석

<지도교수 김준명 >

연세대학교 대학원 의학과

박 윤 선

항에이즈 치료로서 HAART(highly active antiretroviral therapy)가 도입된 이후 HIV 에 대한 치료는 발전을 거듭하고 있다. Efavirenz는 NNRTI(nonnucleoside reverse transcriptase inhibitor) 중 대표적인 제제로서 주로 CYP2B6에 의해, 일부는 CYP2D6에 대사되며, MDR1유전자에 의해 발현되는 P-glycoprotein이 약물수송단백으로서 역할을 하게 된다.

본 연구에서는 CYP2B6 등의 산화대사효소와 MDR1 genes의 변이에 따른 치료 효과 분석 및 약물이상반응과의 관련성을

살펴보고자 한다

총 82명의 HIV 감염자를 치료 반응 유무와 이상반응, 약물 농도의 적절성에 따라 두 군으로 분류하였고 CYP2B6, CYP2D6 및 MDR1에서 11가지 유전자를 선별하여 분석하였다.

C1236T의 변이를 가진 환자에서 치료 후 24주에 좀 더 나은 면역학적 치료 반응을 관찰할 수 있었고 $CD4^+$ T 림프구의 증가량 정도가 높았다. 그러나 바이러스적 치료 반응과 대상 유전자의 변이와의 연관성을 증명할 수는 없었다.

약물이상반응 중 두통, 현기증, 악몽, 자살 충동 등의 중추신경계의 이상반응을 경험한 24명과 그렇지 않은 58명을 비교한 결과는 MDR1 A893S (r.2677G>T) 변이가 관련성이 있음을 보여 주었고(p = 0.007), 적절한 혈중 efavirenz 농도를 유지하고 있는 환자군에서는 MDR1 A893S(d.2677G>T) 변이에서 연관성을 관찰하였다.

위와 같은 결과는 에이즈 치료에서 해당 유전자의 단일 염기 다형성이 efavirenz 가 표함된 치료제에 대한 치료 반응이나 이상반응을 예측하는데 중요한 유전적 표지자 역할을 할 것으로 기대된다.

핵심되는 말: 산화대사효소, 약물수송단백, 변이, HIV, HAART(highly active antiretroviral therapy), 이상반응