846

LIPID, LIPOPROTEIN AND GLUCO-REGULATORY CHANGES DURING SHORT-TERM PROTEASE INHIBITOR AND EFAVIRENZ EXPOSURE IN HIV-SERONEGATIVE SUBJECTS: RESULTS FROM ACTG A5043

S. Rosenkranz[1], K. Yarasheski[2], M. Para[3], R. Reichman[4] and G. Morse[5] and the ACTG A5043 Team.

[1]Harvard School of Public Health, Boston MA; [2]Washington University, St Louis MO; [3]Ohio State University, Columbus OH; [4]University of Rochester, NY; [5]University at Buffalo, SUNY, NY.

Susan L. Rosenkranz, Ph.D.
Frontier Science, Second Floor
900 Commonwealth Avenue
Boston, MA 02215

Phone: 617/632.5915
Fax: 617/632.2001
Email: sue@sdac.harvard.edu

ABSTRACT

Background: Antiretroviral use is associated with altered lipid metabolism. It is unclear whether these alterations result from changes in fat distribution, immune or virologic status or direct actions of antiretrovirals (ARVs). If the last, variation in ARV AUCs, due to individual pharmacokinetics (PK) or drug interactions, could predict lipid levels, a hypothesis we tested in HIV-seronegative adults participating in the PK interaction study ACTG A5043.

Methods: Subjects took efavirenz (E) 600 mg qd on days 1-21, amprenavir (A) 600 mg bid on days 11-21, and were randomized to take a second PI (saquinavir [S], nelfinavir [N], indinavir [I], ritonavir [R]) or none on days 15-21. Fasting serum cholesterol (C), triglyceride (TG), LDL-C and HDL-C were measured on days 0, 14, 21 and 42 (3 weeks after discontinuing all drugs). Nine, 10, 6, 8 and 7 subjects contributed C levels on the S, N, I, R and control arms, respectively. Least-squares means for each day/arm were estimated from mixed-effects models without multiple comparisons adjustment.

Results: Baseline values and BMI did not differ among arms. A+E significantly increased mean C, TG LDL-C and HDL-C (Table 1). Neither A nor E areas under the curve (AUCs) predicted C, TG, LDL-C or HDL-C changes on day 14. C, TG and LDL-C were still higher on day 21 on some arms. Three weeks after drug discontinuation, C and LDL-C remained elevated over baseline. HDL-C increased from day 21 on the control, N and R arms. Body weight was unchanged during the study.

TABLE 1

	Least Squares Mean (mg/dL)													
Day	0		14	21					42				
Drugs	None	(95% CI)	AE	AE	AES	AEN	AEI	AER	None (AE)	None (AES)	None (AEN)	None (AEI)	None (AER)
C	156	(147-164)	[a]170	[b]183	[b]174	[b,c]186	[b,c]191	[b]179	[d]182	[d]183	[d]180	[d]207	[d]176
TG	94	(79-109)	[a]110	[b]132	117	[b,c]139	110	[b,c]141	[e]81	[d]133	[e]101	100	108
LDL	90	(83-98)	[a]100	[b]106	102	[b]110	[b,c]119	102	[d]113	[d]107	[d]105	[d]134	100
HDL	45	(42-49)	[a]48	47	48	44	[b]49	48	[d,e]52	50	[d,e]52	[d]52	[d,e]54

[a] Day 14 differs from day 0 at p<0.05.
[b] Day 21 differs from day 0 at p<0.05.
[c] Day 21 differs from day 14 at p<0.05.
[d] Day 42 differs from day 0 at p<0.05.
[e] Day 42 differs from same arm on day 21 at p<0.05.

Conclusions: As previously reported, varying the second PI taken with A+E yielded differing A but similar E AUCs. Here, lipid changes were also similar across second-PI arms. Lipids did not correlate with A and E AUCs, but increased with the duration of A and E exposure, and remained elevated in some subjects after drug discontinuation.

BACKGROUND

HIV-infection and treatment with antiretroviral medications (ARV) have been associated with the development of metabolic syndromes (HIV+MS or lipodystrophy), a cluster of metabolic, endocrine, body fat composition and distribution complications that occur in ~40-60% of HIV-infected people. Metabolic complications include insulin resistance, hypertriglyceridemia, hypercholesterolemia, low HDL-cholesterol, elevated diastolic blood pressure, adipose tissue maldistribution, lactic acidosis, osteopenia/-porosis, elevated serum biomarkers for prothrombotic events (fibrinolysis) and chronic inflammation.

In HIV-infected people, factors that may contribute to HIV+MS and proatherogenic dyslipidemia include: infection with HIV-1, direct and indirect actions of specific ARVs on tissue and on substrate metabolism, genetic predisposition, activation of immune and complement factors, poor nutrition, physical inactivity, behavioral factors, and advancing age. We examined the relationship between EFV and APV levels and the development of glucose and lipid metabolic alterations in HIV-seronegative people during short-term exposure to these medications and a second PI.

We hypothesized that: (1) alterations in serum triglyceride and cholesterol levels are caused by direct actions of NNRTIs and/or PIs, (2) some PIs may have direct effects and/or may compound other ARV effects through PK drug interactions, and (3) the magnitude of changes in circulating lipid levels is related to serum levels of ARVs. To test these hypotheses, ARV drug and serum lipid/lipoprotein levels were quantified in healthy HIV-seronegative adults enrolled in ACTG A5043, a PK interaction study. Subjects were given a short course of an NNRTI (EFV) plus PI (APV), followed in 4 of 5 arms by the addition of a 2nd PI (SQV, NFV, IDV, RTV; the 5th arm was a control arm with no 2nd PI). The schedule of drug dosing and evaluations for A5043 is shown in Figure 1.

Previously reported effects of co-administered drugs on APV PK exposure (area under the concentration-time curve, AUC) are summarized in Figure 2. Specifically: (1) APV AUCs decreased with EFV co-administration (day 14 above), and (2) APV AUCs increased with addition of the 2nd PIs NFV, IDV and RTV, and decreased with the addition of SQV (day 21 above). Addition of a 2nd PI did not alter EFV PK.

METHODS

Measures
- Fasting samples on days 0, 14, 21 and 42:
  - Total cholesterol (CHOL)
  - Triglycerides (TG)
  - HDL cholesterol (LDL)
  - LDL cholesterol (LDL; calculated as CHOL – HDL – TG/5)
  - C-peptide (CPEP)
  - Blood glucose (GLU)
  - Insulin (INS)
  - An index of insulin sensitivity (IS; glucose homeostasis model [HOMA]; calculated as GLU [mM] * INS [µU/mL] / 22.5)
- Samples considered fasting if collected 7.5-16.5h after last intake (subject self-report)
- For 5 of 222 subjects/days (2%), GLU, INS, CPEP and TG values were clearly non-fasting; these values were also excluded from statistical analyses.
- Post-prandial samples on days 0, 14 and 21:
  - GLU, INS, IS
  - Samples considered post-prandial if collected 1.5-2.5h after ingestion of standardized meal (low-fat for IDV arm, 33% fat for other arms)

Subjects
- Healthy HIV-1 seronegative adult volunteers participating in AACTG PK interaction study A5043
- Normal weight, labs, LFTs
- Women of child-bearing potential excluded
- Included in metabolic analysis if:
  - Provided measure-specific values on 2 or more study days
  - PK evaluable –
    - Not among the 28% discontinuing early for study-defined toxicity (70%), intolerability (30%)
    - Took study drugs throughout as prescribed
    - Provided intensive PK samples on all 3 PK days

STATISTICAL METHODS

Baseline values.
- Descriptive statistics for each measure at baseline (BL; day 0), by arm and pooled across arms
- ANOVA F-test to evaluate differences between arms in BL measure, weight, BMI
- Paired t-test to judge differences between fasting and fed GLU, INS and IS

Changes over time.
- Separate analyses for each measure
- Focus on 3 changes –
  1. Day 14 change from BL: changes due to 14 days EFV, 3 days APV
  2. Day 21 change from day 14: changes due to 7 additional days of APV and EFV, and addition of 2nd PI in 4 of 5 arms
  3. Day 42 changes from BL: differences from BL at visit 2-3 weeks after drug discontinuation
- Mixed-effects linear model fit to all available data (maximize power)
- Fixed effects for Day (0, 14, 21, 42), Arm (Control, SQV, NFV, IDV, RTV), their interaction
- Random intercept for subject, compound symmetry variance structure
- Maximum likelihood estimation via the Newton-Raphson, SAS version 9.1 PROC MIXED
- Summarized as least-squares means of changes and associated 95% Cis
- If Day significant but Arm and interaction not, changes pooled over arms before summarized
- Arm-specific estimates of changes are displayed if:
  1. Arm and/or interaction significant, and/or
  2. For the day 21-14 and/or day 42-0 change, significant difference between the control and at least one 2nd-PI arm
- Comparisons with day 42 not done for post-prandial measures
- Differences considered statistically significant if p ≤ 0.05
- Analyses exploratory rather than confirmatory: no adjustments for multiple comparisons

Relationship between PK parameters and short-term changes in lipid levels

Day 14 change-from-BL **(for measures where day 14 differs significantly from BL)**
- Regression models used to assess linear relationship between changes and APV and EFV exposure (AUCs)
- Initally fit 3 models:
  1. Day 14 APV AUC as the single covariate
  2. Day 14 EFV AUC as the single covariate
  3. Both APV and EFV AUCs; also BL parameter value, weight and BMI
- If Wald p <0.10 for AUC or <0.05 for other predictors, variable tested in a second multivariate model
- Only those significant at p <0.05 retained in the final model

Day 21-14 change (where day 21 and 14 differ significantly)
- Similar stepwise process to select final model
- APV and EFV AUCs measured on days 21 and 14 used as predictors; also 2nd-PI arm, BL parameter, weight and BMI

Day 42 difference-from-BL (where day 42 differs significantly from BL)
- APV and EFV AUCs from day 21 used as predictors; also 2nd-PI arm, BL parameter, weight, BMI and number of days between last dose of study drug and follow-up (nominal day 42) visit

FIGURE 1

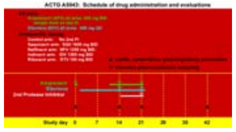
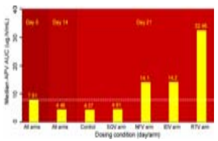


FIGURE 2



RESULTS

TABLE 2

Baseline values for lipids, lipoproteins, C-peptide, gluco-regulatory measures, weight and body mass index (BMI).

Condition	Measure	Arm	N	Mean	(SD)	Median	(Range)	ANOVA F-test arm effect p-value
Fasting	Total cholesterol, mg/dL	All	51	155.53	-25.09	154	(110.00, 200.00)	0.381
	Triglycerides, mg/dL	All	50	93.9	-46.71	79.5	(45.00, 221.00)	0.317
	HDL cholesterol, mg/dL	All	51	45.33	-12.67	43	(19.00, 80.00)	0.935
	LDL cholesterol, mg/dL	All	51	90.4	-25.95	92.8	(33.80, 149.20)	0.577
	C-peptide, ng/mL	All	52	1.61	-0.65	1.5	(0.61, 3.80)	* 0.022
		Control	11	2.05	-0.78	1.9	(0.98, 3.80)	--
		SQV	9	1.39	-0.28	1.5	(0.93, 1.80)	--
		NFV	12	1.71	-0.67	1.5	(0.90, 3.10)	--
		IDV	12	1.55	-0.62	1.4	(0.61, 2.98)	--
		RTV	8	1.19	-0.43	1.05	(0.76, 2.09)	--
	Blood glucose, mg/dL	All	52	89.3	-7.9	89.5	(76.0, 115.0)	0.613
	Insulin, U/mL	All	51	6.64	-3.06	6	(2.00, 16.10)	0.871
	Insulin sensitivity	All	51	1.49	-0.75	1.35	(0.38, 4.05)	0.873
2h post-meal	Blood glucose, mg/dL	All	50	91.5	-11.9	92.5	(62.00, 119.0)	0.745
	Insulin, U/mL	All	50	16.83	-12.39	13.05	(3.00, 72.90)	0.453
	Insulin sensitivity	All	47	4.04	-3.58	3.15	(0.52, 21.04)	0.636
Baseline	Weight, kg	All	51	78.9	-9.1	78.6	(61.8, 104.1)	0.219
	BMI	All	51	23.9	-2.2	23.9	(17.6, 28.7)	0.132

RESULT SUMMARY

- **Day 14** (following EFV for 14d, APV for 3d):
  Significant **elevations** above baseline for: **CHOL, TG, HDL, LDL** and **GLU** (Table 3).
- **Day 21** (additional 7 days of EFV+APV; 2nd PI added in all but Control arm):
  Significant **elevations** above day 14 for: **CHOL, TG** and **LDL** (Table 3).
  - For GLU, 95% CIs around changes include zero on all but the RTV arm, which shows a decrease from day 14 to 21.
- **Day 42** (2-3 weeks after stopping all study drugs):
  Significant **elevations** above baseline for: **CHOL, HDL, LDL, INS** and **IS** (Table 3).
  - IDV arm: significantly higher CHOL elevations than seen on Control arm.
  - NFV arm: significantly lower INS and IS elevations than seen on Control arm.
  - SQV arm: significantly higher TG elevations than seen on Control arm.
  - Day 42 elevations in CHOL and LDL were highest in the IDV arm and lowest in the RTV arm.
- For the most part, changes in lipid, lipoprotein and gluco-regulatory measures are *not* correlated with EFV or APV AUCs (Table 4). However:
  - Higher EFV AUCs are associated with smaller TG elevations on Day 14.
  - Higher APV AUCs are associated with smaller HDL elevations on Day 14.
- Baseline measures were predictive of later measures (Table 4). See Table 2 for summary of baseline values. Also:
  - Weight and BMI did not differ across arms at baseline or change over time.
  - Fed GLU, INS and IS values were 1.71 mg/dL, 10.50 U/mL and 2.67 units higher than corresponding fasted values at baseline; however, only INS and IS differences were statistically significant.

TABLE 3

Summary of significant changes in endocrine measures

Condition	Measure	Significant changes, all arms combined: Least-squares mean (95% CI)			Changes that differ significantly between 2nd-PI and control arm, least-squares means	ANOVA F-test p-values for fixed effects		
						Day	Arm	D*A
Fasting	**Cholesterol**	14 > 0 **13.8 mg/dL** (6.9 to 20.8)	21 > 14 **13.1 mg/dL** (6.3 to 19.9)	42 > 0 **29.2 mg/dL** (21.7 to 36.6)	Day 42-0: IDV, 54.2 mg/dL Control, 24.2 mg/dL	<0.0001	ns	ns
	Triglycerides	14 > 0 **16.0 mg/dL** (2.1 to 29.8)	21 > 14 **18.2 mg/dL** (4.5 to 31.9)	--	Day 42-0: SQV, 32.7 mg/dL Control, –18.4 mg/dL	<0.0001	ns	ns
	HDL	14 > 0 **2.6 mg/dL** (0.7 to 4.5)	--	42 > 0 **7.0 mg/dL** (4.9 to 9.1)	--	<0.0001	ns	ns
	LDL	14 > 0 **8.6 mg/dL** (2.4 to 14.9)	21 > 14 **8.7 mg/dL** (2.4 to 14.9)	42 > 0 **19.9 mg/dL** (13.1 to 26.7)	--	<0.0001	ns	ns
	C-peptide	--	--	--	--	ns	0.0229	ns
	Glucose	14 > 0 **2.65 mg/dL** (0.77 to 4.53)	--	--	Day 21-14: RTV, –4.60 mg/dL Control, 2.03 mg/dL	0.007	-- ns	0.0357
	Insulin	--	--	42 > 0 **1.23 U/mL** (0.40 to 2.06)	Day 42-0: NFV, –0.70 mg/dL Control, 2.86 mg/dL	0.0049	ns	ns
	Insulin sensitivity	--	--	42 > 0 **0.27 units** (0.07 to 0.47)	Day 42-0: NFV, –0.19 mg/dL Control, 0.59 mg/dL	0.0215	ns	ns
Fed	Glucose	--	--	NA	--	--	--	--
	Insulin	--	--	NA	--	--	--	--
	Insulin sensitivity	--	--	NA	--	--	--	--

TABLE 4

Predictors of changes in endocrine measures

Change from baseline on…	Parameter	Significant predictors		
Day 14	Cholesterol	BL cholesterol		
	Triglyceride	**EFV AUC$_{Day 14}$** BL triglyceride	Predicted changes for EFV AUC$_{Day14}$ = [a]	
			58.0 µg.h/mL (25th %ile):	30.0 mg/dL
			79.5 µg.h/mL (50th %ile):	24.4 mg/dL
			105.0 µg.h/mL (75th %ile):	17.7 mg/dL
	HDL	**APV AUC$_{Day 14}$** BL HDL	Predicted changes for APV AUC$_{Day14}$ = [b]	
			2.14 µg.h/mL (25th %ile):	4.3 mg/dL
			4.09 µg.h/mL (50th %ile):	3.0 mg/dL
			5.58 µg.h/mL (75th %ile):	1.1 mg/dL
	LDL	BL LDL		
	GLU	BL glucose		
Day 21	Cholesterol	BL cholesterol		
	Triglyceride	(Intercept only)		
	LDL	BL LDL		
Day 42	Cholesterol	**2nd-PI arm**	Least-square mean	95% CI
		IDV	65.5 mg/dL	(42.5, 88.5)
		Control	26.3 mg/dL	(7.5, 45.1)
		SQV	24.6 mg/dL	(3.2, 45.9)
		NFV	21.8 mg/dL	(4.0, 39.6)
		RTV	15.8 mg/dL	(–4.2, 35.7)
	HDL	(Intercept only)		
	LDL	**2nd-PI arm**	Least-square mean	95% CI
		IDV	51.6 mg/dL	(31.5, 71.7)
		Control	24.1 mg/dL	(6.7, 41.5)
		SQV	12.1 mg/dL	(–6.5, 30.7)
		NFV	11.3 mg/dL	(–4.3, 26.8)
		RTV	4.3 mg/dL	(–13.1, 21.7)
	INS	(Intercept only)		

[a] BL triglyceride set to its median.
[b] BL HDL set to its median.

FIGURE 3B

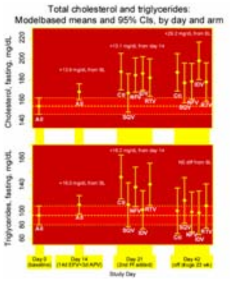


FIGURE 3C

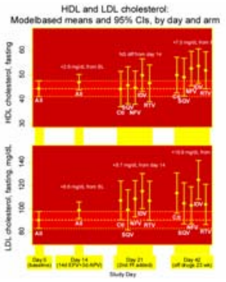
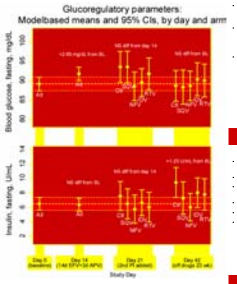


FIGURE 3A



SUMMARY

- In healthy, HIV-1 seronegative subjects, 13 doses of efavirenz and 6 doses of amprenavir (over the preceding 3 days) increased fasting serum total-, HDL- and LDL-cholesterol and triglyceride levels above baseline.
- On day 21, serum total-, LDL-cholesterol, and triglyceride levels increased still further. By day 21, subjects had been exposed to an additional 7 doses of efavirenz, 14 doses of amprenavir (over the preceding 7 days), and 13 doses of a second PI (saquinavir, nelfinavir, indinavir, ritonavir), or no PI (control arm).
- Two to three weeks after stopping all study drugs, total-, HDL- and LDL-cholesterol levels remained significantly elevated above baseline, as did fasting insulin and the HOMA index of insulin sensitivity. Triglycerides and glucose levels had returned to baseline.
- Higher efavirenz and amprenavir AUCs predicted *smaller* increases in triglycerides and HDL-cholesterol, respectively. Despite substantial arm-to-arm variation in amprenavir AUCs (more than sevenfold), day 21 increases in lipid/lipoprotein parameters did not vary across arms; however, the study was not powered to detect such differences.
- Twenty-eight percent of subjects taking A5043 study drug discontinued study early for protocol-defined toxicities (70%) and other reasons (30%), and were thus excluded from analyses. PK measures were not available for all such subjects. If amprenavir AUCs are higher for discontinuers than completers, we may have failed to detect a relationship between amprenavir PK and metabolic changes because subjects with higher amprenavir levels were not available for analysis. It is also possible that amprenavir and/or efavirenz trigger metabolic changes via a threshold mechanism.
- During this short period of drug exposure, neither weight nor BMI changed, despite changes in metabolic measures. Neither baseline weight nor BMI was associated with the magnitude of metabolic alterations.

CONCLUSIONS

- Clinically significant elevations in serum total-, HDL- and LDL-cholesterol, triglycerides and glucose levels were seen in healthy, HIV-1 seronegative subjects after a very short exposure to antiretroviral combination therapy (efavirenz plus amprenavir).
- Elevations in total-, HDL-, and LDL-cholesterol persisted even 2-3 weeks after discontinuing the ARV medications.
- The rapid onset of elevations in serum lipids/lipoprotein levels suggests that exposure to efavirenz and amprenavir of very short duration can trigger pharmacodynamic mechanisms associated with HIV+metabolic syndromes.
- Persistent elevations in fasting serum total- and LDL-cholesterol levels point to a process that is cumulative and not instantaneously reversible.
- For the most part, elevations in lipid and lipoprotein levels were not associated with circulating levels of efavirenz or amprenavir.
- Study design could not separate direct effects of amprenavir from those of efavirenz, nor direct vs. pharmacokinetic-interaction effects of second protease inhibitors. However, the finding of elevated lipids following efavirenz and amprenavir administration is consistent with other published research.

ACKNOWLEDGMENTS

- We would like to thank the subjects who participated in the study, and investigators and staff from contributing sites. Thanks also to Courtney Ashton, Barbara Brizz, Robin DiFrancesco, Yoninah Segal, Leslie Thompson, Ann Walawander and other members of the ACTG A5043 team; and to Peter Bohlin for graphics assistance. Supported in part by the Adult AIDS Clinical Trials Group funded by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U01 AI38558.