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ABCB1 and ABCC1 expression in peripheral mononuclear cells is influenced by gene polymorphisms and atorvastatin treatment

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ABSTRACT

This study investigated the effects of atorvastatin on ABCB1 and ABCC1 mRNA expression on peripheral blood mononuclear cells (PBMC) and their relationship with gene polymorphisms and lowering-cholesterol response. One hundred and thirty-six individuals with hypercholesterolemia were selected and treated with atorvastatin (10 mg/day/4 weeks). Blood samples were collected for serum lipids and apolipoproteins measurements and DNA and RNA extraction. ABCB1 (C3435T and G2677T/A) and ABCC1 (G2012T) gene polymorphisms were identified by polymerase chain reaction-restriction (PCR)-RFLP and mRNA expression was measured in peripheral blood mononuclear cells by singleplex real-time PCR. ABCB1 polymorphisms were associated with risk for coronary artery disease (CAD) ($p < 0.05$). After atorvastatin treatment, both ABCB1 and ABCC1 genes showed 50% reduction of the mRNA expression ($p < 0.05$). Reduction of ABCB1 expression was associated with ABCB1 G2677T/A polymorphism ($p = 0.039$). Basal ABCB1 mRNA in the lower quartile (<0.024) was associated with lower reduction rate of serum low-density lipoprotein (LDL) cholesterol (33.4 ± 12.4%) and apolipoprotein B (apoB) (17.0 ± 31.3%) when compared with the higher quartile (>0.085: LDL-c = 40.3 ± 14.3%; apoB = 32.5 ± 10.7%; $p < 0.05$). ABCB1 substrates or inhibitors did not affect the baseline expression, while ABCB1 inhibitors reversed the effects of atorvastatin on both ABCB1 and ABCC1 transporters. In conclusion, ABCB1 and ABCC1 mRNA levels in PBMC are modulated by atorvastatin and ABCB1 G2677T/A polymorphism and ABCB1 baseline expression is related to differences in serum LDL cholesterol and apoB in response to atorvastatin.

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1. Introduction

Glycoprotein P (MDR1/ABCB1) and multidrug resistance protein 1 (MRP1/ABCC1) are ATP-binding cassette (ABC) multidrug-efflux pumps that play an important role in normal physiology by protecting tissues from toxic xenobiotics and endogenous substrates such metabolites [1]. Expression of these efflux transporters in gastrointestinal tract and brain capillary endothelial cells limits oral absorption and central nervous system uptake of many drugs [2]. Moreover, ABCB1 and ABCC1 transporters involvement in drug transport through cell membranes is likely contribute to variation of drug disposition and response [1].

Single nucleotide polymorphisms (SNPs) in genes encoding the ABC drug-efflux pumps may play a role in responses to drug therapy and disease susceptibility [3,4]. The effect of various genotypes and haplotypes on the expression and function of these proteins is not yet clear, and their true impact remains controversial [5]. Moreover, ABCB1 variants have been implicated in the etiology of several human diseases associated with resistance to pharmacotherapy [2].

Two common polymorphisms at the ABCB1 gene (C3435T and G2677T/A/C) have been associated with differences in gene expression and protein activity, and with variability in disposition of many therapeutic drugs as well [6–8]. The C3435T (rs1045642) is a synonymous polymorphism (Ile1145Ile) located in the exon 26 that was found to be associated with expression levels of ABCB1 [6]. This SNP seems to be a marker of a rare codon that affects the protein conformation and function [9].

The G2677T/A/C (rs2032582) is a non-synonymous polymorphism in the exon 21 with three distinct amino acid changes (Ala893Ser, Ala893Thr, and Ala893Pro, respectively) that is located at the transmembrane domain of the protein and it has a great impact on both the activity and the substrate specificity of ABCB1 toward different test compounds [10].

ABCC1 is expressed in many tissues, and function as an efflux transporter for glutathione-, glucuronate- and sulfate-conjugates as well as unconjugated substrates [11]. It can also confer resistance to a broad range of chemotherapeutic agents and transport a variety of toxicants [11].

Sequence variations within the ABCC1 gene might account for differences in drug response in different individuals. More than 85 polymorphisms have been identified in ABCC1 [4,12]. The ABCC1 G2012T (rs45511401) is a non-synonymous SNP (Gly671Val) located in the exon 16 at the nucleotide binding domain of the protein [13]. G2012T SNP which occur at low frequency in only one or two of four populations examined were predicted to be functionally deleterious and hence are likely to be under negative selection [14,15]. This SNP may be useful for studies associating ABCC1 variants with rare events including adverse drug reactions.

Atorvastatin is a potent inhibitor of the 3-hydroxy-3-methlyglutaryl-coenzyme A reductase (HMGCR), the rate-limiting enzyme in the cholesterol biosynthesis pathway [16]. It plays an important role in reducing plasma low-density lipoprotein (LDL) cholesterol and in preventing the risk for coronary artery disease (CAD) [16,17]. Atorvastatin undergo metabolism largely via the CYP3A family of metabolizing enzyme, mainly CYP3A4/5 [18]. Oxidative and UDP-conjugated products are eliminated via specific membrane transporters, such as ABCB1 [18].

ABCB1 haplotypes have been shown to be associated with differences on atorvastatin pharmacokinetics in healthy Finnish volunteers [19]. Moreover, the potential contribution of ABCB1 polymorphisms to variability on atorvastatin efficacy and safety has been recently reviewed [20]. ABCB1 C3435T and G2677T/A variants were associated with differences in serum total cholesterol, LDL cholesterol and high-density lipoprotein (HDL) cholesterol in response to atorvastatin [21,22].

ABCC1 polymorphisms have been shown to be related with differences in cellular transport that may affect drug disposition [23]. However, the effects of ABCC1 variants on atorvastatin pharmacokinetics and efficacy remain to be investigated.

This study investigated the effects of atorvastatin on ABCB1 and ABCC1 expression in peripheral blood mononuclear cells (PBMC) and their relationship with gene polymorphisms and lowering-cholesterol response in hypercholesterolemic (HC) individuals.

2. Materials and methods

2.1. Subjects and study protocol

The characteristics of study design have been previously reported [24]. Briefly, 136 hypercholesterolemic individuals were selected randomly among the outpatients evaluated for the presence of risk factors for CAD at the Institute Dante Pazzanese of Cardiology and the Hospital of the Sao Paulo University (Sao Paulo City, Brazil). The study protocol was approved by the Ethics Committees of these institutions as well the Committee of the Faculty of Pharmaceutical Sciences (University of Sao Paulo). Individuals diagnosed with thyroid, liver and kidney diseases, diabetes, and triglycerides higher than 400 mg/dL or subjects under treatment with lipid-lowering drugs, hormone replacement or oral contraceptives were not included. Pregnant women or patients with heart disease known previously were not included too.

Information on age, body mass index (BMI) gender, hypertension, obesity, menopause status, cigarette smoking, physical activity and family history of coronary artery disease were recorded. Each individual declared his ethnic group during the interview, recommended by Brazilian Census [101].

HC patients with low-density lipoprotein cholesterol higher than 160 mg/dL, even after a low cholesterol diet [25] during 4 weeks, were started on atorvastatin therapy, 10 mg orally once daily for 4 weeks. Response to atorvastatin was evaluated by reduction of LDL cholesterol after the treatment, and adverse effects were monitored by measuring muscular (CK) and liver (ALT) enzymes.

Blood samples for biochemical profile (lipids, CK, and ALT) measurements and mRNA expression in mononuclear cells were collected after an overnight fast, 1 day before and 4 weeks after atorvastatin treatment. All patients followed exactly the same study protocol. Clinical and laboratory data of the HC individuals are presented in Table 1.

Table 1 – Clinical, laboratory data and polymorphisms of the study group.

Variables	Total	ABCB1 C3435T			ABCB1 G2766TA			ABCB1 haplotypes[a]			ABCC1 G2012T		
		CC	CT + TT	*p*	GG	Non-GG	*p*	T/T	Non-T/T	*p*	GG	GT	*p*
Number of individuals	136	42	94		52	84		70	66		125	11	
Genotype frequency		31%	69%		38%	62%		51%	48%		92%	8%	
Age, years	57 ± 11	57 ± 11	57 ± 11	0.860	59 ± 11	57 ± 11	0.279	57 ± 12	59 ± 11	0.323	57 ± 11	60 ± 12	0.434
BMI, kg/m^2	27.7 ± 4.4	27.3 ± 3.7	27.9 ± 4.7	0.405	28.1 ± 4.2	27.6 ± 4.5	0.472	27.6 ± 4.7	27.9 ± 4.1	0.724	27.8 ± 4.3	28.1 ± 5.4	0.814
Ethnics (European)	68%	60%	72%	0.195	58%	74%	0.051	73%	62%	0.181	69%	73%	1.000[b]
Gender (women)	68%	67%	62%	0.909	65%	70%	0.554	70%	67%	0.676	70%	45%	0.101[b]
Hypertension	58%	58%	58%	0.993	61%	56%	0.534	57%	59%	0.838	57%	64%	0.759[b]
Obesity	29%	24%	31%	0.402	33%	26%	0.398	29%	30%	0.887	29%	30%	1.000[b]
Menopause	86%	82%	88%	0.512	88%	87%	1.000	86%	88%	0.747	85%	100%	1.000[b]
Cigarette smoking	17%	16%	17%	0.494	15%	15%	0.879	15%	15%	0.836	18%	8%	0.286[b]
Alcohol consumption	30%	32%	29%	0.677	29%	30%	0.840	31%	29%	0.791	28%	64%	0.034[b]
Physical activity	46%	43%	48%	0.593	45%	48%	0.732	49%	44%	0.594	45%	54%	0.754[b]
Family history of CAD	57%	44%	63%	0.042	46%	64%	0.043	67%	47%	0.021	56%	54%	1.000[b]
ABCB1 substrates[c]	15%	17%	14%	0.698	10%	19%	0.129	18%	12%	0.350	15%	9%	1.000[b]
ABCB1 inhibitors[d]	14%	12%	16%	0.613	15%	14%	0.863	17%	12%	0.479	14%	9%	1.000[b]
Lipids, mg/dL													
Total cholesterol	281 ± 38	280 ± 33	281 ± 41	0.978	279 ± 34	283 ± 41	0.618	283 ± 43	280 ± 33	0.789	281 ± 38	272 ± 38	0.433
HDL-c	56 ± 14	56 ± 14	55 ± 13	0.807	57 ± 14	55 ± 13	0.400	54 ± 12	58 ± 15	0.207	56 ± 13	48 ± 18	0.015
LDL-c	193 ± 35	193 ± 30	194 ± 37	0.993	192 ± 32	194 ± 37	0.836	196 ± 39	192 ± 30	0.686	194 ± 35	187 ± 25	0.618
VLDL-c	32 ± 13	31 ± 11	32 ± 14	0.803	29 ± 11	33 ± 14	0.081	33 ± 14	31 ± 12	0.301	31 ± 12	37 ± 19	0.337
Triglycerides	160 ± 66	160 ± 58	162 ± 69	0.802	147 ± 57	168 ± 70	0.081	166 ± 71	153 ± 60	0.300	157 ± 62	185 ± 94	0.337
ApoAI	130 ± 25	131 ± 24	130 ± 26	0.658	133 ± 25	128 ± 26	0.249	126 ± 24	1134 ± 26	0.0.78	131 ± 25	121 ± 28	0.205
ApoB	140 ± 22	143 ± 25	139 ± 21	0.555	140 ± 22	140 ± 23	0.948	141 ± 21	140 ± 24	0.751	140 ± 22	142 ± 20	0.746
ApoB/ApoAI ratio	1.13 ± 0.36	1.12 ± 0.27	1.13 ± 0.39	0.964	1.08 ± 0.23	1.16 ± 0.41	0.371	1.17 ± 0.42	1.08 ± 0.27	0.131	1.11 ± 0.36	1.23 ± 0.35	0.233

Number of individuals in parenthesis. ApoAI: apolipoprotein AI; ApoB: apolipoprotein B; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; and VLDL-c: very low-density lipoprotein cholesterol. BMI: body mass index; CAD: coronary artery disease. Hardy–Weinberg equilibrium: C3435T: $\chi^2 = 2.381$, $p > 0.05$; G2766T/A: $\chi^2 = 0.155$, $p > 0.05$; G2012T: $\chi^2 = 0242$, $p > 0.05$.

[a] T/T haplotype (GT/CT, GT/TT, TT/CT, TT/TT, TA/CT) and non-T/T haplotype (GG/CC, GG/CT, GG/TT, GT/CC, GA/CC).

[b] Numerical variables are presented as mean ± S.D. of log transformed data (exception of the age) and were compared by *t*-test. Categorical variables were compared by χ^2 test or Fisher Exact test.

[c] Antiarrhythmics, beta-blockers, diuretics, ACE inhibitors and others (Marzolini et al. [3]).

[d] Antiarrhythmics, calcium antagonists, calcium channel blockers, antidepressants and others (Marzolini et al. [3]).

2.2. Biochemical profile

Total cholesterol, high-density lipoprotein cholesterol and triglycerides serum concentrations were measured using standard enzymatic methods. Plasma apolipoprotein AI (apoAI) and apolipoprotein B (apoB) were determined by nefelometry. Values of low-density lipoprotein, very low-density lipoprotein (VLDL) cholesterol and apoB/apoAI ratio were estimated [26,27]. Serum ALT and CK tests were determined by kinetic methods using the ADVIA®1650 analyzer (Siemens Medical/Bayer Diagnostics, Tarrytown, NY, EUA).

2.3. Genomic DNA analysis

Genomic DNA was extracted from EDTA-anticoagulated blood by a salting-out procedure optimized in our laboratory [28]. ABCB1 (G2677T/A, C3435T) and ABCC1 (G2012T) polymorphisms were detected by fragment analysis after amplification by polymerase chain reaction-restriction (PCR).

ABCB1 genotyping was carried out as described previously [29]. For ABCC1 variant analysis, we designed the forward and reverse primers 5′-TGAACTCAGCAGTAGAAATGGAAGGAATGT-3′ and 5′-GCCCACCACGGCCACCAAAGA-3', respectively. Each 50 μL PCR reaction contained 50 ng DNA, 200 nM primers (Integrated DNA Technologies, Coralville, EUA) and 200 μM DNTPs (Amersham Biosciences, Piscataway, NJ, EUA), 0.5 U DNA polymerase and PCR buffer (50 mM KCl, 20 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 75 mM Tris–HCl, pH 9.0) (Biotools B&M Labs, S.A., Madrid, Spain). The thermal cycling protocol consisted of initial cycle at 98 °C for 3 min followed by 36 cycles at 94 °C for 1 min, 68 °C for 2 min and 72 °C for 2 min, and final extension at 72 °C for 10 min. Amplification was carried out in a thermal cycler, PTC-200 (MJ Research Inc., Walthan, MA, USA).

ABCC1 G2012T PCR products were digested with RsaI endonuclease (1 U) at 37 °C for 1 h (New England Biolabs Inc., MA, USA). Restriction fragments were identified by 8% polyacrylamide gel electrophoresis after silver staining.

2.4. mRNA expression analysis by real-time PCR

Peripheral blood mononuclear cells were isolated from EDTA-anticoagulated blood using Histopaque® (Sigma Co., St. Louis, MO, USA) and total RNA was extracted using Trizol® reagent (Invitrogen, Carlsbad, CA, USA) as previously described [30]. RNA was dissolved in DEPC-treated water and the concentration was measured by UV spectrophotometry. cDNA was produced from 1 μg of total RNA using 200 ng random hexamers (Invitrogen), 200 μM dNTP (each) (Amersham Biosciences, Piscataway, NJ) and 200 U SuperScript II RT transcriptase (Invitrogen) according to the manufacturer's protocol.

ABCB1 and ABCC1 mRNA were measured by TaqMan quantitative PCR assay, using glyceraldehyde-3-phosphate dehydrogenase gene (GAPD) as endogenous reference (Human GAPD Endogenous control, Applied Biosystems). The real-time PCR assays were carried out in 96-well plates using a 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The thermal cycling protocol consisted of 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min. The primers and probe sequences used for ABCB1 mRNA detection were described elsewhere [30]. For ABCC1 mRNA quantification, the sequences were as follows: forward primer 5′-TCCGCTTCAAGATCACCATCATC-3′, reverse primer 5′-AACCTGGACCCATTCAGCC-3′ and probe 5′-FAM-CCCCAGGACCCTGTTTT-MGB-NFQ-3′ (Applied Biosystems, Foster City, CA, USA).

2.5. Statistical analysis

Individuals carrying the rare allele for ABCB1 C3435T (CT and TT genotypes) or G2677T/A (GT, GA, TT, TA genotypes) were combined. ABCB1 haplotypes were formed by grouping C3435T and G2677T/A genotypes: T/T (GT/CT, GT/TT, TT/CT, TT/TT, TA/CT) and non-T/T (GG/CC, GG/CT, GG/TT, GT/CC, GA/CC) haplotypes as reported previously [28]. The agreement of genotypes frequencies with Hardy–Weinberg equilibrium expectations was tested by χ^2 test. Relationships between the genotypes or haplotypes and categorical variables were evaluated by the χ^2 test or Fisher Exact test.

Continuous variables are presented as mean ± S.D. (or S.E.M.) and those without normal distribution were log transformed ($\log_{10}$). Numerical variables were compared by *t*-test (two variables) and One-way ANOVA (three or more variables) and Tukey's test was used for multiple comparisons. Spearman's correlation coefficients (R_s) were used to estimate the association between numerical variables. Logistic regression analysis was used to evaluate the relationships between reduction of serum LDL cholesterol and other variables after treatment with atorvastatin. Statistical tests were performed using the SAS System for Windows version 8.02 (SAS Institute Inc., 1999–2001, Cary, NC, USA). Significance was considered $p < 0.05$.

Table 2 – Univariate logistic regression analysis of the variables associated with atorvastatin-induced LDL cholesterol reduction.

Variables	*p* value	Odds ratio	95% Confidence interval
Age	0.067	1.03	0.99–1.07
Gender (women)	0.188	1.81	0.75–4.40
Ethincs (African)	0.947	0.97	0.43–2.22
Hypertension	0.508	1.31	0.59–2.88
Obesity	0.676	1.20	0.52–2.76
No cigarette smoking	0.051	4.58	0.99–21.11
Alcohol consumption	0.334	0.64	0.26–1.57
Physical activity	0.368	1.43	0.66–3.13
Family history of CAD	0.671	1.18	0.54–2.59
ABCB1 3435C allele	0.865	1.09	0.42–2.82
3435T allele	0.968	0.98	0.43–2.25
2677G allele	0.333	0.56	0.17–1.81
2677T allele	0.764	0.89	0.41–1.94
2677A allele	0.022	5.69	1.28–25.24
T/T haplotype	0.843	0.93	0.43–2.01
ABCC1 20212T allele	0.553	0.62	0.13–3.02

LDL cholesterol reduction was considered higher than 48% of the basal levels. CAD: coronary artery disease.

3. Results

3.1. *ABCB1 and ABCC1 polymorphisms*

Genotype and haplotype frequencies for ABCB1 and ABCC1 polymorphisms are presented in Table 1. As expected, allele frequencies of these variants were in Hardy–Weinberg equilibrium confirming the random selection of the samples. Allele frequencies of the ABCB1 G2677T/A (G: 64%, T: 33%, A: 4%) and C3435T (C: 55%, T: 45%) variants were similar to that found in other populations [31–34]. ABCC1 2012T allele frequency (4.0%) was similar to that found in Caucasian (2.8%) and Indian (1.4%) populations [15].

The variables age, BMI, ethnicity, gender, hypertension, obesity, menopause, cigarette smoking, alcohol consumption, physical activity, and use of ABCB1 substrates and inhibitors have similar frequencies between ABCB1 genotypes and haplotypes, and ABCC1 as well (Table 1). These results suggest that ABCB1 and ABCC1 variants were not associated with these variables in this sample.

Individuals carrying the rare alleles for C3435T (CT + TT genotype: 63%) and G2677T/A (non-GG genotypes: 64%) had higher frequency of family history of CAD than the 3435CC (44%, $p = 0.042$) and 2677GG (46%, $p = 0.043$) carriers. In accordance with these results, the frequency of CAD in those carrying the T/T haplotypes (67%) was higher than that found in non-T/T individuals (47%, $p = 0.021$). Therefore these ABCB1 variants seem to be related to increased risk for CAD.

ABCB1 genotypes and haplotypes were also not associated with variations in serum concentrations of lipids and apolipoproteins. Interestingly, individuals carrying the ABCC1 2012GT genotype had lower serum HDL cholesterol than the GG genotype carriers ($p = 0.015$).

3.2. *Effects of atorvastatin on serum lipids*

After atorvastatin treatment, LDL cholesterol serum concentrations varied largely from reduction of 64% to increase of 8.1%. Therefore, individuals with LDL cholesterol in the first quartile (reduction higher than 48%) were compared with

Table 3 – Relationship between ABCB1 expression in PBMC and response to atorvastatin.

Variables		ABCB1 basal expression levels				
		<0.024 (28)	0.024–0.045 (29)	0.045–0.085 (27)	>0.085 (29)	*p*
ABCB1 mRNA	Atorva	0.03 ± 0.04^{a}	$0.06 \pm 0.10^{b,d}$	$0.04 \pm 0.03^{b,c}$	$0.08 \pm 0.08^{b,d}$	<0.001
	%	$-90 \pm 235^{a,b}$	$-64 \pm 311^{a,b}$	$+37 \pm 39^{c}$	$+47 \pm 39^{c}$	<0.001
ABCC1 mRNA	Basal	0.34 ± 0.97	0.27 ± 0.30	0.61 ± 1.27	0.45 ± 0.03	0.178
	Atorva	0.16 ± 0.18	0.19 ± 0.19	0.25 ± 0.43	0.26 ± 0.38	0.781
	%	-11 ± 156	$+10 \pm 56$	0.00 ± 149	$+22 \pm 89$	0.168
TC, mg/dL	Basal	278 ± 45^{a}	$271 \pm 33^{a,b}$	$304 \pm 42^{a,c}$	278 ± 31^{a}	0.008
	Atorva	206 ± 31^{a}	$186 \pm 25^{a,b}$	$212 \pm 37^{a,b}$	195 ± 29^{a}	0.015
	%	-25.9 ± 8.2	-30.7 ± 9.6	-30.3 ± 7.9	-29.3 ± 11.5	0.159
HDL-c, mg/dL	Basal	56 ± 14	55 ± 12	58 ± 13	56 ± 16	0.866
	Atorva	54 ± 12	54 ± 11	54 ± 13	55 ± 14	0.985
	%	-3.3 ± 10.7	0.20 ± 7.06	-5.6 ± 10.9	-0.8 ± 12.7	0.084
LDL-c, mg/dL	Basal	192 ± 41^{a}	$186 \pm 29^{a,b}$	$214 \pm 41^{a,c}$	$189 \pm 28^{a,b,d}$	0.012
	Atorva	127 ± 29^{a}	$106 \pm 21^{b,c}$	$128 \pm 30^{a,c}$	112 ± 28^{a}	0.004
	%	-33.4 ± 12.4^{a}	-42.1 ± 12.3^{b}	$-39.9 \pm 8.9^{a,b,c}$	$-40.3 \pm 14.3^{b,c}$	0.023
VLDL-c, mg/dL	Basal	31 ± 14	31 ± 12	32 ± 12	32 ± 14	0.869
	Atorva	26 ± 9	26 ± 8	29 ± 12	28 ± 14	0.759
	%	-10.6 ± 27.9	-10.5 ± 27.7	-8.9 ± 25.5	-12.4 ± 26.7	0.912
TG, mg/dL	Basal	155 ± 70	154 ± 34	163 ± 61	163 ± 71	0.870
	Atorva	128 ± 45	128 ± 39	145 ± 60	140 ± 70	0.756
	%	-10.6 ± 27.9	-10.5 ± 27.7	-8.9 ± 25.5	-12.2 ± 26.5	0.919
ApoAI, mg/dL	Basal	127 ± 30	134 ± 20	135 ± 23	131 ± 27	0.534
	Atorva	134 ± 24	137 ± 24	135 ± 26	132 ± 29	0.881
	%	12.9 ± 52.7	3.02 ± 11.9	0.3 ± 9.3	2.2 ± 15.4	0.664
ApoB, mg/dL	Basal	135 ± 27	136 ± 18	149 ± 21	146 ± 20	0.051
	Atorva	107 ± 22^{a}	91 ± 16^{b}	101 ± 20	98 ± 20	0.038
	%	-17.0 ± 31.3^{a}	-32.8 ± 11.0	-31.9 ± 10.6	-32.5 ± 10.7^{b}	0.029
ApoB/ApoAI	Basal	1.07 ± 0.62	1.04 ± 0.18	1.14 ± 0.26	1.17 ± 0.31	0.555
	Atorva	0.82 ± 0.21	0.68 ± 0.15	0.77 ± 0.20	0.78 ± 0.22	0.113
	%	-22.2 ± 27.7	-34.2 ± 10.9	-31.7 ± 11.2	-32.7 ± 13.9	0.730

Number of individuals in parenthesis. ApoAI: apolipoprotein AI; ApoB: apolipoprotein B; TC: total cholesterol; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; TG: triglycerides; and VLDL-c: very low-density lipoprotein cholesterol. Values are presented as mean ± S.D. and compared by One-way ANOVA performed on the log transformed variables. Values in a row with different superscript letters are significantly different, $p < 0.05$ (Tukey's test).

those with lower response. Results from univariate logistic regression analysis showed that no smokers had high probability of increased LDL cholesterol response (OR: 4.58, 95% CI: 0.99–21.11, $p = 0.051$) (Table 2). Moreover, increased response to atorvastatin was also found in individuals carrying the ABCB1 2677A allele (OR: 5.69, 95% CI: 1.28–25.24, $p = 0.022$). Multivariate logistic regression analysis using step-wise criteria revealed only the ABCB1 2677A allele as determinant of increased LDL cholesterol response to atorvastatin (data not shown).

ABCB1 and ABCC1 mRNA basal levels were also related to serum lipids in response to atorvastatin. Due to the variation found in ABCB1 mRNA levels the values were separated in quartiles and comparisons among them for other variables are shown in Table 3. Differences in ABCB1 mRNA, total and LDL cholesterol and apoB were found after atorvastatin treatment ($p < 0.05$). Lower basal ABCB1 mRNA levels were associated with lower reduction rates for LDL cholesterol (basal < 0.024: 33.4 ± 12.4% reduction; basal > 0.085: 40.3 ± 14.3% reduction, $p < 0.05$) and apoB (basal < 0.024: 17.0 ± 31.3% reduction;

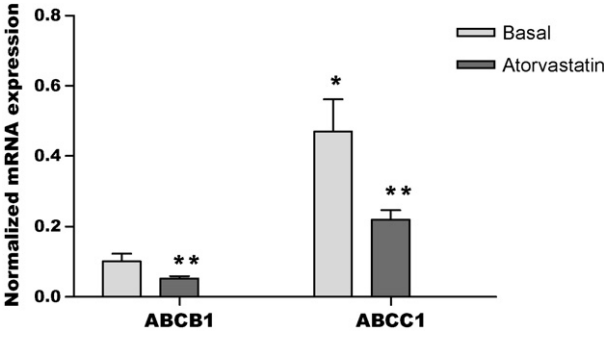

Fig. 1 – Effects of atorvastatin on ABCB1 and ABCC1 mRNA levels in peripheral blood mononuclear cells. Hypercholesterolemic individuals were treated with atorvastatin (10 mg/day/4 weeks) and mRNA expression was analyzed by real-time PCR. Values are represented as mean ± S.E.M. *$p < 0.001$ as compared to ABCB1 values and **$p < 0.05$ as compared to baseline values as indicated by *t*-test.

Table 4 – Relationship between ABCC1 expression in PBMC and response to atorvastatin.

Variables		ABCC1 basal expression levels				
		<0.088 (31)	0.088–0.202 (30)	0.202–0.419 (31)	>0.419 (31)	*p*
ABCC1 mRNA	Atorva	0.06 ± 0.13^{a}	0.17 ± 0.13^{b}	$0.20 \pm 0.12^{b,c}$	0.44 ± 0.49^{d}	<0.001
	%	-23 ± 172^{a}	$-28 \pm 114^{a,b}$	$+26 \pm 46^{a,b}$	$+46 \pm 84^{c}$	<0.001
ABCB1 mRNA	Basal	0.05 ± 0.04^{a}	$0.05 \pm 0.04^{a,b}$	0.10 ± 0.15^{a}	0.22 ± 0.44^{c}	<0.001
	Atorva	0.04 ± 0.03^{a}	0.04 ± 0.02	0.04 ± 0.03	0.09 ± 0.13^{b}	0.017
	%	-38 ± 222	-15 ± 113	$+19 \pm 51$	-29 ± 320	0.315
TC, mg/dL	Basal	292 ± 41	280 ± 44	274 ± 34	280 ± 36	0306
	Atorva	203 ± 30	205 ± 34	188 ± 36	200 ± 28	0.097
	%	-30.3 ± 7.0	-25.9 ± 12.2	-31.7 ± 8.2	-28.3 ± 9.5	0.216
HDL-c, mg/dL	Basal	54 ± 14	55 ± 12	56 ± 15	57 ± 15	0.928
	Atorva	53 ± 12	53 ± 11	54 ± 14	54 ± 14	0.989
	%	-1.0 ± 9.0	-2.0 ± 12.2	-2.2 ± 9.4	-3.5 ± 12.4	0.771
LDL-c, mg/dL	Basal	206 ± 38	194 ± 40	186 ± 30	192 ± 32	0.127
	Atorva	122 ± 25	124 ± 30	109 ± 32	117 ± 25	0.093
	%	-40.2 ± 8.4	-34.8 ± 16.4	-41.5 ± 11.7	-38.3 ± 11.4	0.347
VLDL-c, mg/dL	Basal	33 ± 13	32 ± 15	32 ± 13	32 ± 12	0.961
	Atorva	28 ± 12	28 ± 10	24 ± 9	28 ± 11	0.256
	%	-13.7 ± 19.0	-2.2 ± 32.1	-22.4 ± 21.3	-7.9 ± 29.1	0.109
TG, mg/dL	Basal	163 ± 63	160 ± 74	162 ± 65	159 ± 74	0.962
	Atorva	139 ± 62	142 ± 49	120 ± 43	140 ± 55	0.268
	%	-13.7 ± 19.0	-2.2 ± 32.1	-22.2 ± 21.3	-7.9 ± 29.1	0.119
ApoAI, mg/dL	Basal	125 ± 19	134 ± 21	128 ± 33	131 ± 27	0.559
	Atorva	128 ± 17	132 ± 24	136 ± 31	136 ± 30	0.820
	%	3.7 ± 10.1	-1.2 ± 11.5	12.2 ± 48.7	3.78 ± 13.9	0.165
ApoB, mg/dL	Basal	139 ± 24	141 ± 21	140 ± 20	143 ± 26	0.960
	Atorva	99 ± 16	101 ± 22	95 ± 20	103 ± 21	0.371
	%	-27.8 ± 12.2	-28.1 ± 15.2	-32.0 ± 11.4	-24.9 ± 29.6	0.471
ApoB/ApoAI	Basal	1.14 ± 0.26	1.09 ± 0.26	1.19 ± 0.54	1.13 ± 0.30	0.883
	Atorva	0.79 ± 0.18	0.79 ± 0.21	0.73 ± 0.20	0.80 ± 0.22	0.470
	%	-29.7 ± 15.3	-27.0 ± 13.1	-35.9 ± 13.3	-27.3 ± 24.3	0.087

Number of individuals in parenthesis. ApoAI: apolipoprotein AI; ApoB: apolipoprotein B; TC: total cholesterol; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; TG: triglycerides; and VLDL-c: very low-density lipoprotein cholesterol. Values are presented as mean ± S.D. and compared by One-way ANOVA performed on the log transformed variables. Values in a row with different superscript letters are significantly different, $p < 0.05$ (Tukey's test).

basal > 0.085: 32.5 ± 10.7% reduction, $p < 0.05$) concentrations induced by atorvastatin.

3.3. *Effects of atorvastatin on mRNA expression*

Baseline levels of ABCC1 transcripts in PBMC were four times higher than that of ABCB1 ($p < 0.001$) (Fig. 1). After atorvastatin treatment, both genes showed 50% reduction of mRNA expression ($p < 0.05$), but the differences between ABCB1 and ABCC1 remain. Weak correlations were found between ABCB1 and ABCC1 mRNA levels in PBMC before ($R_s = 0.294$, $p < 0.002$) and after treatment with atorvastatin ($R_s = 0.266$, $p = 0.005$). In spite of these interesting results, variations in gene expression were not correlated with differences in serum lipids or apolipoproteins after treatment with atorvastatin (Low R_s, $p > 0.05$, data not shown).

We also investigated whether the ABCB1 and ABCC1 mRNA basal levels were related to gene expression in response to atorvastatin. Individuals with the lowest basal ABCB1 mRNA had higher reduction in gene expression than whose with the highest basal levels (basal < 0.024: 90 ± 235% reduction vs basal > 0.085: 53 ± 39% increase, $p < 0.05$) (Table 3).

After atorvastatin treatment, variations in ABCC1 mRNA levels also correlated negatively with the basal expression (basal < 0.088: 23 ± 72% reduction vs basal > 0.419: 46 ± 84% increase, $p < 0.05$) (Table 4). Baseline levels of ABCC1 transcripts were also associated with differences in ABCB1 mRNA expression before ($p < 0.001$) and after ($p = 0.017$) treatment with atorvastatin.

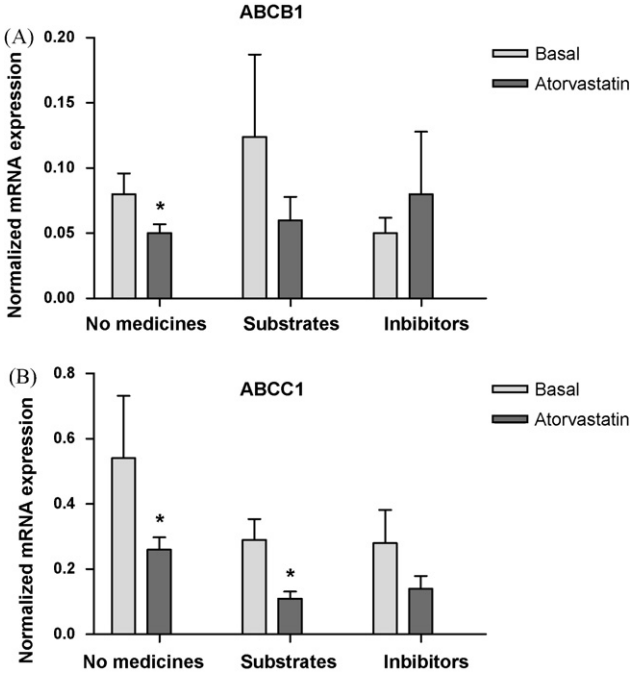

Fig. 2 – Effects of atorvastatin on ABCB1 (A) and ABCC1 (B) mRNA levels in peripheral blood mononuclear cells. Hypercholesterolemic individuals were treated with atorvastatin (10 mg/day/4 weeks) alone ($n = 85$) or in combination with concomitant ABCB1 substrates ($n = 14$) or inhibitors ($n = 11$). Values of mRNA expression measured by real-time PCR are represented as mean ± S.E.M. $^*p < 0.05$ as compared to baseline values as indicated by t-test.

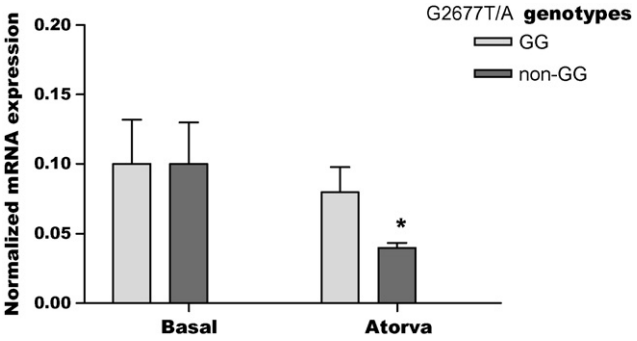

Fig. 3 – Effects of ABCB1 G2677T/A polymorphism on gene expression in peripheral blood mononuclear cells. Hypercholesterolemic individuals were treated with atorvastatin (10 mg/day/4 weeks) and mRNA expression was analyzed by real-time PCR. Values are represented as mean ± S.E.M. of the GG genotype ($n = 52$) and non-GG (GA + GT + TA + TT) genotypes ($n = 84$) carriers. $^*p = 0.039$ as compared to GG genotype values as indicated by t-test.

As shown in Table 1, 15% of HC patients were medicated with concomitant ABCB1 substrates (antiarrhythmics, beta-blockers, diuretics, ACE inhibitors, and others) and 14% with concomitant ABCB1 inhibitors (antiarrhythmics, calcium antagonists, calcium channel blockers, antidepressants and others). Baseline transcripts levels of ABCB1 and ABCC1 in PBMC were similar among individuals taking ABCB1 substrates and inhibitors (Fig. 2). On the other hand, ABCB1 substrates reversed the inhibitory effect of the atorvastatin effects on ABCB1 expression, while ABCB1 inhibitors reversed this effect on both ABCB1 and ABCC1 expression (Fig. 2). Exclusion of data from individuals taking concomitant medicines did not modify significantly the previous results of this study.

3.4. *ABCB1 and ABCC1 polymorphisms and gene expression*

Analysis of the relationship between polymorphisms and mRNA expression showed that individuals carrying ABCB1 2677 non-GG genotypes had 50% reduction of ABCB1 transcripts in response to atorvastatin when compared to the GG genotype carriers ($p = 0.039$) (Fig. 3).

4. Discussion

In this study, baseline ABCC1 mRNA expression in PBMC of hypercholesterolemic individuals was higher than that of the ABCB1. Similar differences were found in PBMC samples from patients with liver and gastrointestinal neoplasms [35] and in PBMC used to evaluate the effects of liponavir and other antivirals in vitro [36]. On the other hand, ABCB1 expression level was shown to be the predominant form among three major multidrug-resistant efflux pumps in lymphocytes of healthy volunteers [37].

The effects of atorvastatin on mRNA expression were dependent on the basal levels of both ABCB1 and ABCC1 genes. Results from correlation analysis are suggestive that ABCB1 and ABCC1 genes are coordinately down-regulated by atorvastatin in PBMC. It has been shown that basal expression of both ABCB1 and ABCC1 genes was positively correlated with expression of the pregnane X receptor (PXR), a key regulator in drug metabolism and efflux, in PBMCs [35]. PXR is a nuclear receptor that belongs to a family of transcription factors that function as heterodimers to regulate target promoters [38,39]. ABCB1 promoter has also been shown to be regulated by the interaction of the farnesoid receptor (FXR) and the steroid-activated receptor (SXR) heterodimer [39,40].

Other transcription factors have also been implicated in modulation of ABCB1 and ABCC1 transcription, such as Sp1. The Sp1 belongs to a family of transcription factors that interacts with GC rich element in the promoter region regulating the constitutive expression of several drug transporters [41]. Sp1 seems to interact with the promoter region of both ABCB1 and ABCC1 genes [42,43] and may explain the coordinated variation of mRNA levels in response to atorvastatin found in this study. It has been suggested that ABCB1 expression in leukemic cells is also regulated by mRNA stabilization and translational initiation [44]. However, the involvement of these mechanisms on ABCB1 expression in PBMC remains to be elucidated.

The effect of atorvastatin on ABCC1 mRNA levels in PBMC remains to be elucidated. It is possible mediated by PPARalpha that has been shown to down-regulate ABCC1 expression in mouse small intestine [45]. Atorvastatin-induced changes in hepatic HNF-4 and PPARalpha may be responsible for the improvement of the lipid metabolic phenotype produced by atorvastatin administration to senescent male rats [46].

In this study, low LDL-c and apoB reduction was found in HC individuals with lower ABCB1 baseline levels. This result is suggestive that the inhibitory effect of the atorvastatin is more effective in the ABCB1 high-expressors than in low-expressors. Even though it has been reported that high plasma LDL cholesterol concentration does not correlate with P-gp activity in PMBC [47], we suggest that ABCB1 basal expression in hypercholesterolemic tate may be an important marker to predict the lowering-cholesterol response to atorvastatin.

Expression of the ABCB1 was shown to be reduced in PBMCs of hypertensive Wistar Kyoto rats [48]. Therefore, differences in blood pressure may influence atorvastatin-induced ABCB1 down regulation in PBMC of the HC individuals. However, we did not find differences in ABCB1 or ABCC1 mRNA expression between hypertensive and non-hypertensive HC individuals before and after atorvastatin treatment.

Concomitant use of ABCB1 substrates and inhibitors did not effect the baseline expression of the ABCB1 and ABCC1 in PBMC. However, the down regulation of the mRNA expression induced by atorvastatin was not found in those individuals taking ABCB1 inhibitors. These results are suggestive that the interaction between atorvastatin and ABCB1 inhibitors affects transporter expression in PBMC and it may be modify the lowering-cholesterol response. However, the serum lipid profile after atorvastatin treatment was not altered by concomitant medicines in this sample.

Baseline ABCB1 mRNA expression in PBMC was not related with ABCB1 polymorphisms as it was demonstrated previously in patients with liver and gastrointestinal tumors [35] and in hypercholesterolemic individuals [30]. Interestingly, the down regulation of the ABCB1 mRNA induced by atorvastatin seems to be dependent on G2677T/A polymorphism suggesting that this SNP plays an important role in regulating ABCB1 expression. On the other hand, reduction of ABCB1 mRNA levels in PBMC in response to lipopolysaccharide (LPS)-induced experimental acute inflammation was found to be associated with ABCB1 C3435T SNP [49].

Genetic factors as contributors to patient's intervariability in lipid-lowering response to statins have been investigated in aspects of pharmacokinetics (e.g. ABC and SLC transporters, CYPs enzymes, UGT enzymes), pharmacodynamics (e.g. HMGCR, LDLR, CETP, APOA1, APOE, etc.) and disease-related genes (e.g. NOS3, ESR1, LOX-1) [reviewed in [20]]. With respect to ABCB1 and ABCC1 genes, the association found between polymorphisms in ABCB1 and ABCC1 and differences in serum LDL cholesterol and HDL cholesterol, respectively, indicates their potential role in the pharmacogenetics of the atorvastatin. It has been shown that ABCB1 is also involved in the regulation of cholesterol trafficking in cells mediating actively cholesterol redistribution in the cell membrane [50].

In conclusion, ABCB1 and ABCC1 mRNA levels in PBMC are modulated by atorvastatin and ABCB1 G2677T/A/C polymorphism and ABCB1 baseline expression is related to differences in serum LDL cholesterol and apoB in response to atorvastatin.

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FURTHER READING

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