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Gene Expression Changes in Peripheral Blood Mononuclear Cells from Cynomolgus Monkeys Following Astemizole Exposure

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Abstract

Surrogate tissue analysis incorporating -omics technologies has emerged as a potential alternative method for evaluating toxic effect of the tissues which are not accessible for sampling. Among the recent applications, blood including whole blood, peripheral blood lymphocytes and peripheral blood mononuclear cells (PBMCs) was suggested as a suitable surrogate tissue in determining toxicant exposure and effect at the pre- or early clinical stage. In this application, we investigated transcriptomic profiles in astemizole treated *Cynomolgus* monkey's PBMCs. PBMCs were isolated from 4-6 years old male monkeys at 24 hr after administration45 Helvetica Light (10 mg/kg, 30 mg/kg). Gene expression profiles of astemizole treated monkey's PBMCs were determined using Affymetrix GeneChip® Human Genome U133 plus 2.0 arrays. The expression levels of 724 probe sets were significantly altered in PBMCs at 10 or 30 mg/kg after astemizole administration following determination of paired *t*-test using statistical criteria of ≥1.5-fold changes at $P<0.05$. Gene expression patterns in PBMCs showed a considerable difference between astemizole 10 and 30 mg/kg administration groups in spite of an administration of the same chemical. However, close examination using Ingenuity Pathway Analysis (IPA) software revealed that several gene sets related to cardiotoxicity were deregulated at astemizole 10 and 30 mg/kg administration groups. The deregulation of cardiac hypertrophy related genes such as TXN, GNAQ, and MAP3K5 was observed at 10 mg/kg group. In astemizole 30 mg/kg group, genes involved in cardiotoxicity including cardiac necrosis/cell death, dilation, fibrosis, and hypertrophy were also identified. These results suggest that toxicogenomic approach using PBMCs as surrogate tissues will contribute to assess toxicant exposures and identify biomarkers at the pre-clinical stage.

Keywords: Surrogate, Astemizole, Monkey, Cardiotoxicity, Peripheral blood monocyte cell

Toxicogenomics is one of the postgenomic approaches which use primarily genomic technique to elucidate mechanisms of toxicant action by studying the genome-wide effects of xenobiotics. The changes in gene expression associated with toxicity are often more sensitive and occur before clinical manifestation of toxicity. Therefore, gene expression profiling would provide a window of opportunity for preclinical diagnosis of possible toxic endpoints[1]. Recent studies have demonstrated that gene expression profiling can offer the potential to classify toxicant exposures[2-5], predict clinical outcome of such exposures[6], and provide mechanistic data useful for risk assessments[7]. However, the determination or prediction of possible toxic endpoints using gene expression profiling is largely limited to the use of accessible biospecimens. Surrogate tissue analysis (STA) has been proposed as one possible solution to offer a convenient biomonitoring method providing insight into the effects of toxicants on target tissues, since gene expression changes in surrogate tissues often reflect those in inaccessible target tissues[8]. A number of tissues have been applied to toxicogenomic study to investigate the potential utility of STA. Currently, blood has been considered as the most practical tissue for STA because it can be easily and routinely obtained from human and experimental animals and contains live and nucleated cells providing high-quality of RNA for gene expression profiling. Recent

studies have demonstrated that gene expression profiling of peripheral blood cells are significantly different, depending on varying environmental, physiological or pathological insults or perturbations. Indeed, it is reported that more than 80% of transcriptome in peripheral blood cells share with each of that in brain, colon, heart, kidney, liver, lung, prostate, spleen and stomach[9].

Drug-inducible cardiotoxicity is a serious safety issue in the pharmaceutical industry since it is one of major reasons of attrition of compounds in preclinical and clinical development. Drug-induced polymorphic ventricular tachyarrhythmia, known as torsades de pointes (TdP), is a rare but potentially life threatening arrhythmia. TdP has been associated with delayed cardiac repolarization, as manifested by a prolongation of the QT interval on the electrocardiogram and thus QT prolongation is generally considered as a surrogate marker for proarrhythmia. However, all the drugs that induce QT prolongation are not always induce arrhythmia in clinical use[10]. Therefore, the definite correlation between the drug-induced prolongation of ventricular repolarization and the risk of proarrhythmia is not fully understood[11]. For this reason, there has been needed more crucial screening assays for assessing arrhythmogenicity.

In this study, we applied toxicogenomics technology using blood samples to assess drug-inducible cardiotoxicity in monkey model system. Cardiotoxicity in cynomolgus monkeys was induced by astemizole, which a well-known second-generation H_1-receptor blocker inducing QT prolongation and torsade des pointes[12,13]. Here, we demonstrate for the first time a comprehensive gene expression and discuss our results in the context of gene expression alteration as elicited by astemizole in peripheral blood mononuclear cells (PBMCs) of cynomolgus monkey.

Microarray Analysis

The gene expression profiles in peripheral blood mononuclear cells of cynomolgus monkeys were made up at two dosage points using Affymetrix GeneChip® Human genome U133 plus 2.0 array. To investigate whether gene expression profile reflect the cardiotoxic effects of astemizole, two RNA samples from PBMCs in each group were analyzed for the expression of whole genes oligonucleotide array. Gene expression data were evaluated first for quantitative changes between control and treated groups and further evaluated for statistical significance using paired *t*-test according to the statistical criteria of $\geq$ 1.5-fold changes at $P < 0.05$. To identify gene expression patterns both 10 mg/kg and 30 mg/kg group, significantly changed 724 probe sets were analyzed

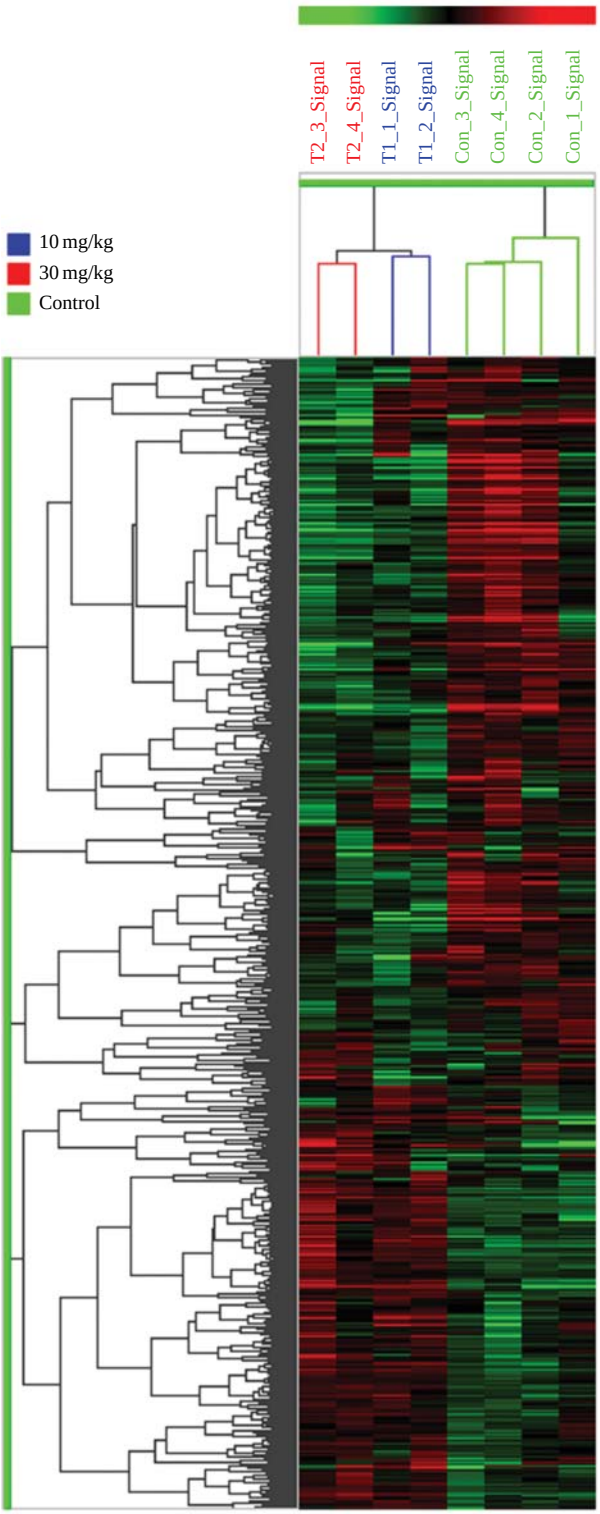

Figure 1. Hierarchical clustering of genes with statistically significant expression changes by astemizole 10 and 30 mg/kg administration. The heat map color depicts the gene expression level from low (green) to high (red). Each row represents the selected genes and each single column represents individual sample.

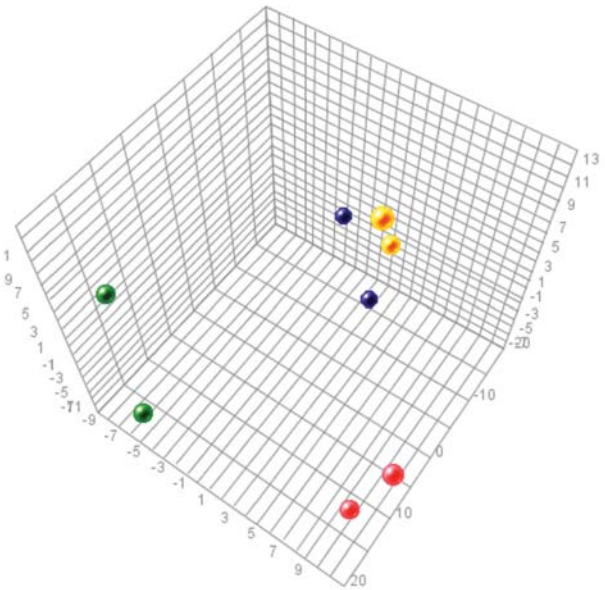

Figure 2. Principal Components Analysis using significantly altered 724 probe sets. The colored circles represent tested groups as follows: 10 mg/kg control, yellow; 30 mg/kg control, blue; 10 mg/kg astemizole administration, red; 30 mg/kg astemizole administration, green.

by unsupervised two-dimensional (2-D) hierarchical clustering with Pearson's correlation. Two dosage groups showed considerable difference in gene expression profile (Figure 1). Moreover, there were a few of probe sets which were deregulated at both dosage points. The discrepancy between two dosage points was also shown at the result of principle components analysis (Figure 2).

Differentially Expressed Genes in PBMCs of Astemizole Treated Monkey

Using the criteria above on the PBMCs data at the dosage of astemizole 10 mg/kg administration group, the expression of 254 probe sets was found to be significantly deregulated compared to control group. The expression level of 124 probe sets was increased and that of 130 probe sets was decreased. At the dosage of astemizole 30 mg/kg, the expression of 490 probe sets, 169 probe sets were increased and 321 probe sets were decreased, was significantly deregulated. Table 1 represents the top 20 up- and down-regulated genes at each dosage point. In astemizole 10 mg/kg group, genes involved in morphology and differentiation (Vsx1 and BMP5), regulation of transcription (ZNF587, PAF1, and RPS11), transport (ATP 11A and SLC4A4), response of stimulus (213642_at and SCD5), and microtubule activity (KNS2 and DN AH3) were up-regulated. On the other hand, genes involved in regulation of transcription (ZNF311), transport (CHRNE, SLC35E3, PRRG4, and RAB3D), metabolism (PNPLA8, MGC26963, B4GALT1, and GDPD3), and glycolysis (ENO3) were down-regulated. In the case of astemizole 30 mg/kg group, genes involved in regulation of transcription (RUNX1), metabolism (KIAA1161), regulation of cell cycle (214019_at), cytoskeleton/cell motility (FRMD6 and CALD1), response to stimulus (FLJ13236), and repair (RAD50) were up-regulated, but genes related to metabolism (CDA, ACSL1, and CYP3A7), inflammation (ORM1, FCAR, S100AB, and PTX3), transport (LTF, AQP9, and SLC25A37), and cytoskeleton/cell adhesion (TMOD3, LIMK2, and CSPG2) were down-regulated.

Toxicological Classification of Differentially Expressed Genes

The differentially expressed genes that might be relevant to toxicity of astemizole were investigated using the Ingenuity pathway analysis (IPA) software. An analysis of 724 significantly deregulated genes by IPA revealed that several genes involved in cardiotoxicity and hepatotoxicity were altered in PBMCs cell. Among genes classified with toxicological criteria, genes related to cardiotoxicity were shown in Table 2. In astemizole 10 mg/kg group, genes involved in cardiac hypertrophy (GNAQ, MAP3K2, MAP3K5, and TXN) were differentially expressed, whereas genes involved in cardiac necrosis or cell death (ADM, NRG1, SOCS3, and TLR4), cardiac dialation (SOD2 and SPP1), cardiac fibrosis/transformation (ACSL1 and VEGFA), and hypertrophy (HIF1A) were differentially expressed at 30 mg/kg group.

Discussion

The results of this investigation showed that the gene expression profile following astemizole administration was significantly altered in PBMCs of cynomolgus monkeys. This investigation is the first toxicological evaluation through gene expression profiles in PBMCs of cynomolgus monkeys following cardiotoxicant administration.

To date, there have been several reports that gene expression in peripheral blood cells was altered significantly following toxicant exposure. The discovery of radiation-response biomarker candidates through *ex vivo* study using irradiated human white blood cells was successful, and those candidates were proved to be useful biomarkers for radiation exposure in human[14]. Additional investigations on peripheral blood cell gene expression profiling of environmental

Table 1. List of top 20 probe sets up- and down-regulated by 10 and 30 mg/kg of astemizole administration.

Probe ID	Gene symbol	Gene description	Fold change
		10 mg/kg of astemizole administration	
Up-regulated			
222972_at	VSX1	visual system homeobox 1 homolog, CHX10-like (zebrafish)	10.23
1558787_a_at	no information	CDNA FLJ43445 fis, clone OCBBF2031366	8.23
213642_at	no information	no information	4.62
233164_x_at	RHBDD1	rhomboid domain containing 1	4.21
239311_at	DHX57	DEAH (Asp-Glu-Ala-Asp/His) box polypeptide 57	4.08
1555373_at	C21orf114	chromosome 21 open reading frame 114	4.07
240526_at	ATP11A	ATPase, Class VI, type 11A	4.03
242862_x_at	LOC643749	hypothetical LOC643749	3.95
1560659_at	KIAA1641	KIAA1641	3.52
221963_x_at	ZNF587	zinc finger protein 587	3.49
233056_x_at	DLGAP4	discs, large (Drosophila) homolog-associated protein 4	3.36
1556834_at	no information	CDNA clone IMAGE:5296106	3.34
202093_s_at	PAF1	Paf1, RNA polymerase II associated factor, homolog (S. cerevisiae)	3.31
205431_s_at	BMP5	bone morphogenetic protein 5	3.17
220232_at	SCD5	stearoyl-CoA desaturase 5	3.16
210739_x_at	SLC4A4	solute carrier family 4, sodium bicarbonate cotransporter, member 4	2.93
232814_x_at	KNS2	kinesin 2	2.84
239845_at	no information	Transcribed locus	2.76
213350_at	RPS11	ribosomal protein S11	2.69
220725_x_at	DNAH3	dynein, axonemal, heavy chain 3	2.65
Down-regulated			
236551_at	ZNF311	zinc finger protein 311	−9.56
215916_at	CHRNE	cholinergic receptor, nicotinic, epsilon	−8.46
235568_at	C19orf59	chromosome 19 open reading frame 59	−6.12
223982_s_at	PNPLA8	patatin-like phospholipase domain containing 8	−3.41
218988_at	SLC35E3	solute carrier family 35, member E3	−3.01
238513_at	PRRG4	proline rich Gla (G-carboxyglutamic acid) 4 (transmembrane)	−2.91
226747_at	KIAA1344	KIAA1344	−2.88
228799_at	TMEM22	transmembrane protein 22	−2.75
224861_at	GNAQ	guanine nucleotide binding protein (G protein), q polypeptide	−2.75
227038_at	MGC26963	hypothetical protein MGC26963	−2.72
225001_at	RAB3D	RAB3D, member RAS oncogene family	−2.66
216627_s_at	B4GALT1	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 1	−2.54
204483_at	ENO3	enolase 3 (beta, muscle)	−2.51
219732_at	RP11-35N6.1	plasticity related gene 3	−2.51
1556185_a_at	no information	CDNA clone IMAGE:5260162	−2.48
222496_s_at	FLJ20273	RNA-binding protein	−2.48
222692_s_at	FNDC3B	fibronectin type III domain containing 3B	−2.43
223131_s_at	TRIM8	tripartite motif-containing 8	−2.39
219722_s_at	GDPD3	glycerophosphodiester phosphodiesterase domain containing 3	−2.34
216650_at	no information	no information	−2.33
		30 mg/kg of astemizole administration	
Up-regulated			
217263_x_at	RUNX1	runt-related transcription factor 1 (acute myeloid leukemia 1; aml1 oncogene)	11.78
233204_at	FLJ11903	similar to hypothetical protein MGC40405	4.91
234753_x_at	no information	no information	3.91
232244_at	KIAA1161	KIAA1161	3.85
1566658_at	RP11-151A6.2	hypothetical protein BC004360	3.66
214019_at	no information	no information	3.59
219392_x_at	PRR11	proline rich 11	3.40
225464_at	FRMD6	FERM domain containing 6	3.37
1553654_at	SYT14; CHR415SYT	synaptotagmin XIV; chr415 synaptotagmin	3.13
223836_at	KSP37	Ksp37 protein	3.08
206323_x_at	OPHN1	oligophrenin 1	3.06
215467_x_at	LOC647070	hypothetical LOC647070	3.04

Table 1. Continued.

Probe ID	Gene symbol	Gene description	Fold change
210739_x_at	SLC4A4	solute carrier family 4, sodium bicarbonate cotransporter, member 4	2.97
241816_at	C14orf106	chromosome 14 open reading frame 106	2.96
244193_at	FLJ13236	hypothetical protein FLJ13236	2.87
209813_x_at	TARP; TRGC2	TCR gamma alternate reading frame protein; T cell receptor gamma constant 2	2.80
230405_at	RAD50	RAD50 homolog (S. cerevisiae)	2.70
205525_at	CALD1	caldesmon 1	2.69
1570257_x_at	no information	Homo sapiens, clone IMAGE:4780252, mRNA	2.67
217052_x_at	no information	no information	2.63
Down-regulated			
205627_at	CDA	cytidine deaminase	−57.56
205040_at	ORM1	orosomucoid 1	−47.69
202018_s_at	LTF	lactotransferrin	−27.59
244703_x_at	no information	MRNA; cDNA DKFZp686P1617 (from clone DKFZp686P1617)	−22.87
205568_at	AQP9	aquaporin 9	−20.97
221920_s_at	SLC25A37	solute carrier family 25, member 37	−19.24
226018_at	C7orf41	chromosome 7 open reading frame 41	−18.95
220800_s_at	TMOD3	tropomodulin 3 (ubiquitous)	−17.45
206683_at	ZNF165	zinc finger protein 165	−13.53
211307_s_at	FCAR	Fc fragment of IgA, receptor for	−12.59
207275_s_at	ACSL1	acyl-CoA synthetase long-chain family member 1	−12.05
211843_x_at	CYP3A7	cytochrome P450, family 3, subfamily A, polypeptide 7	−11.09
217475_s_at	LIMK2	LIM domain kinase 2	−10.83
202917_s_at	S100A8	S100 calcium binding protein A8	−10.58
228950_s_at	GPR177	G protein-coupled receptor 177	−10.19
206157_at	PTX3	pentraxin-related gene, rapidly induced by IL-1 beta	−10.07
212158_at	SDC2	syndecan 2 (heparan sulfate proteoglycan 1, cell surface-associated, fibroglycan)	−9.76
231274_s_at	no information	Transcribed locus	−9.69
227697_at	SOCS3	suppressor of cytokine signaling 3	−8.95
204619_s_at	CSPG2	chondroitin sulfate proteoglycan 2 (versican)	−8.83

Statistical significance of represented genes in the table was assigned at $P < 0.05$ (paired *t*-test).

toxicants including benzene, arsenic and heavy metals provide informative findings for potential mechanistic pathway information or biomarkers for toxicants exposure[15-17]. In addition to toxicology field, the evaluation of blood cell gene expression profiling based on microarray technology have been applied to assess human disease, healthy status, environmental and behavioral factors, cancers, neurologic disease, cardiovascular disease and autoimmune diseases[18]. In these applications, the preparation of qualified RNA samples from blood cells without *ex vivo* change has been issued. Several studies shown that whole blood RNA including high proportion hemoglobin RNA has low detection sensibility with high noise compared with fractionated blood samples such as PBMCs[19]. By contrast, the extra isolation procedures for fractionated blood cell involve a time delay, which might cause in *ex vivo* changes in gene expression profiles[19]. In this study, we isolated RNA from fractionated PBMCs cell and performed following microarray procedures because we could afford to collect and fractionate blood samples without a time delay.

The gene expression profile in PBMC following astemizole administration showed considerable difference between two dosage points. The numbers of differentially expressed probe sets at astemizole 10 and 30 mg/kg administration group were 254 and 490, respectively. Although a number of genes were significantly altered following astemizole administration, only nine of common probe sets were deregulated at both dosage points. The results of 2-D hierarchical clustering and PCA also showed a significant discrepancy between two dosage points (Figures 1, 2). These results suggest that different gene expression data in PBMC can provide valuable information to predict exposure level of known and unknown toxicants. Indeed, recent study reveals that blood gene expression data can provide signatures that are useful predictors to classify of exposure level to toxic doses of acetoaminophen[20].

Table 2. List of cardiotoxicity related genes in PBMC of cynomolgus monkey following astemizole administration.

Probe ID	Gene symbol	Gene title	Fold change
		10 mg/kg of astemizole administration group	
Cardiac hypertrophy			
224861_at	GNAQ	guanine nucleotide binding protein (G protein), q polypeptide	−2.75
221695_s_at	MAP3K2	mitogen-activated protein kinase kinase kinase 2	1.53
203837_at	MAP3K5	mitogen-activated protein kinase kinase kinase 5	−2.20
208864_s_at	TXN	thioredoxin	−2.07
		30 mg/kg of astemizole administration group	
Cardiac necrosis/cell death			
202912_at	ADM	adrenomedullin	−2.92
208230_s_at	NRG1	neuregulin 1	−2.79
227697_at	SOCS3	suppressor of cytokine signaling 3	−8.95
1552798_a_at	TLR4	toll-like receptor 4	−4.78
Cardiac dilation			
215223_s_at	SOD2	superoxide dismutase 2, mitochondrial	−3.35
209875_s_at	SPP1	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	2.35
Cardiac fibrosis/transformation			
207275_s_at	ACSL1	acyl-CoA synthetase long-chain family member 1	−12.05
210512_s_at	VEGFA	vascular endothelial growth factor A	−3.54
Cardiac hypertrophy			
200989_at	HIF1A	hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	−2.32

Statistical significance of represented genes in the table was assigned at $P < 0.05$ (paired *t*-test).

Astemizole, the first compound which caused ventricular tachyarrhythmias (*torsade de pointes*) among the antihistamines, was withdrawn from the market worldwide in 1988[12]. It has known that antihistamines block the delayed rectifier potassium channel (IKr) of heart and thus cause QT-prolongation and cardiac- and ventricular arrhythmias[21]. Our investigation using IPA software revealed that several genes associated with cardiotoxicity were significantly altered in PBMCs (Table 2). In astemizole 10 mg/kg group, the identified genes which might be related cardiotoxicity at astemizole 10 mg/kg group, TXN, GNAQ, and MAP3K5, were involved in cardiac hypertrophy and shown decrease in their expression level. TXN is an essential component to regulate oxidative stress in the mouse heart, and its activity plays an important role in stimulation of cardiac hypertrophy in vivo[22]. GNAQ has been known to participate in initiation of cardiac hypertrophy also. The investigations using cardiomyocyte-specific transgenic and knock-out mouse models revealed that gnaq-mediated signal pathway was essential for cardiac hypertrophy under pressure overload[23,24]. MAP3K5 has been reported playing a pivotal role in the impairment of vascular endothelial function and cardiac hypertrophy[25]. In astemizole 30 mg/kg group, the identified cardiotoxicity genes were involved in cardiac necrosis/cell death (ADM, NRG1, SOCS3, TLR4), dilation (SOD2, SPP1), fibrosis (ACSL1, SPP1), and hypertrophy (HIF1A). In previous reports, ADM and NRG1 have known as antiapoptotic factors against xenobiotic-induced cardiac myocyte apoptosis. In cultured rat cardiac myocytes, ADM has shown that a protective role against doxorubicin-induced apoptosis by inhibiting caspase-3 activation via a cAMP-dependent pathway[26]. NRG1 has also a prosurvival effect on cardiac mytocytes via inhibition of xenobiocs and oxidative stress induced myocyte apoptosis via PI3-kinase/Akt pathways[27,28]. SOCS3 has known as a mechanical stress-inducible gene in cardiac muscle cells, and which suppresses cytokine-induced cardiac myocyte hypertrophy and antiapoptosis[29].

In summary, our investigation showed that astemizole cardiotoxicity was associated with gene expression change in PBMC of cynomolgus monkey and considerable difference in gene expression patterns according to dosage points in spite of an administration of the same chemical. These results suggest the possibility that gene expression profiling in PBMC can be applied to assess toxicant exposures or to differentiate the host response to different dosage of toxicant exposures.

Materials & Methods

Experimental Animals

Four male cynomolgus monkeys (4-6 years old) were used in this study. Cynomolgus monkeys were purchased from Guangxi Grandforest Scientific Primate Company (Ping Nan, China) when they were 3-5 years old. All purchased monkeys had been acclimated and checked medical inspection for 36 days. The monkeys were housed individually in suspended stainless-steel cages. The animal room was environmentally controlled to maintain 23 ± 3°C, a relative humidity of 50 ± 10%, the air ventilation frequency of 10-20 times/hr, and a light intensity of 150-300 Lux. The monkeys were fed daily certified primate diet (Oriental Yeast Co., Ltd., Japan) correspond to 4 % of body weight and water *ad libitium*. Four male monkeys were divided into two groups of two monkeys and given an oral administration of vehicle. And then, 10 mg/kg and 30 mg/kg of astemizole (Sigma-Aldrich, USA) suspended in 0.5% w/v methyl cellulose solution were administrated, respectively. The blood was collected at 2 hours after vehicle administration and 24 hours after astemizole administration. This experiment was conducted in facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). All procedures were approved by Institutional Animal Care and Use Committee (IACUC).

PBMC Preparation and RNA Isolation

For gene expression analysis, a volume of 4 mL blood from individual animals was collected into vacutainer tubes containing EDTA (Becton Dickenson, USA). Each anticoagulated blood sample was diluted 1 : 2 in PBS Dulbecoo's layered onto the density gradient medium, Ficoll-Hypaque Plus (GE Healthcare, Sweden) and centrifuged for 30 min at $400 \times g$, 18°C. The PBMC were harvested by careful pipetting of the corresponding density band and then were rinsed twice with PBS solution for 10 min at $100 \times g$, 18°C. Isolated PBMC were stored in RNAlater (Qiagen, USA) until RNA isolation. Total RNA was extracted RNeasy mini kit (Qiagen, USA) according to manufacturer instructions. Isolated total RNA was quantified using NanoDrop spectrophotometer (NanoDrop Technologies, USA), and the quality of RNA was evaluated using 2100 Bioanalyzer (Agilent Technologies, USA) for DNA chip experiments.

Microarray Analysis

Affymetrix GeneChip® Human Genome U 133 plus 2.0 array covering whole genome was used for microarray experiment. Two samples from cynomolgus monkeys' PBMCs in each group were diluted at 3 μg/8 μL concentration and used for produce gene expression data. All microarray experiment include cDNA synthesis, synthesis of biotin-labeled cRNA, fragmentation, hybridization, washing, and scanning were performed according to the manufacturer's protocols. Raw image data was processed using Affymetrix GeneChip Operating System and resultant cell intensity files was normalized by MARS 5.0 algorithm. Following statistical analysis and differentially expressed genes selection were performed using GenPlex software version 2.8 (ISTECH Inc., Korea). Statistical analysis was performed to evaluate global gene expression pattern using paired *t*-test and statistical significant assigned $P < 0.05$. The differentially expressed genes in astemizole administration groups were selected at a minimum 1.5-fold change compared to each control group. All selected genes were analyzed by two-dimensional hierarchical clustering based on Pearson correlation and Complete Linkage. Principal component analysis (PCA) was applied to further elucidate discriminate gene sets. The toxicity function of interesting genes was classified by Ingenuity Pathway Analysis software. The selected genes were annotated based on NetAffyx, linked at http://www.affymetrix.com.

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