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Gene Expression Analysis of Hepatic Response Induced by Gentamicin in Mice

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Abstract

Gentamicin is a broad-spectrum aminoglycoside antibiotic used in the treatment of bacterial infection. Although side effects of gentamicin such as nephrotoxicity and ototoxicity have been investigated, the information on the hepatic effects of gentamicin is still limited. In the present study, gene expression profiles were analyzed in the liver of gentamicin treated mice using Affymetrix GeneChip® Mouse Expression 430A 2.0 Array. Totally, 400 genes were identified as being either up- or down-regulated over 1.5-fold changes ($P < 0.01$) in the liver of gentamicin treated mice. Among these deregulated genes, 16 up-regulated genes mainly involved in transport (*Kif5b, Pex14, Rab14, Clcn3*, and *Necap1*) and 20 down-regulated genes involved in lipid and other metabolisms (*Hdlbp, Gm2a, Uroc1*, and *Dak*) were selected using *k*-means clustering algorithm. The functional classification of differentially expressed genes represented that several stress-related genes were regulated in the liver by gentamicin treatment. This data may contribute in understanding the molecular mechanism in the liver of gentamicin treated mice.

Keywords: Gentamicin, Gene expression profiling, Hepatic response

Gentamicin has been used as aminoglycoside antibiotic which is widely used in the treatment of bacterial infections, but it has been known to induce nephrotoxicity and ototoxicity[1,2]. Gentamicin-induced renal damage is mainly confined to proximal tubule but the mechanism of cellular toxicity is not clear, although several hypotheses of nephrotoxicity have been presented, such as lysosomal alteration and phospholipidosis. In our previous report, the gene expression profiling of gentamicin-induced nephrotoxicity was analyzed using mircoarray to identify the genes associated with renal injury and understand the mechanism of cellular toxicity in the kidney[3].

However, the damaging effects on the liver of treatment of gentamicin alone have not been reported so far, although it was recently reported that the combined treatment with cisplatin and gentamicin induced the hepatotoxicity[4]. Other investigations represented that a common characteristic of kidney and liver is the abundance of blood vessels, indicating that the liver could be target of both cisplatin and gentamicin[5-7]. The liver is one of the most important organs, owing to its biological functions such as drug metabolism, amino acid metabolism, lipid metabolism and glycolysis. The biotransformation of xenobiotics is mainly occurred in the liver by metabolizing enzymes and the liver is considered as the most common target organ for xenobiotic toxicity. The genomic approach of the hepatic effects of gentamicin could permit the evaluation of change in cellular process based on transcriptional level.

Recently, the genomic approaches in the field of toxicity have been expanded to characterize how genome responds to toxicants and to understand the cellular mechanism in disease and dysfunction. For the hepatotoxicity, genomic approaches have been performed using many hepatotoxicants such as acetaminophen[8], carbon tetrachloride[9], tetracycline[10], and amiodarone[11]. In this report, we describe the global gene expression patterns in the liver to understand the molecular mechanism of hepatic effects of gentamicin. Using Affymetrix GeneChip system, the gene expression profile at different dose- and time- points has been analyzed after administration of gentamicin. Genes that are differentially expressed according to the dose- and time- points were analyzed using hierarchical and *k*-means clustering and the functional classification of deregulated genes were also analyzed. In brief, we report on the gene expression profiling of hepatic response induced by gentamicin.

Blood Biochemistry and Histopathology

As shown in Figure 1, AST and ALT activity was analyzed in all dose of gentamicin at 1, 3, and 7 day.

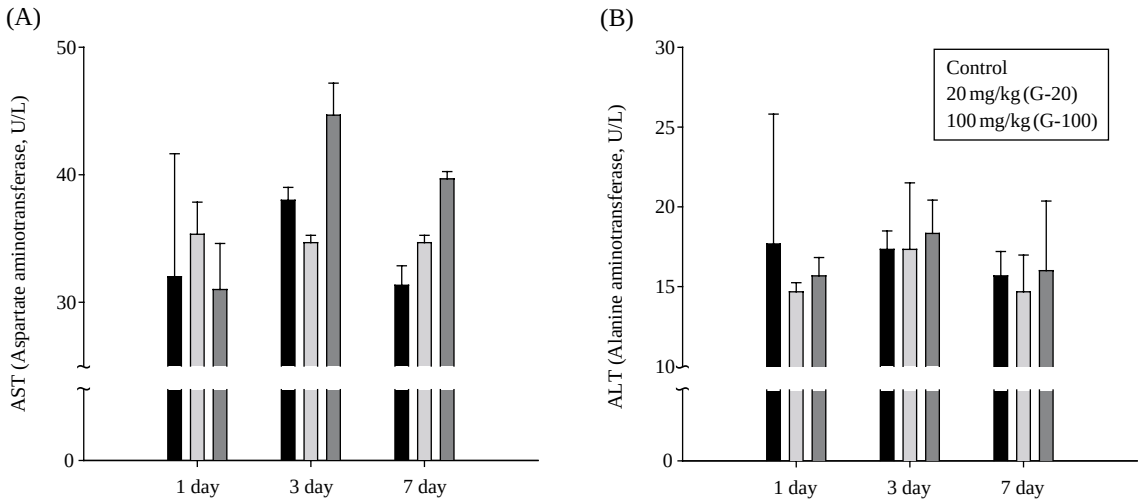

Figure 1. Blood biochemical data in the liver of gentamicin treated mice (A) AST (B) ALT.

AST activity was slightly elevated in 100 mg/kg dose group (G-100) at 7 day, but no significant change was observed in ALT activity. Histopathological observation of liver showed that the administration of gentamicin exhibited no discernable hepatic pathology at the entire tested groups (data not shown), while the renal lesion was observed at 7 day after 100 mg/kg administration of gentamicin, as described in the previous study[3].

Microarray Analysis

Gene expression profiling of gentamicin in the liver was analyzed using Affymetrix GeneChip system. Although the hepatic lesion was not observed in histopathology, all dose- and time- point groups were analyzed to track the gene expression change in the liver after administration of gentamicin.

As shown in Table 1, differentially expressed genes (DEGs) in each time point were selected. In each time point, 137, 82, and 154 genes were differentially expressed at 1, 3, and 7 day, respectively, based on significance analysis ($P < 0.01$, > 1.5-fold). For all time points, 400 genes were differentially expressed and were analyzed using hierarchical clustering (Figure 2(A)). Hierarchical clustering showed that all dose groups at 7 day were closely clustered and were branched off separately from other groups. The gentamicin treated group at 1 and 3 day was closely clustered with controls. This result represents that a 7-day administration of gentamicin has an influence on the liver in transcriptional level, although histopathological analysis could not show the lesion of liver in this group.

Table 1. Number of differentially expressed genes in the liver of gentamicin treated mice.

	1 day	3 day	7 day	All time points
ANOVA ($P < 0.01$)	500	546	952	3,240
> 1.5-fold Change	596	494	810	1,730
$P < 0.01$ & > 1.5- fold	137	82	154	400

Differentially Expressed Genes in the Liver of Gentamicin Treated Mice

Among 400 deregulated genes as mentioned above, we focused on the gene sets at 7 day showing the significant gene expression changes. Hierarchical clustering and *k*-means clustering of 154 deregulated genes at 7 day was performed, as shown in Figure 2 (B) and Figure 3, respectively. Figure 2(B) showed that samples corresponding to control and treated group were clustered in a dose dependent manner and many of genes were down-regulated in gentamicin-treated groups comparing to controls. Based on *k*-means clustering (Figure 3), 16 up-regulated (Cluster 2) and 20 down-regulated (Cluster 4) genes were selected. As shown in Table 2, the up-regulated genes at treated groups included known genes involved in transport (*Kif5b, Pex14, Rab14, Clcn3*, and *Necap1*), protein dephosphorylation (*Ptp4a2*), actin cytoskeleton organization (*Epb4.1*), and signaling cascade (*Jak1*). On the other hand, genes representing a down -regulated expression included those involved in lipid metabolism (*Hdlbp* and *Gm2a*), other metabolism

Table 2. Up-regulated genes at 7 day in the liver of gentamicin treated mice.

Gene symbol	Gene title	Acc. No	GO
Kif5b	kinesin family member 5B	NM_008448	mitochondrial transport
Pex14	peroxisomal biogenesis factor 14	NM_019781	protein transport
Rab14	RAB14, member RAS oncogene family	NM_026697	protein transport
Ptp4a2	protein tyrosine phosphatase 4a2	NM_008974	protein amino acid dephosphorylation
Tex261	testis expressed gene 261	NM_009357	
Epb4.1	erythrocyte protein band 4.1	NM_183428	actin cytoskeleton organization and biogenesis
Tprkb	Tp53rk binding protein	NM_176842	biological process unknown
Pb1	polybromo 1	XM_619217	regulation of transcription
1427422_at	Mus musculus, clone IMAGE:5050186		
4921506J03Rik	RIKEN cDNA 4921506J03 gene	NM_001033474	
Jak1	Janus kinase 1	NM_146145	intracellular signaling cascade
1438120_x_at			
Clcn3	chloride channel 3	NM_007711	ion transport
Necap1	NECAP endocytosis associated 1	NM_026267	protein transport
Plac8	placenta-specific 8	NM_139198	biological process unknown
Cyba	cytochrome b-245, alpha polypeptide	NM_007806	superoxide metabolism

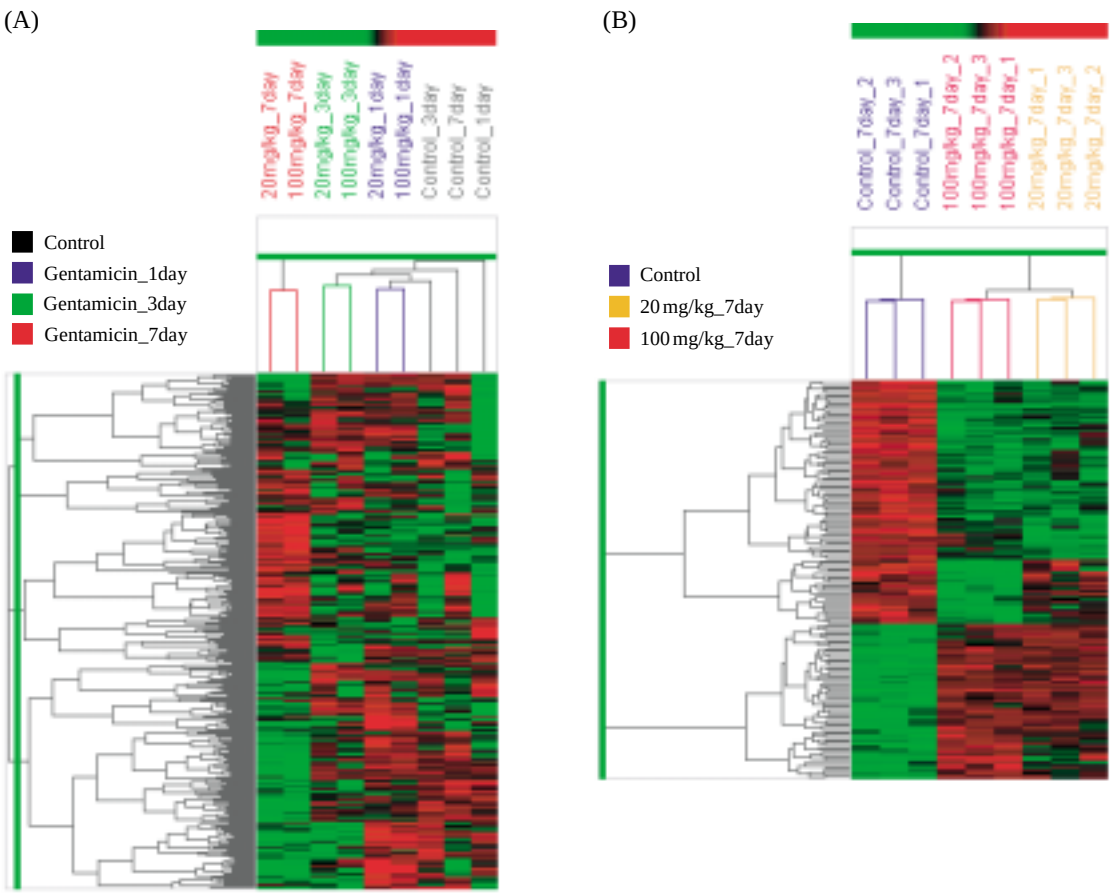

Figure 2. Hierarchical clustering of differentially expressed genes in the liver of gentamicin treated mice. (A) Gene expression profile at all dose- and time- points. 400 deregulated genes were analyzed based on statistical significance as described in the Methods section. Heat map was represented by calculating the average value of 3 individual arrays belonged to the group. (B) Gene expression profile of 154 deregulated genes at 7 day in gentamicin treated group. Heat map was represented with expression value of each individual array.

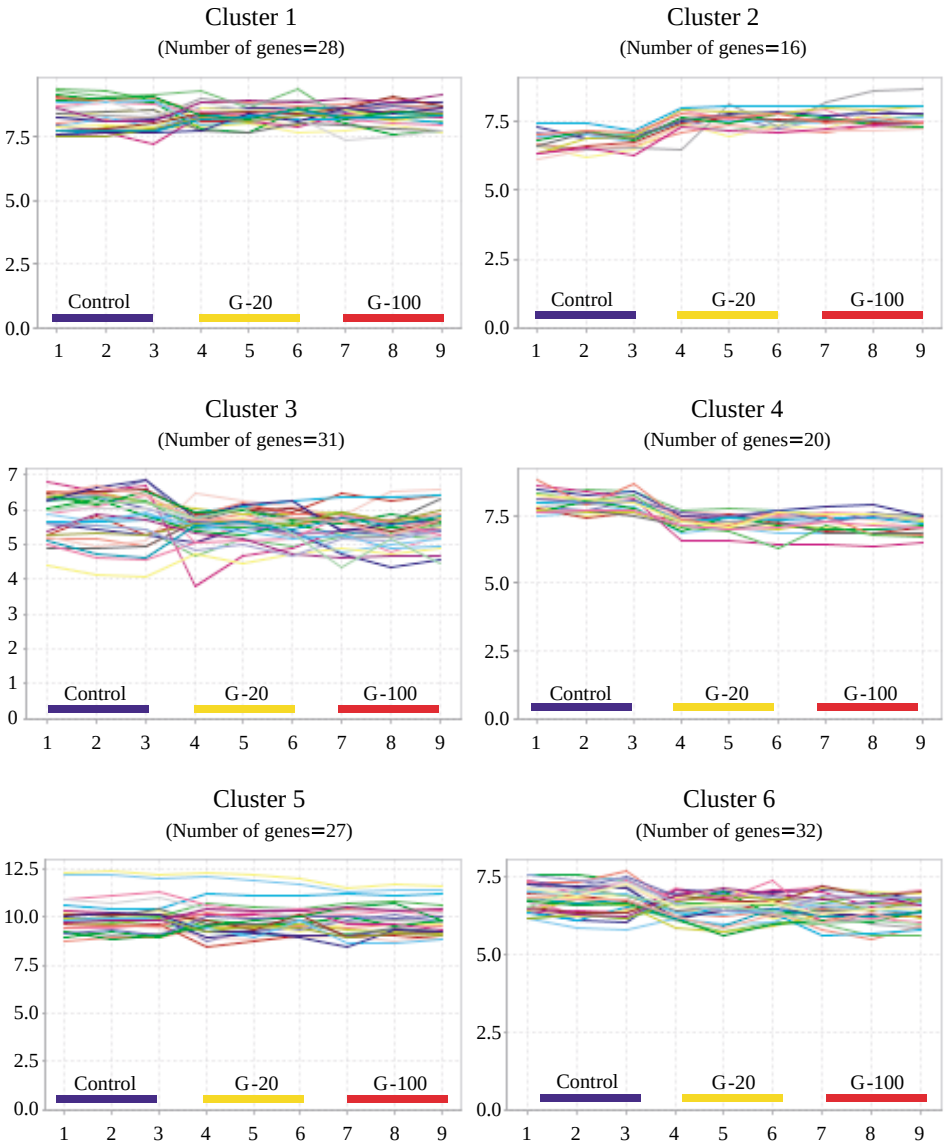

Figure 3. *k*-means clustering of 154 deregulated genes at 7 day. Control and gentamicin treated group including 3 individuals were represented as the colored bar. G-20 and G-100 indicate the dose of gentamicin such as 20 mg/kg and 100 mg/kg, respectively.

(*Uroc1* and *Dak*), regulation of transcription (*Bpnt1* and *Rnf141*), and protein transport (*Rab5c* and *Sybl1*) (Table 3).

Functional Classification of Differentially Expressed Genes

To analyze the functional classification of differentially expressed genes in the liver of gentamicin treated mice, 154 deregulated genes at 7 day were analyzed using Gene Ontology, as described in the Methods section. The results for the biological process and molecular function with 4th GO terms are given in Figure 4. In the biological process category, most of up-regulated genes were assigned into the term of transport, signal transduction, and regulation of cellular process, such as 32.1%, 28.6%, and 21.4 %. In the case of down-regulated genes, most of genes were assigned into the term of regulation of cellular process, nucleic acid metabolic process, and transport, such as 46.3%, 35.2%, and 24.1%, respectively. As shown in Figure 4, the GO terms showing different distribution between up-regulated and down-regulated in treated group were observed in the both categories. In the biological process category (Figure 4(A)), a kind of genes in involved in phosphorus metabolic process (10.1%), generation of precursor metabolites and energy (7.1%), ion homeostasis (7.1%), and ROS metabolic process (7.1%) are highly observed in up-regulated genes comparing to down-regulated genes, whereas a kind of genes involved in

Table 3. Down-regulated genes at 7 day in the liver of gentamicin treated mice.

Gene symbol	Gene title	Acc. No	GO
Hdlbp	high density lipoprotein (HDL) binding protein	NM_133808	lipid metabolism
Gm2a	GM2 ganglioside activator protein	NM_010299	lipid metabolism
Pdia4	protein disulfide isomerase associated 4	NM_009787	
Bpnt1	bisphosphate 3′-nucleotidase 1	NM_011794	regulation of transcription
Clec2d	C-type lectin domain family 2, member d	NM_053109	negative regulation of osteoclast differentiation
Yme1l1	YME1-like 1 (S. cerevisiae)	NM_013771	proteolysis
Rab5c	RAB5C, member RAS oncogene family	NM_024456	protein transport
Uroc1	urocanase domain containing 1	NM_144940	histidine metabolism
Dak	dihydroxyacetone kinase 2 homolog (yeast)	NM_145496	glycerol metabolism
Col3a1	procollagen, type III, alpha 1	NM_009930	cell adhesion
Aplp2	amyloid beta (A4) precursor-like protein 2	NM_009691	extracellular matrix organization and biogenesis
Rnf141	ring finger protein 141	NM_025999	regulation of transcription
Ik	IK cytokine	NM_011879	
Sash1	SAM and SH3 domain containing 1	NM_175155	cell cycle
Gpiap1	GPI-anchored membrane protein 1	NM_016739	
2010106G01Rik	RIKEN cDNA 2010106G01 gene	NM_023220	proteolysis
Rad23b	RAD23b homolog (S. cerevisiae)	NM_009011	response to DNA damage stimulus
Sybl1	synaptobrevin like 1	NM_011515	protein transport
2700089E24Rik	RIKEN cDNA 2700089E24 gene	XR_002351	

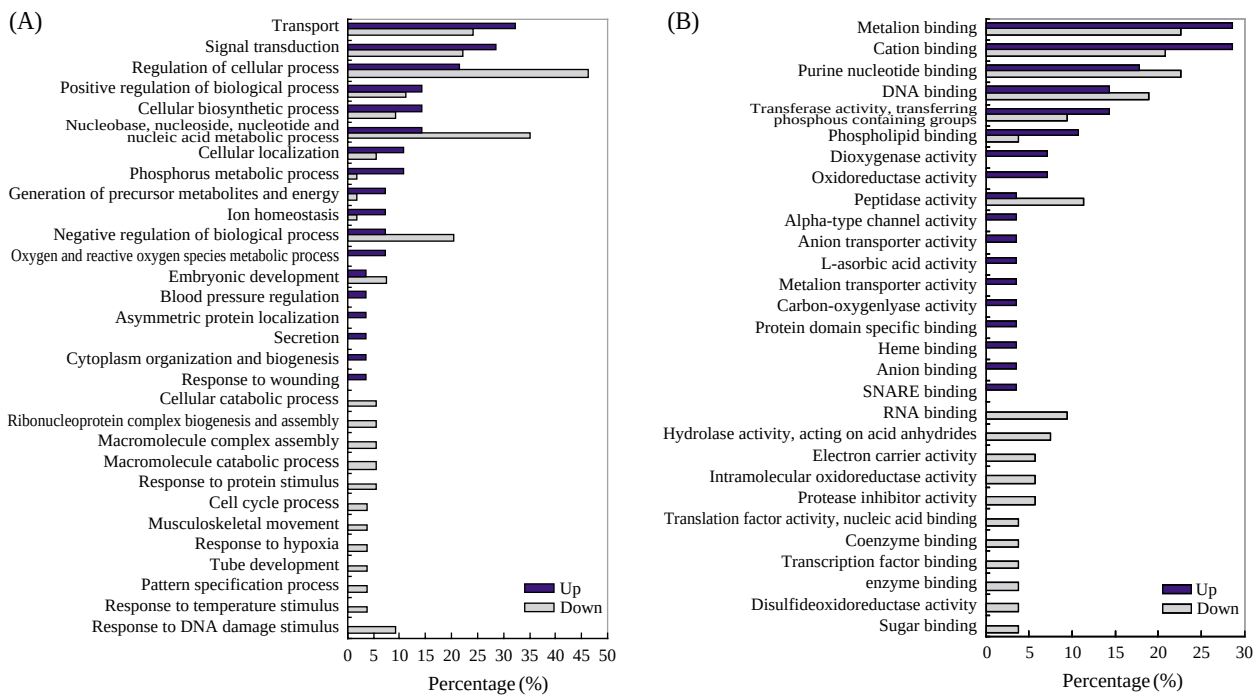

Figure 4. Functional classification of 154 deregulated genes at 7 day using Gene Ontology. (A) Biological process category, (B) Molecular function category; dark bar indicates the up-regulated genes and gray bar indicates the down-regulated genes in the liver of gentamicin treated mice.

regulation of cellular process (46.3%), nucleic acid metabolism (35.2%), and negative regulation of biological process (20.4%), are highly observed in down -regulated genes comparing to up-regulated genes. In

the molecular function category (Figure 4(B)), genes involved in phospholipids binding (10.7 %), dioxygenase activity (7.1%), and oxidoreductase activity (7.1%) are highly observed in up-regulated genes comparing to down-regulated genes, while a kind of genes involved in peptidase activity (11.3%), RNA binding (9.4%), and hydrolase activity (7.6%), electron carrier activity (5.7%), and intramolecular oxidoreductase activity (5.7%) are highly observed in down-regulated genes comparing to up-regulated genes.

Discussion

In recent decades, high-throughput approaches for genomics, proteomics, and metabolomics have been carried out in the field of toxicology to investigate the toxicological mechanism and assess the drug safety. In several studies using-omic technologies, the toxicological mechanism or potential biomarkers were revealed, considerably focused on heptaotoxicity[12-15]. In our previous study, the gene expression profiles during early renal injury induced by gentamicin were analyzed[3]. Along with this investigation, we were concerned with seeing how genes are regulated in the liver, which plays a major role in xenobiotic metabolism, after administration of gentamicin. In the present study, the blood biochemical data showed that AST value was slightly evaluated in the gentamicin treated mice. This result is concordant with other observation of mild elevation of AST in the patient, as treatment with aminoglycosides[16]. Although no pathological lesion was observed, we postulated that gene expression change could occur in the liver before the onset of histopathologic change.

Here, microarray analysis showed that gene expression change in the liver of gentamicin treated mice at all dose- and time- points. Among the deregulated genes in gentamicin treated group, genes involved in transport such as *Kif5b, Pexl4, Rab14 Clcn3*, and *Necap1* were overexpressed. *Clcn3*, chloride channel 3, plays a role in ion transport. Activation of chloride channel in Kupper cells participate in regulating the intracellular calcium concentration. Corcoran *et al.*[17] reported that early increase of calcium concentration in the liver during acetaminophen-induced hepatotoxicity. *Rab14* is associated with GTPase ligand and these up-regulated transport genes may be concerned in intracellular signaling cascade following to gentamicin treatment. On the other hand, genes involved in lipid metabolism such as *Hdlbp* and *Gm2a* were down-regulated in gentamicin treated group. Several investigations showed that lipid metabolism is associated with hepatotoxicity such as steatosis. Gene expression analysis of hepatotoxicity induced by carbon tetrachloride represented that the expression of sterol-CoA desaturase and fatty acid synthases were decreased. The formation of carbon tetrachloride radicals induced the steatosis by inhibiting of lipoprotein secretion of fatty acid flow. The down-regulation of lipid metabolic pathway may attribute to feedback from lipid accumulation in the liver.

The functional classification of deregulated genes in gentamicin treated group showed that genes involved in ROS metabolic process such as *Txnip* and *Cyba* were highly observed in up-regulated genes comparing to down-regulated genes, while genes involved in intramolecular oxidoreductase activity such as *Hpgd, Pdia4*, and *Pdia3* were observed in down-regulated genes. Genes involved in response to other stimulus such as *Serpinh1, Hspa1b, Epas1, Hif1a*, and *Hspa5* were also observed in down-regulated genes in gentamicin treated group. Likewise, the expression of these gene set associated with cellular stress was concurrently and sophistically regulated in the liver by gentamicin treatment.

Using the microarray analysis, we examined the difference in gene expression profiles in the liver of gentamicin treated mice. The information on differentially expressed genes in the liver could help to understand the genetic events in the hepatic response induced by aminoglycosides including gentamicin.

Methods

Animal Treatment

Female C57BL/6 (25-27 g), approximately 10-week-old, mice were used and gentamicin was purchased from Sigma (G4918). Animal care and gentamicin treatment of mice was performed, as described in the previous study[3]. Gentamicin was administered once daily at 20 mg/kg (G-20) and 100 mg/kg (G-100) up to 7 day, and time-matched control animals was administrated with received corresponding quantities of the vehicle. Necropsies were then performed at 1, 3 and 7 day.

Tissue Collection

At necropsy, each mouse was anesthetized with diethyl ether and the blood was collected for blood biochemistry analysis. The liver tissue submerged in an appropriate volume of RNAlater (Qiagen, Germany) for RNA extraction. The liver tissues in RNAlater were kept at 4˚C for overnight and then discarded the reagent, and stored at −80˚C until the RNA

was extracted.

Blood Biochemistry and Histopathology

Blood biochemistry was estimated after administration of gentamicin at 1, 3, and 7 day. The value of AST and ALT, biochemical markers of hepatotoxicity, were measured using Fuji Automated Clinical Chemistry Analyzer (Fujifilm, Japan). Average value was presented and statistical significance was calculated using two-tailed, unpaired *t* test for comparison between two groups. For histopathology, formalin-fixed liver tissues were embedded in paraffin, cut into 4-μm sections, and stained with hematoxylin and eosin (H&E), and analyzed by light microscopy.

RNA Extraction

Liver samples were homogenized in Trizol reagent (Molecular Research Center, Inc., USA) and total RNA was isolated using Trizol reagent and purified using RNeasy mini kit (Qiagen, Germany) according to manufacturer instructions. Total RNA was quantified using NanoDrop (NanoDrop, USA) and the quality of RNA was evaluated using 2100 Bioanalyzer (Agilent Technology, USA).

Microarray Analysis

Sample labeling, microarray hybridization, washing, and scanning were performed according to the manufacturer's protocols (Affymetrix, Inc., USA) as described previously[18]. The preprocessing procedure of resultant cell intensity files (CEL) and following microarray analysis were performed using GenPlex software (Istech Inc., Korea). Data normalization was performed using quantile normalization. The differentially expressed genes on each time point were selected based on statistical ANOVA test ($P < 0.01$) and 1.5-fold change. The selected deregulated genes were analyzed by hierarchical clustering algorithm and the *k*-means partitioning clustering algorithm. The functional classification of differentially expressed genes was performed based on GO database. The assigned genes were analyzed against two categories of biological process and molecular function. The comparison of GO terms for deregulated genes in gentamicin treated group was analyzed using GO analysis tools, linked at http://www.fatigo.org/.

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