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Gene Expression Profiling of 6-MP (6-mercaptopurine) in Liver

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Abstract

The KFDA (Korea Food & Drug Administration) has performed a collaborative toxicogenomics project since 2003. Its aim is to construct a toxicology database of 12 compounds administered to mice at initial phase. We chose 6-MP (6-mercaptopurine) which has been used in the treatment of childhood leukemia. It was administered at low (0.224 mg/kg) and at high (2.24 mg/kg) dose (5 mice per group) intraperitonealy to the postnatal 6 weeks mice, then the serum and liver were collected at the indicated time (6, 24 and 72 h) after scarification.Serum biochemical markers for liver toxicity were measured and histopathologic studies also were carried out. The gene expression profiling was carried out by using Applied Biosystems 1700 Full Genome Expression Mouse. By self-organization maps (SOM), we identified groups with unique gene expression patterns, some of them are supposed to be related to 6-MP induced toxicity, including lipid metabolism abnormality, inflammatory response, oxidative stress, ATP depletion and cell death. The potential toxic effects appearing as gene expression changes are dependent of the time of 6-MP but independent of the dosage of it. This study would contribute to establishment of international database as well as national one about hepatotoxicity.

Keywords: 6-Mercaptopurine (6-MP), acute hepatoxicity, microarray analysis, gene expression

Introduction

So far, the field of toxicogenomics has mainly concentrated on hepatotoxicity, since the liver is the primary target of most toxic responses. The liver is the first organs to be exposed when chemicals are administered via the portal vein or perorally. Chemical concentrations in the liver are often a lot higher than the peak plasma concentration. The liver is also the main site for metabolizing xenobiotics and diverse chemicals can cause the formation of active metabolites which have an effect on toxicity.

The TGRC (Toxicogenomics research Consortium) hosted by KFDA (Korea Food & Drug Administration) consists of 6 laboratory to construct database of acute and subchronic toxicity of chemical entities. The 12 chemicals have been selected for the project and 2 compounds per laboratory were assigned. The following are examined for each. The *in vivo* test using mice consists of a single administration test (6, 24 and 72h with 2 dose levels including vehicle control) and the data of histopathological examination of liver and blood biochemistry are obtained from each animal.

We chose 6-Mercaptopurine (6-MP) out of 12 compounds. The drug affects mainly bone marrow suppression but its hepatotoxicity has also been demonstrated in many researches (Tapner *et al.*, 2004; Berkovitch *et al.*, 1996; Schmiegelow *et al.*, 1992) to prove an animal modelof liver toxicity. 6-MP is one of a large series of purine analogues which interfere with nucleic acid biosynthesis and has been used in the treatment of childhood leukemia since 1953 (Burchenal *et al.*, 1953). 6MP acts as a prodrug, which is metabolized through different steps into 6TG (6-thioguanine) nucleotides. These are incorporated into DNA, leading to replicative error and cell death. 6MP is also a substrate for thiopurine methyltransferase (TPMT), which inactivates 6MP into methylmercaptopurine. Interindividual variation in TPMT activity is great, and patients with high enzyme activity accumulate lower concentrations of 6TG nucleotides (Lennard *et al.*, 1993). The clinical symptoms and histologycal experiment about toxicity by 6-Mercaptopurine have been reported (Berkovitch *et al.*, 1996; Michael *et al.*, 2004; Nygaard *et al.*, 2004), but gene expression change analysis that use DNA Microarray has never been attempted. The purpose of the current study is to investigate the correlation between biochemical markers and gene expression profile at 6-MP-treated mice, and to construct a toxicology database about 6MP.

Methods

Animals and 6-MP administration

Approximately 6-week-old SLC-ICR male mice were assigned

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to 9 groups (5 mice/group). Mice were administration a single dose of 6MP (Sigma) dissolved in distilled water by intraperitoneal injection. The dosing solutions were prepared to deliver a volume of 5 ml/kg as follows; 2.24 mg/kg high dose, 0.224 mg/kg low dose. At the indicated time (6, 24 and 72h after the administration), blood samples were collected from the atrium, and then the mice were sacrificed and the livers collected. At the same time a cross-section of the left lateral lobe of the liver was collected in 10% neutral buffered formalin for histopathology. The remaining portions of liver were collected in RNase-free tubes and snap-frozen in liquid nitrogen. Frozen tissues were stored at -70 °C to extract RNA

Biochemical and histopathological examination

After the blood serum was isolated by centrifusion at 3000 rpm for 20 min, it was store at -70 °C. Two typical biochemical markers for hepatotoxicity (alanine aminotransferase, ALT; aspartate aminotrasnferase, AST) were measured using an autoanalyzer (Prestige 24i, Tokyo Boeki Medical System, Japan).

The liver tissues to be examined histologycally were fixed in 5% neutral-buffered formalin, embedded in paraffin and subsequently sectioned. The tissues stained with hematoxylin/eosin were conducted by pathologist.

Microarray analysis

Total RNA from the liver tissues was extracted with Trizol® reagent (Invitrogen, Carlsbad, USA) and RNeasy kits (Qiagen, U.K) according to the manufacturer's protocol.

Equal amounts of total RNA from samples of each administration were used for the microarray analysis and reverse transcripatase (RT)-PCR.

Microarray experiments were performed using an Applied Biosystems 1700 Full Genome Expression Mouse Microarray (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. The array consisted of 33315 probes including ESTs. Array hybridization, chemiluminescence detection, image acquisition and analysis were performed using an Applied Biosystems Chemiluminescence Detection Kit (Applied Biosystems, Foster City, CA) and Applied Biosystems 1700 Chemiluminescent Microarray Analyzer following the manufacturer's protocol.

Semi-quantitative RT-PCR was performed as follows: 2 μg of total RNA were transcribed into cDNA by reverse transcription with AMVReverse Transcriptase (Intron, Korea) using an oligo $(dT)_{15}$ primer in a total volume of 20 μl. Reverse transcription was performed for 60 min at 42 °C in a thermocycler (Biometra, Göttingen, Germany) followed by 5 min at 70°C. 1 μl of the RT-reaction were used for PCR experiments. Primer sequences were listed in Table 1.The PCR conditions consisted of an initial denaturation 95 °C for 2 min, 30 cycles at 95 °C for 30 sec, 45~60 °C for 30 sec, and 72 °C for 30 sec, and a final extension at 72 °C for 10 min. The PCR products were separated on 1.5% agarose gels and visualized using ethidium bromide staining under UV light. These samples were also amplified with primers for β-actin, housekeeping gene.

Bioinformtics analysis

To minimize number of falsely significant genes, we performed multiple testing. The genes were filtered by flag threshold (flag smaller than 100) and performed quantile normalization. Two-way ANOVA (analysis of variatio) was used to identify differentially expressed genes. Using SOM cluster classified each group which had similar expression pattern. The significant genes were classified by Gene ontologyand pathway.

Results and Discussion

The ultimate purpose of this study is to establish a gene expression database for prediction of acute hepatotoxicity after 6MP-administered. The 6-MP induced acute hepatotoxic response by a variety of observed mechanisms, including necrosis, steatosis, inflammatory response and ATP depletion. An earlier time point would likely have included immediate early response genes. Hepatic centrilobular necrosis was found after 6 h in mice at the two doses, but not in 72 h. Gene expression profile also changed regardless of dose. This suggests that hepatotoxicity induced by 6MP is not dose-dependant but time-dependant.

Table 1. Primer sequences for the genes with representive expression patterns that were used to validate the microarray analysis by semi-quantitative RT-PCR

Gene	Genbank Acc. No.	Forward primer sequence	Reverse primer sequence
Hsd3b4	NM_008294	CAGACCATCCTAGATGTC	AGGAAGCTCACAGTTTCCA
Krt2	NM_033073	GTTTCAGACTATCTTCCAGG	TATCTCTGTTGTGAATTCCA
Tuba1	NM_011653	AAACCCATAAGTGAAATGGG	RGCTTCCACAGGGATGTTTAT
Myo1f	NM_053214	CGGGCACTGTACCAATACAT	GGAGACTTAGATAGCAAGTC

Histopathologic and biochemical analysis

H & E evaluation of stained liver sections revealed a little histopathological difference in mice treated 6MP (Fig. 1). There were some changes in the livers treated with 2.24 mg/kg and 0.224 mg/kg 6MP at 6 h regardless of dose. The minimal disarray of lobular architecture with mild degree of hepatocellular necrosis was accompanied by infiltration of lymphocytes. The 24 h-livers were similar with 6 h-liver, but their cells were more swollen compared with 6 h. The livers of 72 h after the administration were recovered cell to swell.

The serum transferase levels in the 6MP-administered groups were measured at 6, 24 and 72 h after administration (Fig. 2) Measure of AST and ALT enables to assess hepatocellular injury and death (Scheig, 1996). Both AST and ALT levels were the highest in the high dose-administered groups at 6 h, but little difference in the low doseadministered groups. Taking these results into consideration, the maximal toxic times of each 6MP dosage were estimated as 6h in high dose.

Gene expression profile in 6MP-administered mice

The gene expression profiles in the two dose and five time points were determined using the data from the DNA microarray. Scatter plot of geneexpression between vehicle controls at each time point revealed that most of the genes distributed within a 1.5 fold range of their 45° line, meaning that few gene were affected by the vehicle (data not shown).

Gene expression profiles of interest were significantly up- or down- regulated in mice treated with 6MP when compared to control. Table 2 shows the list of genes that showed at least a 1.5 fold difference in at least one time point with $p<0.01$. Based on each gene annotation, 8 categories were classified; lipid metabolism, lipid transport, cytoskeleton organization and biogenesis, inflammatory response, response to stress, oxidation, cell death and ribosome biogenesis. Though very low dose was treated, hepatotoxic-associated genes that were presentedby acute exposure were expressed differently. Many of the genes are related lipid metabolism. As shown in Table 2, the maximal up-or down-regulated time points in almost all the individual gene expression profiles reflected the maximal toxic time.

We also performed hierarchical clustering based on each dosage (data not shown). Because 6MP was treated very low dose, the gene expression changes were small

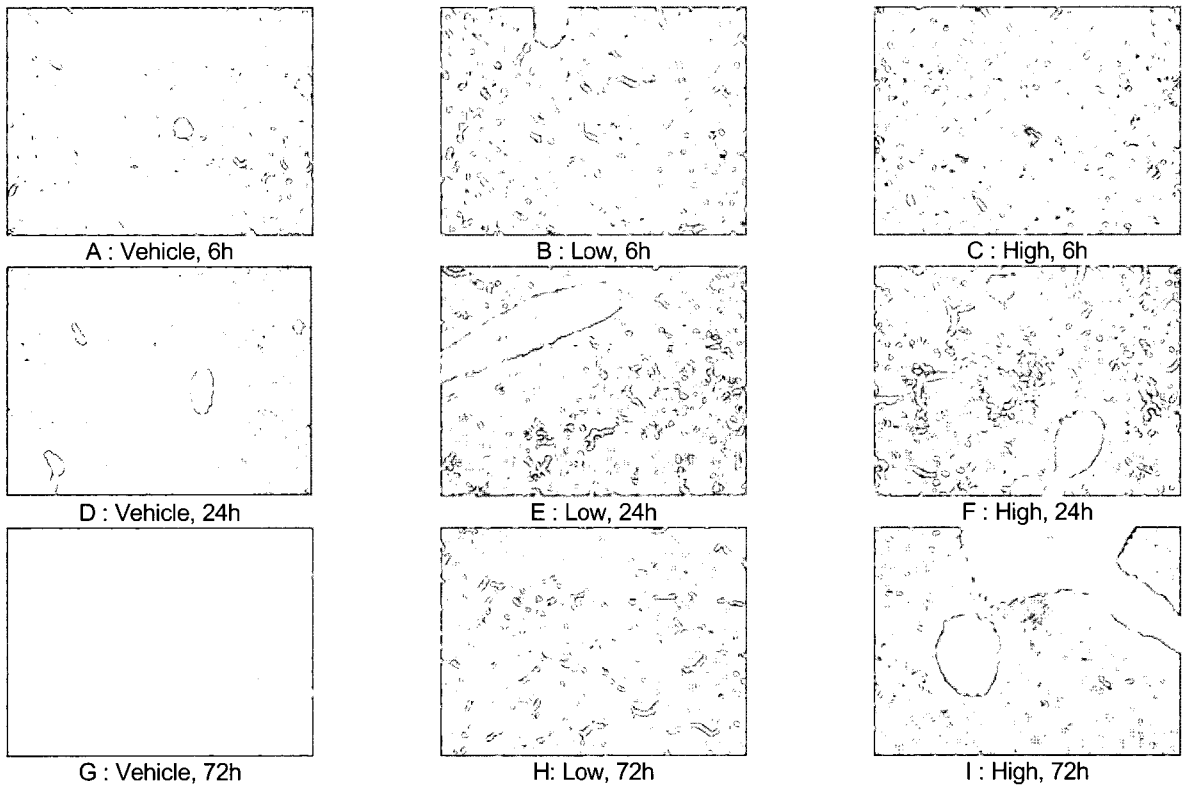

Fig. 1. Histopathologic analysis of livers from a control (vehicle) mice and mice treated with 6-MP. H&E staining 100X. (A,D,G) control liver (B,E,H) Liver tissue of 0.224mg/kg 6MP-treated mice respectively 6h, 24h and 72h after administration. (C,F,I) Liver tissue of 2.24mg/kg 6MP-treated mice, respectively 6 h, 24 h and 72 h after administration

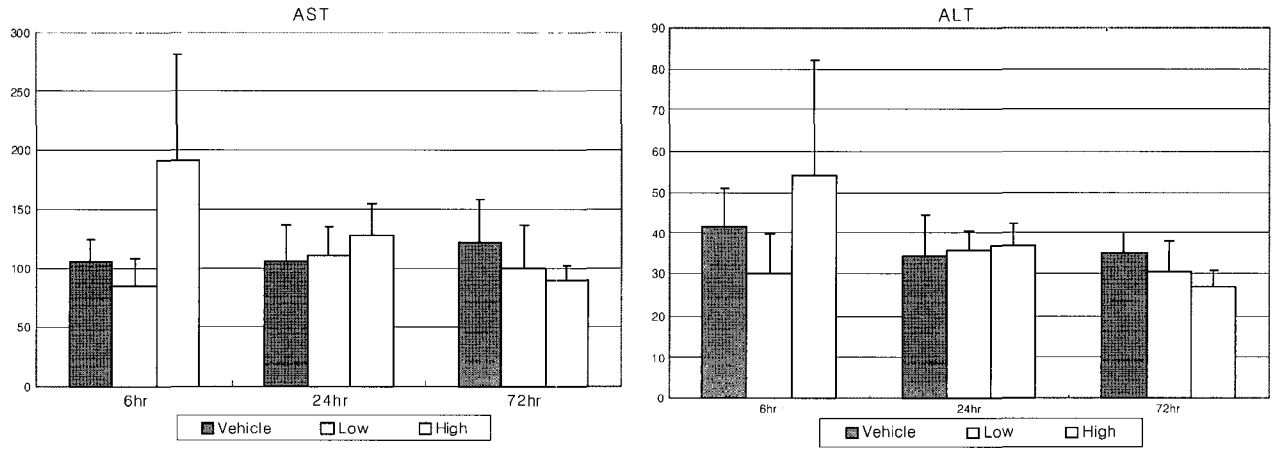

Fig. 2. Serum levels of AST and ALT enzymes, expressed as means and standard deviation of 5 mice/ group. AST: aspartate aminotransferase; ALT: alanine aminotransferase

Table 2. Gene expression changes after 6-mercaptopurine dosage.

Function	Gene	Gene Symbol	L_6*	L_24	L_72	H_6	H_24	H_72
Lipid metabolism								
	sulfotransferase, hydroxysteroid preferring 2	Sth2	16.37	0.16	0.72	4.62	0.44	0.26
	isopentenyl-diphosphate delta isomerase	Idi1	6.22	0.47	1.06	5.93	0.49	1.02
	elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 3	Elovl3	4.49	1.21	2.68	1.41	0.90	6.93
	aldehyde dehydrogenase family 1, subfamily A3	Aldh1a3	4.15	1.14	1.00	1.32	2.10	1.55
	hydroxysteroid dehydrogenase-4, delta<5>-3-beta.	Hsd3b4	4.00	1.24	1.06	4.04	0.95	2.67
	hydroxysteroid dehydrogenase-5, delta<5>-3-beta.	Hsd3b5	3.89	1.14	1.09	3.13	0.83	1.55
	prostaglandin D2 synthase (brain)	Ptgd	3.30	1.38	0.14	0.84	1.00	0.38
	aldehyde dehydrogenase family 1, subfamily A7	Aldh1a7	2.37	1.15	0.84	1.66	1.15	0.82
	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	Hmgcs1	2.05	0.38	1.46	3.69	0.78	1.46
	acyl-Coenzyme A thioesterase 2, mitochondrial;acyl-Coenzyme A thioesterase 3, mitochondria1	Acate3;Acate2	1.82	4.85	1.56	1.02	4.65	1.28
	glycerol-3-phosphate acyltransferase, mitochondrial	Gpam	1.79	0.52	2.52	1.53	0.78	1.21
	sterol-C5-desaturase	Sc5d	1.54	0.62	0.77	2.92	0.75	0.67
	oxysterol binding protein-like 8	Osbpl8	1.48	2.27	1.61	0.94	1.61	0.67
	pyruvate dehydrogenase kinase, isoenzyme 4	Pdk4	1.00	1.15	0.85	2.02	1.82	0.72
	lipase, endothelial	Lipg	0.90	0.80	0.45	1.62	1.05	0.37
	fatty acid synthase	Fasn	0.89	0.50	1.05	1.33	0.56	1.16
	cytosolic acyl-CoA thioesterase 1	Cte1	0.60	1.78	1.36	0.28	3.31	1.22
	cytochrome P450, family 46, subfamily a, polypeptide 1	Cyp46a1	0.42	0.74	0.63	0.95	1.26	1.15
Cytoskeleton organization and biogenesis								
	keratin complex 2, basic, gene 7	Krt2-7	3.37	2.77	2.11	3.06	3.99	3.50
	tubulin, alpha 1	Tuba1	2.72	0.95	0.97	5.02	1.37	0.87
	myosin IF	Myo1f	2.38	1.59	0.70	14.76	1.20	0.55
	dynein, cytoplasmic, light chain 1	Dnclc1	0.81	1.10	0.54	0.68	2.23	0.87
	myosin light chain, regulatory B	Mylc2	0.76	1.10	0.62	1.02	2.23	0.77
	cell division cycle 42 homolog (S. cerevisiae)	Cdc42	0.58	0.89	1.41	1.43	1.10	2.57
	gamma-aminobutyric acid receptor associated protein	Gabarap	0.46	1.18	2.76	0.49	1.01	1.68
Inflammatory respons								
	retinoic acid early transcript delt	Raet1b	7.59	4.97	1.07	0.76	1.51	0.59
	chemokine (C-X-C motif) ligand 9	Cxcl9	1.97	0.91	1.55	1.96	1.12	0.82
	regenerating islet-derived 4 alpha	Reg3a	1.88	0.47	0.73	5.21	0.69	0.73
	chemokine (C-X-C motif) ligand 11	Cxcl11	1.52	0.81	1.15	0.12	1.53	0.43
	chitinase 3-like 3;chitinase 3-like 4	Chi3l3;Chi3l4	0.58	0.24	0.49	3.53	0.86	0.85
	chemokine (C-X-C motif) ligand 1	Cxcl1	0.20	0.79	0.15	1.02	1.19	2.51

*fold change: 1 L_6 : low dose 6hr / vehicle 6 h, L_24 : low dose 24 h / vehicle 24 h, L_72 : low dose 72 h / vehicle 72 h , H_6 : High dose 6 h / vehicle 6 h
H_24 : High dose 24 h / vehicle 24 h, H_72 : High dose 72 h / vehicle 72 h
List of genes showing at least a 1.5 fold difference with $p < 0.01$.

Table 2. Continued

Function	Gene	Gene Symbol	L_6*	L_24	L_72	H_6	H_24	H_72
Response to stres								
	2`-5` oligoadenylate synthetase 3	Oas3	2.04	0.18	0.18	3.10	0.84	0.27
	regenerating islet-derived 3 alpha	Reg3a	1.88	0.47	0.73	5.21	0.69	0.73
	vomeronasal 1 receptor, H12	V1rh12	1.63	1.34	0.59	3.40	0.94	0.66
	B-cell linker	Blnk	1.41	2.80	1.31	2.07	3.33	0.94
	killer cell lectin-like receptor, subfamily A, member 6; member 1	Klra6;Klra19	0.99	1.27	0.63	1.41	2.18	1.18
	RAD54 like (S. cerevisiae)	Rad54l	0.91	1.10	0.75	1.80	2.12	1.47
	chemokine (C-C motif) ligand 17	Ccl17	0.83	1.40	0.30	1.81	0.74	0.41
	tumor necrosis factor (ligand) superfamily, member1	Tnfsf11	0.74	0.28	1.09	1.35	0.28	3.12
	calsenilin, presenilin binding protein, EF hand transcription factor	Csen	0.61	0.64	0.69	2.49	1.12	0.37
	solute carrier family 26, member 4	Slc26a4	0.52	0.60	1.46	4.96	0.60	1.19
	liver-expressed antimicrobial peptide 2	Leap2	0.47	1.05	0.51	0.66	0.90	0.88
Oxidation								
	lysyl oxidase-like 1	Loxl1	1.46	2.92	1.13	1.01	2.03	1.06
	leucine rich repeat containing 16	Lrrc16	1.36	1.45	1.13	1.92	1.35	1.01
	malic enzyme, supernatant	Mod1	1.28	0.89	0.55	1.21	0.95	0.62
	3-oxoacid CoA transferase 2A; 2B	Oxct2a;Oxct2	0.81	0.78	0.92	2.98	1.00	1.11
	glycogen synthase 3, brain; 1, muscle	Gys3;Gys1	0.80	0.81	1.47	0.47	0.72	1.31
Cell deat								
	BCL2-antagonist/killer 1	Bak1	2.02	1.69	2.83	0.92	1.84	2.96
	E2F transcription factor 1	E2f1	1.46	2.92	1.13	1.01	2.03	1.06
	caspase 6	Casp6	1.36	1.45	1.13	1.92	1.35	1.01
	Bcl-2 binding component 3	Bbc3	1.28	0.89	0.55	1.21	0.95	0.62
	interleukin-1 receptor-associated kinase 2	Irak2	0.81	0.78	0.92	2.98	1.00	1.11
	programmed cell death protein 11	Pdcd11	0.80	0.81	1.47	0.47	0.72	1.31
	growth arrest and DNA-damage-inducible 45 beta	Gadd45	0.73	2.49	1.29	0.26	3.23	0.26
	CASP8 and FADD-like apoptosis regulator	Cflar	0.64	0.87	0.94	0.98	1.14	0.45
	BCL2/adenovirus E1B 19kDa-interacting protein 1, NIP 3	Bnip3	0.61	1.23	1.01	0.45	0.92	1.15
	granzyme C	Gzmc	0.55	3.66	1.18	0.82	4.81	1.36
	deoxyribonuclease I	Dnase1	0.34	1.35	5.35	0.30	0.57	2.78
Ribosome biogenesis								
	ribosomal protein S6 kinase, polypeptide 2	Rps6ka2	2.36	0.40	0.32	1.66	0.59	0.75
	ribosomal protein S14	Rps14	1.54	1.54	0.42	1.45	2.18	1.61
Other								
	tissue inhibitor of metalloproteinase 4	Timp4	0.33	0.47	2.34	2.50	1.16	1.24
	extracellular matrix protein 1	Ecm1	1.00	1.44	0.90	0.42	0.97	1.44
	sialyltransferase 7 ((alpha-N-acetylneuraminyl 2,3-beta-galactosyl-1,3)-N-acetyl galactosaminde alpha-2,6-sialyltransferase) B	Siat7b	0.95	0.59	2.34	1.01	1.20	0.28

and no changes between the two dosage groups. However, in time-dependent manner, the distance between the early (6 h) and the late (24 h and 72 h) phase was increased (Table 2).

Lipid metabolism

Table 2 shows that treatment with 6MP resulted in regulation of a number of genes in the cholesterol and fatty acid pathways, including an up-regulation of Sth2 (sulfotransferase, hydroxysteroid preferring 2), Idi1 (isopentenyl-diphosphate delta isomerase), Elovl3 (elongation of very long chain fatty acids-like 3), Aldh1a3 (aldehyde dehydrogenase family 1, subfamily A3), Hsd3b4 (hydroxysteroid dehydrogenase-4), Hmgcs1 (aldehyde dehydrogenase family 1). They were usually over-expressed at 6h after administered and returned to the same level or lower at 24 h: Sth2. Idi1. Elovl3, Hsd3b4, Aldh1a7. A few of these genes involve in steatosis. Steatosis is a common response to acute exposure to many but not all hepatotoxins (Farrell, 1994)

Among cytosolic sulfotransferases, hydroxysteroid sulfotransferase termed Sth2 (SULT2A) is known to catalyze sulfation of bile acids. The biological functions of Sth2 include hormone regulation/metabolism and xenobiotic detoxification. There are reports showing high excretion of sulfated bile acids in liver disorders (Takikawa *et al.*, 1984; Makino *et al.*, 1974). In this data Sth2 gene was expressed highly at 6 h in both low and high dose and decreased rapidly at 24 h and 72 h. These results suggest the possibility that Sth2 induction is a general adaptive response to extranormal levels of bile acids in liver of mammals.

Structure and cytoskeleton

Hepatotoxicants disrupt the integrity of hepatocyte cytoskeleton by affecting proteins that are vital to its dynamic nature (Phillips *et al.*, 1986; Runnegar *et al.*, 1995). The genes related cytoskeleton organization and biogenesis were also up-regulated: Krt2-7, Tuba1, Myo1f. The induction of both the protein synthesis and the cell-structure genes suggests that enhanced protein synthesis and/or proliferation occur, which aids in hepatic tissue remodeling and recovery (Heijne *et al.*, 2004) after 6MP-induced hepatocellular injury.

Acute phase response

The acute phase response is elicited by various types of stress like mechanical damage and inflammation (Ramadori and Christ, 1999; Suffredini *et al.*, 1999). Especially in the liver, changes in many genes provide a protective response and re-establish cellular homeostasis. We identified that the acute phase response was elicited upon an intraperitoneal administration of 6MP. Oas3 (2'-5' oligoadenylate synthetase 3), Reg3a (regenerating islet-derived 3 alpha), V1rh12 (vomeronasal 1 receptor, H12) and Blnk (B-cell linker) were expressed largely in both low dose and high dose group at 6 h. Rad54l (RAD54 like), Ccl17 (chemokine (C-C motif) ligand 17), Tnfsf11(tumor necrosis factor superfamily), Csen (calsenilin) and Slc26a4(solute carrier family 26, member 4) were up-regulated by 6MP in only high dose.

Raet1b (retinoic acid early transcript delta), Cxcl9 (chemokine (C-X-C motif) ligand 9) and Reg3a (regenerating islet-derived 4 alpha) associated with inflammation were up-regulated significantly at 6h after low and high dose. Theinflammation results in cirrhosis. All tissues were not severe, but some of them may have indicated slight cirrhosis.

Oxidative stress

Clinically relevant concentrations of 6MP are toxic to rat hepatocyte cultures by a mechanism that involves oxidative stress, mitochondrial injury and ATP depletion (Tapner *et al.*, 2004). Our result also showed genes associated with oxidation, Loxl1 (lysyl oxidase-like 1) and Lrrc16 (leucine rich repeat containing 16), up-regulated at 6 h and 24 h. This can lead to irreversible de-energization and cell death by necrosis.

Apoptosis and necrosis

At histopathologic analysis, livers of 6 h after dosing 6MP appeared necrosis. Many genes associated with cell death were expressed at the livers, too. For 6MP treating, oxidant stress can activate cell death signaling pathways such as Bak1 (BCL2-antagonist/killer 1) and Casp6 (caspase 6). Although the increase in gene expression does not necessarily indicate that the relevant gene products are involved in apoptosis, these results are suggestive that the pathway involving Bak1, Casp6 and DNase 1 participates in 6MP induced apoptosis. Since apoptosis is controlled by interactions between pro- and anti-apoptotic machineries, the up-regulation of negative effectors of apoptosis may also contribute to regulating 6MP-induced apoptosis (Kim *et al.*, 2005).

Rivosome biogenesis

In the acute model, many genes associated with ribosome biogenesis have been reported to be up-regulated during the injury stage (Chung *et al.*, 2005). Rps6ka2 (ribosomal protein S6 kinase, polypeptide 2) was expressed highly at 6h in both low dose and high dose, while was down-regulated at 24 h and 72 h. Rps14 (ribosomal protein S14) was up-regulated in all except 72 h in the low dose.

Recovery

Most of gene expression profiles obtained from livers isolated 72 h after both low and high 6MP dose resembled the profiles of the control-livers. This suggests that the mice in the 72 h group did not suffer, or recovered from the toxic stimulus.

Validation of gene expression changes

To confirm the microarray results, we selected 5 (Hsd3b4, Krt2, Tuba1, Myo1f) known genes that are related to hepatotoxicity for semi-quantitative RT-PCR confirmation of differential expression. In general, the semi-quantitative RT-PCR results were similar to time-dependent results obtained with the DNA microarrays (Fig 3).

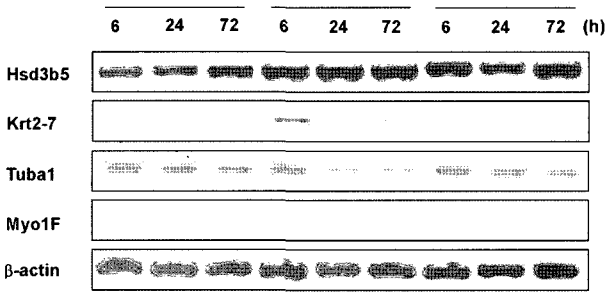

Fig. 3. Semi-quantitativeRT-PCR analysis of genes differentially expressed after 6MP-administered. The relative expression level of each gene was normalized by that of β-actin. From the list in Table 2, four of the genes involved in hepatotoxicity were selected for semi-quantitative RT-PCR. The primer sets used for RT-PCR are shown in Table 1. The RNA samples used for semi-quantitative RT-PCR were identical to those used for the microarray analysis.

Conclusion

In summary, acute exposure of 6-MP administered mice resulted in toxic manifestations and numerous gene expression changes. Mechanisms of 6MP-induced injury of liver cells includelipid peroxidation, mitochondrial damage and disruption of the cytoskeleton. These effects may play a role in 6-MP-induced hepatotoxicity and may be of particular toxicologicalrelevance. We expect that drug action related to hepatotoxicity may be detected in detail using our database.

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