Original Article

Induction of apoptosis and CYP4A1 expression in Sprague-Dawley rats exposed to low doses of perfluorooctane sulfonate

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ABSTRACT — In previous studies, perfluorooctane sulfonate (PFOS), an environmental organic compound, was reported to cause hepatotoxicity and hypolipidemia in rodents. However, the low dose toxicity of PFOS and the toxic mechanisms involved remain to be determined. To clarify the low dose toxicity and action mechanism in the target organ toxicity, Sprague-Dawley (SD) rats were orally administered with PFOS at the doses of 0, 1.25, 5, 10 mg/kg/day for 28 days. As a result, no death or abnormal symptoms were observed in all groups. The significant loss of mean body weight was observed in female rats treated with 10 mg/kg PFOS and the relative liver weight of 10 mg/kg PFOS-treated group was significantly greater compared to control. Histopathological examination revealed that fatty change was evident in the liver of male rats treated with PFOS (5 and 10 mg/kg) and hypertrophy and cellular swellings in females at the dose of 10 mg/kg, which showed different pattern of pathological lesions. In addition, we demonstrated the expression induction of hepatic caspase-3 and cytochrome P450 4A1 (CYP4A1) related with apoptosis and lipid metabolism, respectively. This study suggested that no-observed-adverse-effect level (NOAEL) of PFOS was 1.25 mg/kg in 28-day repeated toxicity study and, however, the toxic response showed gender differences. The possible toxic mechanism of PFOS was the induction of apoptosis and altering lipid metabolism which resulted in hepatotoxicity.

Key words: PFOS, General toxicity, Hepatotoxicity, Apoptosis, CYP4A1

INTRODUCTION

Since the 1950s perfluorooctane sulfonate (PFOS) and other perfluorinated alkyl acids (PFAAs) that metabolize into PFOS have been widely used for industrial applications that include carpet, floor polishes, coating approved for food contact and insecticide formulations (3M Company, 1999). Previous studies have revealed accumulation of PFOS in a variety of wildlife species including marine mammals, fishes and birds due to the widespread use and its chemical property which is resistant to environmental and metabolic degradation (Giesy and Kannan, 2000; Hansen *et al.*, 2001; Kannan *et al.*, 2001, 2002). Also, pharmacokinetic studies have shown that once absorbed PFOS is distributed and accumulat-

ed in the serum and liver but poorly eliminated (Seacat *et al.*, 2003). In rats, a serum elimination half-life of 7.5 days was reported after an oral treatment of PFOS (Johnson *et al.*, 1979); in cynomolgus monkeys, a half-life of 200 days was described (Seacat *et al.*, 2002); and in humans, a mean half-life of approximately 8.7 years was recently estimated from retired production workers (Thibodeaux *et al.*, 2003).

Toxicological profiles of PFOS have been reported in several animal species on repeated dose toxicity, genotoxicity and developmental toxicity. In the rat subchronic study, Goldenthal *et al.* (1978a) administered CD rats dietary levels of 0, 30, 100, 300, 1,000 or 3,000 ppm PFOS for 90 days. As a result, all of the rats in the 300, 1,000 and 3,000 ppm groups died. Death occurred between days

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13-25 and days 18-28 for the males and females, respectively, in the 300 ppm group. Similar evidence of cumulative toxicity was observed in primates after repeated dosing with PFOS in 90-day toxicity studies. Rhesus monkeys receiving 0.5, 1.5 or 4.5 mg/kg/day for 90 days showed lowering of total serum cholesterol and signs of liver toxicity at the 1.5 mg/kg/day dose level, and death occurred within 7 weeks in all monkeys in the 4.5 mg/kg/day dose-group (Goldenthal *et al.*, 1978b).

Otherwise, developmental or reproductive toxicities have been rather clearly evaluated in laboratory rodents (Lau *et al.*, 2003; Thibodeaux *et al.*, 2003; Yahia *et al.*, 2008). Timed-pregnant SD rats and CD-1 mice were given 1, 5, or 10 mg/kg PFOS by gavage daily beginning on GD 2 until term. In rats, significant deficits of maternal weight gain were seen in the 5 and 10 mg/kg PFOS groups. Lower maternal serum cholesterol and triglycerides were noted at 10 mg/kg, but liver weights were unaffected. In mice, PFOS did not alter the maternal weight gain appreciably, although liver weight of the dams was markedly elevated in the 5 and 10 mg/kg groups. In contrast to rats, PFOS did not significantly affect the mouse fetal weight, although the incidence of fetal mortality was slightly increased in the 10 mg/kg group. And PFOS is generally considered non-genotoxic and non-mutagenic. However, the general toxicity data focused on low dose exposure has been insufficient, although there have been developmental toxicity studies exposed to low doses of PFOS.

In regard to toxic mechanisms, PFOS has been suggested to interfere with mitochondrial bioenergetics, gap junctional intercellular communication, and fatty acid-protein binding in livers and cause peroxisomal proliferation in rodents (Berthiaume and Wallace, 2002; Hu *et al.*, 2002). Perfluorooctanoate (PFOA), another major perfluorooctanoate compound, induced apoptosis, as well as perturbed the cell cycle in vitro system (Shabalina *et al.*, 1999) and also caused increased beta-oxidation of fatty acids, increases in several cytochrome P450 (CYP)-mediated reactions and inhibition of the secretion of very low-density lipoproteins and cholesterol from the liver (Kennedy *et al.*, 2004). Although PFOS is expected to cause similar toxic effects as PFOA, the possible toxic mechanisms remain to be unclear compared with PFOA.

Recently, Cui *et al.* (2009) demonstrated toxicological profiles based on histological study in only male SD rats administered with 5 and 20 mg/kg of PFOS. Although obvious pathological alterations were observed in rats dosed at 20 mg/kg, all animals of the group died within 26 days and slight tumefaction and dark color were observed in the liver of 5 mg/kg group. Despite no-

observed-adverse-effect level (NOAEL) of PFOS would be expected to be lower than 20 mg/kg in rodent repeated dose study based on previous reports, the available low dose toxicity profile of PFOS has been extremely limited. Short-term exposure to high doses of PFOS induces various metabolic changes and toxic responses, however, extrapolation of these findings to the long-term and low-dose exposures to which humans are subject is highly problematic (Qazi *et al.*, 2010). Since additional experiments to investigate NOAEL and its toxic mechanism in the target organ are indispensable, SD rats were orally administered with 1.25, 5 and 10 mg/kg of PFOS for 28 days. In parallel, we focused on the involvement of apoptosis and CYP4A as toxic mechanisms in SD rats exposed to low doses of PFOS.

MATERIALS AND METHODS

Animals husbandry and maintenance

Five-week old male and female SD rats were purchased from Orient Bio Inc. (Gapyeong, Korea) and acclimated for 1 week prior to commencement of the study. Animals were randomly allocated to 4 groups, each consisting of 12 males and 12 females, housed in a room with barrier system, and maintained under following conditions: temperature of $23 \pm 1^\circ\text{C}$, relative humidity $55 \pm 5\%$, and 12 hr light-dark cycle. The animals were housed in plastic cages (three rats per cage) on soft chip bedding. The protocol for this study was approved by the committee of National Institute of Toxicological Research and rats were handled in an accredited Korea Food and Drug Administration animal facility in accordance with the AAALAC International Animal Care Policies (accredited unit; Korea Food and Drug Administration; Unit Number 000996).

Chemicals and experimental design

PFOS (Perfluorooctane sulfonic acid potassium salt, CAS No. 2795-39-3, > 98% pure) obtained from Fluka was dissolved in dimethylsulfoxide (DMSO, <0.1%) and adjusted to 1.25, 5, and 10 mg/kg PFOS using saline. PFOS was administered to the animals by gavage at volume of 2 ml/kg/day for 28 days. DMSO and Hoechst 33258 were purchased from Sigma (St. Louis, MO, USA). Clinical signs were observed daily and body weight and food consumption were measured twice a week. Prior to an autopsy, blood samples were collected from abdominal aorta under carbon dioxide anesthesia after starvation.

Cell culture and cytotoxicity assay

HepG2 cells were cultured in DMEM (Gibco, Grand Island, NY, USA) medium supplemented with 10% fetal

bovine serum (FBS; Gibco) and antibiotics (penicillin and streptomycin, Gibco) under a humidified atmosphere with 5% CO₂ at 37°C. The cell cultures were incubated with serum-free media containing PFOS (1, 3, 10, 30, 100, 300, 500 and 1,000 µM) in 96 well plate. The cytotoxicity of PFOS for 24 hr was quantified by MTT assay in HepG2 cells. After incubation with PFOS for 24 hr, MTT solution was added to each well.

Detection of apoptosis by fluorescence microscopy

The morphological characteristics associated with apoptosis were analyzed by fluorescence microscopy by staining cells with Hoechst 33258 DNA-binding dye. One day before treatment, cells were plated on 100 mm² dishes and then were treated with PFOS in the absence of FBS. After 24 hr, cells were fixed with 4% paraformaldehyde in phosphate buffered saline (PBS) for 15 min, then rinsed with PBS and stained with 1.0 µg/ml Hoechst 33258 in the dark for 10 min. Cells were observed for fluorescence and photographed using a digital camera (Nikon, Tokyo, Japan).

Hematology, serum chemistry and urinalysis

Whole blood was collected in EDTA-coated tubes and analyzed using ADVIA 120 analyzer (Bayer, UK) for following hematological parameters: total leukocyte count (WBC), erythrocyte count (RBC), platelet count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), RBC distribution width (RDW) and mean platelet volume (MPV). The serum biochemistry parameters including aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphate (ALP), albumin, triglyceride (TG), gamma glutamyl transpeptidase (γ-GT) and creatinine were evaluated using an autoanalyzer (Prestige 24i, Boeki Medical System, Tokyo, Japan). Urine samples collected by refrigerating metabolic cages (Tecniplast, Buguggiate, Italy) were analyzed for pH, protein, glucose, ketone body, nitrite, occult blood, bilirubin and leukocytes using dipsticks (Multisatix 10 SG, Bayer). Urine specific gravity was determined with a refractometer.

Histopathology

For light microscopy, following organs were excised, trimmed and weighed prior to fixation: adrenals; brain; pituitary; kidneys; heart; lung; spleen; liver; testes; epididymis; ovary; and uterus. All organs sampled were fixed in 10% neutral buffered formalin and were processed and embedded in paraffin, sectioned to the thickness of 5

µm, and stained with hematoxylin and eosin (H&E) for a microscopic examination (Olympus IX70, Tokyo, Japan).

Western blot analysis

Total proteins were isolated from tissue homogenates using protein extraction solution (iNtRON Biotechnology, Seongnam, Korea). Hepatic proteins (25 µg) were separated by 10% SDS-PAGE (Invitrogen, Carlsbad, CA, Italy), blotted onto a nitrocellulose membrane, blocked with 5% skim milk and incubated with primary antibodies against anti-caspase-3 (1:500), CYP4A1 (1:1,000) or β-actin (1:1,000). The membranes were washed for 15 min with PBS containing 0.1% Tween-20 and incubated with horseradish peroxidase-conjugated anti-goat (1:1,000) or anti-mouse IgG (1:1,000). The blots were detected with ECL plus chemiluminescent detection reagent (Amersham Biosciences, Little Chalfont, UK).

RNA extraction and quantitative real-time PCR

Total RNA was extracted from frozen livers using TRIzol Reagent (Invitrogen) according to the manufacturer's protocol. Quantitative real-time PCR was performed by addition of Universal PCR Buffer and TaqMan primer/probe assay reagent specific for CYP4A1. All probes were purchased from Applied Biosystems as Assay-on-Demand™ Gene Expression Products (TaqMan MGB, FAM dye-labeled). Thermal cycling was performed with a real-time sequence detection platform (ABI Prism 7500, Applied Biosystems, Foster City, CA, USA) with cycling parameters of 50°C for 2 min for activation, 95°C for 10 min, and 40 cycles of 95°C for 15 sec and 60°C for 1 min. The Ct values obtained for the genes were normalized to GAPDH, and the derived fold increases compared with the controls.

TUNEL staining

To detect apoptosis, paraffin-embedded sections were stained with terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) kits (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. Then the sections were examined using light microscopy (Olympus IX70).

Statistical analysis

Statistical analyses were performed using SigmaStat package (Version 10, Systat software). Treatment effects were analyzed using one-way ANOVA, followed by Dunnett's tests. Data comparisons were considered significant if $p < 0.05$.

RESULTS

General observations and body weight

During the study, no remarkable changes in general appearance were observed and all rats survived until scheduled necropsy. Likewise, there were no significant differences in the body weight of male rats between control and treatment groups. However, in females, body weight significantly decreased in rats administered with 10 mg/kg PFOS in a time-dependent manner (Fig. 1).

Hematology, clinical biochemistry and urinalysis

The results of hematology and serum biochemical examinations are summarized in Tables 1 and 2. Triglyceride (TG) was significantly decreased by PFOS administration in male rats exposed to 10 mg/kg. However, urine analysis results showed no statistically significant changes in urine parameters for any of the endpoints measured (data not shown) as well as hematological analysis (Table 2).

Organ weights and histopathology

Relative liver weight significantly was increased in both male and female rats administered with 10 mg/kg PFOS ($p < 0.05$), however, the relative kidney weight was not changed (Fig. 2). All other relative organ weights measured of both male and female rats were not affected by doses of PFOS up to 10 mg/kg for 28 days. The histopathological study revealed morphological alterations mainly in the liver tissue due to the treatment with PFOS, however, it showed gender differences (Table 3). In the liver of male rats, the presence of fatty change was observed in a dose-dependent manner (Fig. 3B). Hepat-

ic hypertrophy and cellular swellings were clearly detected in 10 mg/kg PFOS-treated female rats. Except livers, there was no significant change in the incidence between control and PFOS-treated groups.

TUNEL assay and caspase-3 cleavage

To investigate whether PFOS induces apoptosis in the rat liver, the tissue sections were labeled with an *in situ* TUNEL assay. Apoptotic signals were markedly observed in the liver of rat administered with 10 mg/kg PFOS compared to control group (Figs. 3C and D). To examine the involvement of caspase pathway in the apoptosis, Western blot analysis for procaspase-3 was carried out. As a result, we observed marked decrease in the expression of procaspase-3 protein in the groups of PFOS treatment as the result of TUNEL assay (Fig. 4).

Hoechst 33258 staining

To determine the concentration of PFOS-treatment, cell viability was measured using the MTT assay in HepG2 cells. The cell viability was decreased dose-dependently in HepG2 cells and no significant cytotoxicity was not observed by 30 μ M PFOS following 24 hr treatment. To characterize the involvement of the apoptotic process in the cell death by PFOS, we analyzed cells by staining with Hoechst 33258 dye. An increased incidence of profound chromatin condensation and nuclear fragmentation was observed in HepG2 cells treated with 30 μ M of PFOS (Fig. 5B), however, vehicle control group did not induced these morphological changes relating to apoptosis triggered by PFOS (Fig. 5A).

Fig. 1. Body weight change in SD rats administered with PFOS (0, 1.25, 5, 10 mg/kg) for 4 weeks. (A), male; (B), female.

Low dose toxicity of PFOS in SD rats

Table 1. Serum biochemical values of SD rats administered with PFOS for 4 weeks

<i>Males</i>	0	1.25 mg/kg	5 mg/kg	10 mg/kg
AST (IU/l)	56.8 ± 16.4	64.2 ± 18.2	63.9 ± 12.8	89.4 ± 7.7*
ALT (IU/l)	41.0 ± 6.5	46.7 ± 13.3	47.8 ± 11.8	58.4 ± 17.0
ALP (IU/l)	170.8 ± 26.5	164.2 ± 31.7	187.8 ± 45.5	244.3 ± 45.7
γ-GT (IU/l)	1.75 ± 0.71	1.50 ± 0.55	1.67 ± 0.82	1.62 ± 0.74
CRE (mg/dl)	1.25 ± 0.08	1.27 ± 0.10	1.31 ± 0.08	1.15 ± 0.10
TG (mg/dl)	58.7 ± 34.8	59.9 ± 19.8	30.8 ± 14.2	12.9 ± 3.4*
ALB (g/dl)	4.11 ± 0.19	3.56 ± 1.32	4.13 ± 0.16	3.99 ± 0.23
<i>Females</i>	0	1.25 mg/kg	5 mg/kg	10 mg/kg
AST (IU/l)	80.8 ± 14.1	43.1 ± 17.5*	70.1 ± 19.9	87.3 ± 3.9
ALT (IU/l)	26.3 ± 3.9	36.5 ± 6.3	43.0 ± 9.9	49.3 ± 17.9
ALP (IU/l)	101.3 ± 31.3	87.1 ± 13.5	121.3 ± 25.3	141.1 ± 27.9
γ-GT (IU/l)	2.17 ± 0.8	1.86 ± 0.9	2.17 ± 0.9	2.12 ± 0.9
CRE (mg/dl)	1.23 ± 0.08	1.31 ± 0.09	1.28 ± 0.07	1.35 ± 0.09
TG (mg/dl)	26.8 ± 6.4	36.2 ± 6.4	33.4 ± 9.7	26.0 ± 9.2
ALB (g/dl)	4.27 ± 0.27	4.38 ± 0.16	4.27 ± 0.21	4.55 ± 0.26

*, Significantly different from the control group at the level of $p < 0.05$.**Table 2.** Hematological findings for SD rats administered with PFOS for 4 weeks (n = 8~10)

<i>Males</i>	0	1.25 mg/kg	5 mg/kg	10 mg/kg
WBC ($10^3/\text{mm}^3$)	9.4 ± 2.3	10.2 ± 3.7	10.6 ± 2.4	11.4 ± 3.4
RBC ($10^6/\text{mm}^3$)	8.1 ± 0.6	8.4 ± 0.6	7.9 ± 0.3	7.6 ± 0.9
Hemoglobin (g/dl)	16.2 ± 1.2	17.1 ± 1.1	16.5 ± 0.7	15.4 ± 1.9
Hematocrit (%)	47.4 ± 4.5	49.9 ± 4.0	47.5 ± 2.1	47.5 ± 3.3
MCV (fl)	58.6 ± 2.2	59.1 ± 1.6	60.5 ± 1.3	59.4 ± 1.3
MCH (pg)	20.0 ± 0.5	20.3 ± 0.4	21.0 ± 0.3	20.3 ± 0.5
MCHC (%)	34.2 ± 1.0	34.3 ± 0.8	34.7 ± 0.6	34.3 ± 0.9
RDW (%)	11.5 ± 0.4	11.5 ± 0.4	11.9 ± 0.4	11.5 ± 0.4
MPV (fl)	7.6 ± 0.6	8.1 ± 0.5	8.2 ± 0.3	8.6 ± 0.5
Platelet ($10^4/\text{ul}$)	105 ± 11.8	93 ± 14.2	95 ± 14.7	108 ± 13.2
<i>Females</i>	0	1.25 mg/kg	5 mg/kg	10 mg/kg
WBC ($10^3/\text{mm}^3$)	7.6 ± 1.8	8.3 ± 3.0	9.4 ± 3.6	8.5 ± 2.6
RBC ($10^6/\text{mm}^3$)	7.6 ± 0.2	7.8 ± 0.4	7.7 ± 0.3	7.7 ± 0.5
Hemoglobin (g/dl)	15.6 ± 0.5	16.0 ± 0.8	16.1 ± 0.6	15.4 ± 1.0
Hematocrit (%)	43.1 ± 1.9	45.1 ± 2.2	44.4 ± 2.5	42.7 ± 2.9
MCV (fl)	57.0 ± 0.9	58.2 ± 1.9	57.8 ± 1.9	55.5 ± 1.1
MCH (pg)	20.6 ± 0.4	20.6 ± 0.7	21.0 ± 0.4	20.0 ± 0.4
MCHC (%)	36.1 ± 0.7	35.3 ± 1.1	36.4 ± 1.0	36.0 ± 0.6
RDW (%)	10.5 ± 0.4	10.8 ± 0.3	10.6 ± 0.4	10.5 ± 0.6
MPV (fl)	7.0 ± 0.7	7.6 ± 0.2	7.9 ± 0.3	7.5 ± 2.8
Platelet ($10^4/\text{ul}$)	99 ± 5.4	105 ± 13.8	112 ± 8.6	113 ± 13.4

Fig. 2. Relative weight of liver (A) and kidney (B) in SD rats administered with PFOS for 4 weeks.

Table 3. Histopathological findings for SD rats exposed to PFOS for 4 weeks

Dose of PFOS (mg/kg/day)	Males				Females			
	0	1.25	5	10	0	1.25	5	10
Liver								
Hypertrophy	0	0	0	0	0	0	0	8 ^a (67) ^b
Necrosis	1(8)	1(8)	0	2(17)	0	0	0	0
Microgranuloma	0	1(8)	0	2(17)	0	0	0	0
Fatty change	0	0	4(33)	8(67)	0	0	0	0
Cellular swelling	0	0	0	0	0	0	0	6(50)
Kidney								
Tubular basophilia	2(17)	2(17)	3(25)	1(8)	0	0	0	0
Spleen								
Fibrosis	0	1(8)	0	0	0	0	0	0
Epididymis								
Focal inflammation	0	0	1(8)	0	0	0	0	0
Uterus								
Dilatation	0	0	0	0	1(8)	0	4(33)	1(8)

^aNumber of animal with lesion

^bValues are processed as percentage

Expressions of CYP4A1 mRNA and protein in the liver

The effect of PFOS on CYP4A1 mRNA and protein expression was investigated, and GAPDH and β -actin were used as internal reference genes, respectively. PFOS significantly induced expression level of hepatic CYP4A1 mRNA at the doses of 5 and 10 mg/kg (Fig. 6B). In addition, the expression of CYP4A1 protein also increased in a dose-dependent manner (Fig. 6A).

DISCUSSION

Toxicological evaluations by repeated dose studies have been generally accepted, which has been advocated as a fundamental test for safety assessment. Although there have been previous reports on the toxicological profile of PFOS, however, available toxicological profiles of low dose PFOS including NOAEL have been limited.

In a recent, Cui *et al.* (2009) has clearly demonstrated histopathological observations and distribution results

Low dose toxicity of PFOS in SD rats

Fig. 3. Representative photographs of liver sections from the vehicle control (A and C) and 10 mg/kg PFOS (B and D) stained with hematoxylin-eosin (A and B) and TUNEL (C and D) in male rats (X100).

Fig. 4. Decrease of procaspase-3 expression in the liver of male SD rat exposed to PFOS. A, Densitometry analysis of procaspase-3 expression. B, Western blot analysis for procaspase-3 in the liver of SD rat. Western blot analysis for procaspase-3 showed a similar result between caspase cleavage and TUNEL staining. Details are described in Materials and Methods.

in only male SD rats administered with two doses of PFOS (5 and 20 mg/kg/day for 4 weeks). Although obvious pathological alterations were observed in rats dosed at 20 mg/kg, all animals of the group died within 26 days and slight tumefaction and dark color were observed in the liver of 5 mg/kg PFOS-treated group (Cui *et al.*, 2009). There is another report on consecutive low dose treatment study of PFOS, which was focused on neuroendocrine effects (Austin *et al.*, 2003). They have reported that PFOS induced significant body weight loss and increased norepinephrine concentrations in the paraventricular nucleus of the hypothalamus at dose of 10 mg/kg in the female rats. But, they used intraperitoneal route and exposed for 14 days, relatively short period, resulted in no significant changes was observed at the lower dose of 1 mg/kg PFOS in the parameters measured. One of the early study on subchronic toxicity published over 30 years ago showed the toxicity exposed to 0, 30, 100, 300, 1,000 or 3,000 ppm PFOS by feed. All of the rats in 300, 1,000 and 3,000 ppm groups died due to high dosing (Goldenthal *et al.*, 1978a). Since toxicological profiles of low dose PFOS in rodent repeated dose studies have been unclear, we aimed to demonstrate NOAEL of PFOS at low doses ranging from 1.25 to 10 mg/kg. During the study, body weights significantly decreased only

Fig. 5. Apoptotic morphological changes of nuclei in HepG2 cells treated with PFOS for 24 hr. HepG2 cells were exposed to test compounds and nuclear condensation was monitored with Hoechst 33258 staining as described in Materials and Methods. (A) Vehicle control; (B) 30 μ M PFOS. Arrows indicate apoptotic features of chromatin condensation. Magnification 100 $\times$.

Fig. 6. The expression levels of CYP4A1 protein (A) and mRNA (B) in male rat livers exposed to PFOS.

in 10 mg/kg-exposed female rats (Fig. 1). Since food consumption was significantly reduced at the dose of 10 mg/kg PFOS, this body weight decrease partially corresponded to the reduction in food intake as reported previously (data not shown). In serum biochemistry examinations, the significant elevation of AST and significant decrease in TG value was observed only in male rats exposed to 10 mg/kg PFOS (Table 1). These results were consistent that the associations between exposure and cholesterol and triglyceride levels have been observed

(Olsen *et al.*, 2003). Also, it is plausible that PFOS modified lipid metabolism, it could be related to the reduced body weight (Apelberg *et al.*, 2007). Histopathological findings revealed that fatty change was evident in the liver of male rats treated with 5 and 10 mg/kg PFOS. In contrast, hypertrophy and cellular swelling of hepatocytes were mostly observed only in the group of 10 mg/kg PFOS-treated female rats, which showed different pattern of pathological lesions. According to the previous report on gender differences, centrilobular to midzonal cytoplasmic hypertrophy of hepatocytes and focal necrosis was observed in the liver and the incidence and relative severity were greater in the males, and three males and two females in the 100 ppm group died prior to scheduled sacrifice (Goldenthal *et al.*, 1978a). In this study, we demonstrated the significant decrease of body weight only in highest female group (10 mg/kg) and different patterns of pathological lesions in the target organ in terms of gender difference and these results supported that NOAEL value was lower in the male rather than in females.

In order to investigate action mechanisms of PFOS in the target organ, we carried out microarray analysis using male liver samples. As a result, apoptosis and lipid/fatty acid metabolism-related genes were significantly changed by PFOS administration (fold change > 1.5) (data not shown). Based on these results, to investigate whether PFOS induces apoptosis in the liver, we conducted *in situ* TUNEL assay and Western blot analysis for caspase-3. We demonstrated that PFOS induced apoptosis in the rat liver via caspase pathway (Figs. 3 and 4). In addition, to prove the involvement of apoptosis *in vitro* system, HepG2 cell line with a wide variety of signal responses to different kinds of drugs was exposed to PFOS. As a result, morphological changes such as chromatin con-

densation were observed in HepG2 cells exposed to 30 μ M PFOS (Fig. 5). Recently, it has been reported that 50-200 μ M PFOS also induced dissipation of mitochondria membrane potential, boost ROS generation and affect genes expression of apoptotic regulators in HepG2 cells (Hu and Hu, 2009). PFOS is a structurally diverse class of chemicals that induce hepatomegaly in rodents which is characterized by the subcellular proliferation of organelles such as smooth endoplasmic reticulum, mitochondria, but most notably peroxisomes (Kennedy *et al.*, 2004; Vanden Huevel *et al.*, 2006). Maggiora *et al.* (2010) demonstrated that peroxisome proliferator-activated receptor α (PPAR α) protein content increased in HepG2 cells treated with clofibrate, causing apoptosis and it may be concluded that PPAR α is chiefly related to apoptosis and PPAR γ to cell proliferation.

PPARs and another nuclear receptors superfamily, liver X receptor, are particularly important as master regulators of lipid and lipoprotein metabolism (Li and Glass, 2004). The PPAR α agonist includes β -oxidation of fatty acid, increase in several CYP-mediated reactions, and inhibition of secretion of very low density lipoproteins and cholesterol from the liver. The reduction in serum TG, hypolipidemia, which was observed in this study was consistent to the previous reports. Hypolipidemic effects have been known as sensitive changes observed in response to PFOS exposure (Seacat *et al.*, 2002; Thibodeaux *et al.*, 2003). To figure out causes which are related with the hypolipidemia by PFOS, we examined CYP4A1, a major hepatic enzyme responsible for lipid metabolism. The result showed significant increase of CYP4A1 expression in a dose-dependent manner (Fig. 6). This result supports that PFOS induced the CYP4A1 isozyme, which resulted in disturbing lipid metabolism through the peroxisome proliferation in the rat liver.

In summary, exposure to low dose PFOS for 28 days led to body weight loss, an increase in relative liver weight, a decrease in the level of triglyceride and specific histopathological hepatic lesions as previously reported above 10 mg/kg PFOS. PFOS revealed a significant hepatotoxicity in SD rats at the dose level of 5 and 10 mg/kg, however, the toxic response to PFOS showed the apparent gender difference. Finally, PFOS primarily affected liver by inducing apoptotic signals and CYP4A1 expression which might be a potent cause of blood hypolipidemia. Further study on other genes responsible for lipid metabolism is promising to elucidate the mechanism of hypolipidemia by PFOS-treatment and is needed to examine molecular mechanisms involved in gender differences.

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