Free Rad. Res., Vol. 31, pp. 181–190
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Effect of Peroxisome Proliferator on Extracellular Glutathione Peroxidase in Rat

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Accepted by Prof. B. Halliwell

(Received 10 February 1999; In revised form 18 March 1999)

Glutathione peroxidase (GPX) activity measured using *tert*-butyl hydroperoxide as a substrate detects solely cellular/classical GPX (cGPX) in rat liver and kidney, and extracellular/plasma glutathione peroxidase (EC-GPX) in rat serum. To investigate the effect of peroxisome proliferator on EC-GPX, we measured activities of GPX and catalase in rat liver, kidney and serum, and then we performed immunoblot and Northern blot analyses in the kidney. Rats were fed on a diet containing either 2% (w/w) di-2-ethylhexyl phthalate (DEHP) or 0.25% (w/w) clofibrate for two or three weeks, respectively. Catalase activity was increased 1.4-fold ($p < 0.001$) in the treated liver, but not in the kidney. GPX activity was decreased to 59.2% (DEHP) and 70.4% (clofibrate) of the control ($p < 0.001$) in the serum but was unaltered in the liver and kidney. The immunoreactivity for EC-GPX was also significantly decreased in the DEHP-treated kidney compared with the control. The mRNA levels of EC-GPX and cGPX were unaltered. The immunostaining for 4-hydroxy-2-nonenal, a maker of lipid peroxide, was more intense in the treated kidney compared with the control. These results suggest that EC-GPX is post-transcriptionally decreased by peroxisome proliferator through the oxidative stress in the renal tubules. This may be a new deleterious effect of an endocrine disruptor DEHP.

Keywords: Glutathione peroxidase, microbodies, diethylhexyl phthalate, clofibrate, lipid peroxide, rat kidney

INTRODUCTION

Antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase (GPX) and catalase, play an important role in tissue protection against oxidative stress induced by reactive oxygen species (ROS). Extracellular/plasma glutathione peroxidase (EC-GPX), an isoenzyme of selenium (Se)-containing GPX, is predominantly produced in renal proximal tubules[1,2] and secreted to the blood.[1] Rodent liver abundantly expresses the cellular/classical GPX (cGPX), but not EC-GPX.[3,4] Only a trace amount of EC-GPX remains in renal tubules, and thus cGPX represents > 99% of GPX activity in kidneys.[4] On the other hand, serum GPX activity is solely

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represented by EC-GPX, but not by cGPX.[4] Thus, tissue distribution of EC-GPX is different from that of cGPX.

EC-GPX and cGPX are homotetramers consisting of subunits with a similar molecular size.[2,5] EC-GPX has a similar catalytic action to that of cGPX because of high homology in the active site.[6] They catalyze the reduction of organic hydroperoxides and hydrogen peroxide (H_2O_2) using reduced glutathione as a substrate. We previously demonstrated that the expression of both EC-GPX and cGPX decreased in a parallel manner in Se deficient rat.[4]

Peroxisome proliferators include a wide range of chemicals such as hypolipidemic drugs, industrial plasticizers, halogenated hydrocarbon solvents and herbicides.[7–9] These structurally diverse compounds produce similar effects of proliferation of peroxisomes and a differential increase in peroxisomal enzymes, most markedly in rodent liver and also significantly in the kidneys.[10] Peroxisome proliferators are non-genotoxic but induce hepatocellular hypertrophy, hyperplasia and, if chronically exposed, carcinoma in rodents. Peroxisomal proliferator induces the β-oxidation (H_2O_2 producing) enzyme fatty acyl-CoA oxidase by 30-fold,[11] and also increases lipid peroxide,[12] leading to formation of 8-hydroxydeoxyguanosine in liver DNA.[13] Thus, the oxidative stress through overproduction of H_2O_2 and other ROS can mediate hepatic or renal cellular damage by peroxisome proliferator.

Phthalate esters, widely used as a plasticizer, draw attention as one of the "environmental estrogens/endocrine disruptors", which are toxic to sex organs.[14–16] Not only phthalate but also other chemical types of peroxisome proliferator, WY-14643 and gemfibrozil, show such endocrine-disrupting effect.[17] Adverse effect of phthalate on sex organ function is only one example of a diverse biological function of this compound. Elucidating the mechanism of biohazard caused by plasticizers is of critical importance, because such compounds prevail widely in the environment. Peroxisome proliferation as a potential source of free radicals may play a key role in such biohazard.

To our knowledge, the effect of peroxisome proliferators on EC-GPX has not yet been reported. In the present study, two peroxisome proliferators, DEHP and clofibrate, were tested. To determine the effects of peroxisomal proliferator on the level of EC-GPX, we measured the enzyme activity of GPX in serum, and the immunoreactive protein and the mRNA for EC-GPX in kidney. To visualize the free radical injury in kidney by DEHP, we immunostained renal tissue section for 4-hydroxy-2-nonenal (HNE).

The peroxisome proliferators induced a selective decline of serum EC-GPX level, without affecting the level of cGPX in the liver and kidney. The decrease was post-transcriptional modification, possibly by free radical protein damage, suggesting a new effect of peroxisome proliferator on antioxidant enzyme defense system *in vivo*.

MATERIALS AND METHODS

Animal Treatments

DEHP was purchased from Kanto Chemical Co., Tokyo, Japan. Clofibrate was kindly provided from Yamanouchi Pharmaceutical Co., Tokyo, Japan. Six-week-old male Sprague-Dawley rats were purchased from Japan SLC Inc., Shizuoka, Japan, and 6 rats each were assigned to the drug-treated and the control groups. In experiment 1, one group was fed on a diet containing 2% (w/w) of DEHP, and the other the same chow without DEHP, for two weeks. In experiment 2, the drug-treated group was fed on a diet containing 0.25% (w/w) of clofibrate, and the controls the same chow without clofibrate, for three weeks.

After the treatment with peroxisome proliferator animals were sacrificed under pentobarbital anesthesia (40 mg/kg). Total RNA was extracted from the freshly excised kidney. Blood was collected from the aorta. For assays of enzyme

activities, freshly excised tissue was homogenized in 10 volumes (w/v) of 10 mM ice-cold potassium phosphate/0.01% digitonin buffer, pH 7.4. The enzyme activity was assayed without freezing storage. Tissue was homogenized with 5 volumes (w/v) of 50 mM Tris-HCl/2 mM EDTA/2 mM 2-mercaptoethanol buffer (pH 7.4) for immunoblot analysis.

All animals received humane care in compliance with the Yamanashi Medical University's guidelines and the National Research Council's criteria for humane care as outline in "Guide for the Care and Use of Laboratory Animals".

Assay Methods for the Activities of Catalase and Glutathione Peroxidase

The catalase activity was measured by the method of Baudhuin *et al.*[18] One unit of activity is defined as the amount of enzyme causing the destruction of 90% of hydrogen peroxide per minute spectrophotometrically at 405 nm. The GPX activity in tissue homogenate and serum was assayed by the coupled enzyme method using *tert*-butyl hydroperoxide as a substrate.[19] One unit of enzyme is defined as μmol of NADPH oxidized per min per mg protein. The contribution of GSH transferase as GPX activity in the present system is minimal.[20] Protein was estimated by the method of Bradford.[21]

Immunoblot Analysis

The specificity of the antibodies against rat cGPX and rat EC-GPX has been described previously.[4,22] The polyclonal antibodies were raised against electrophoretically pure antigens by immunizing rabbits. The antibodies were monospecific and did not crossreact each other when evaluated by Ouchterlony plate and immunoblot analysis. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the procedure of Laemmli[23] using a 12.5% slab gel. After electrophoresis, the proteins were transferred onto a polyvinylidene difluoride membrane and incubated 150 min at room temperature with 1 : 3000 diluted cGPX[22] or EC-GPX antiserum,[4] followed by the incubation with [^{125}I]-labeled anti-rabbit IgG (MEM Life Science Products, Du pont Co., Boston, MA, USA; 74 kBq/membrane) for 90 min at room temperature. Antigen–antibody complex was visualized by autoradiography, using the Bio-imaging Analyzer Fujix BAS-2000 system (Fuji Photo Film Co., Tokyo, Japan).

Northern Blot Analysis

The cDNA probe for catalase (Accession number: M11670,[24] National Center for Biotechnology Information, NIH, Bethesda, MD, USA) was obtained from rat liver RNA by reverse-transcription followed by polymerase chain reaction (RT-PCR). Oligonucleotide primers (20 base pairs each) for PCR were designed using Gene Works version 2.4 software (Intelligenetics Inc., Campbell, CA, USA). The PCR product corresponding to the nucleotide position 906–1309 for catalase yielded single bands on 1.5% agarose gel electrophoresis. The cDNA probes for cGPX, EC-GPX and β-actin were described previously.[4]

Total RNA was isolated from the rat kidney using the Ultraspec-II RNA isolation system, Biotecx Laboratories Inc., Houston, TX, USA. Northern-blotting was performed as described previously.[4] In brief, denatured total RNA (20 μg) was subjected to agarose gel electrophoresis in the presence of 7% (v/v) formaldehyde. After alkalization and neutralization, the RNA was transferred onto a nylon filter by aspiration in a Genopirator nucleic acid transfer system, Model AE-6680, ATTO Corp., Tokyo, Japan. The RNA was fixed to the membrane by UV cross-linking. After prehybridization, the membrane was hybridized overnight with a ^{32}P-labeled probe, and then washed. Autoradiography was performed using the Bio-imaging Analyzer Fujix BAS-2000 system.

Immunohistochemical Staining for 4-hydroxy-2-nonenal

A specific monoclonal antibody against HNE is purchased from Japan Institute for the Control of Aging, Shizuoka, Japan.[25,26] The kidneys were fixed with 10% buffered formalin, dehydrated through a graded series of ethanol, and embedded in paraffin. Specimens were then cut into 2 μm thickness and stained according to the indirect immunoenzyme method as described previously.[27] In brief, following deparaffinization and rehydration, tissue sections were exposed to 3% H_2O_2 for 10 min to inactivate endogenous peroxidase activity and then to 10% normal goat serum for 30 min to block non-specific binding. The sections were incubated with 1 : 100 anti-HNE antibody overnight at 4°C. The tissue sections were sequentially exposed to 1 : 1000 peroxidase-conjugated goat anti-mouse IgG Fab (Bio-Rad Laboratories, Hercules, CA, USA) for 1 h. The staining was visualized by diaminobenzidine reaction.

Statistics

Data are expressed as the means and standard deviations (SD). Statistical significance was determined by the unpaired Student's *t*-test using SPSS version 7.5J software (SPSS Inc., Chicago, IL).

RESULTS

Body Weight and Organ Weight

The body weight of the DEHP-treated group was decreased by 6.9% and was significantly lower than that of the controls, while the body weight of the clofibrate-treated group was similar to that of the controls (Table I). The percent liver weight (g)/body weight (g) in both DEHP- and clofibrate-treated groups was increased 1.9- and 1.5-fold ($p < 0.001$) compared with the respective controls. The percent kidney weight (g)/body weight (g) in each treated group was also significantly higher than the respective controls, although the increase was milder in the kidneys than in the liver.

Activities of Catalase cGPX and EC-GPX

Table II summarizes the activities of catalase and cGPX in rat liver and kidneys treated with peroxisome proliferators. The catalase activity was increased 1.4-fold in both DEHP- and clofibrate-treated livers, and was significantly higher than in the respective control livers. The activity of catalase in kidney was unaffected by the treatment with both drugs. The activity of cGPX did not change significantly in the liver and kidneys by the treatment with either DEHP or clofibrate.

Serum GPX activity was significantly decreased in both DEHP- and clofibrate-treated

TABLE I Body weight and organ (liver and kidney) weights in rats treated with DEHP or clofibrate

	Body wt (g)	Liver/body wt (%)	Kidney/body wt (%)
Exp. 1			
Control ($n = 6$)	291 ± 2.5	3.99 ± 0.30	0.345 ± 0.011
DEHP ($n = 6$)	271 ± 10.7	7.59 ± 0.29	0.396 ± 0.032
	$p < 0.005$	$p < 0.001$	$p < 0.005$
Exp. 2			
Control ($n = 6$)	348 ± 7.6	4.16 ± 0.14	0.322 ± 0.009
Clofibrate ($n = 6$)	341 ± 10.0	6.06 ± 0.33	0.417 ± 0.021
	ns	$p < 0.001$	$p < 0.005$

Data are mean ±SD. Statistics: Student's *t*-test (unpaired). ns: not significant. Liver/body weight (wt): the percent of liver weight (g)/body wt (g); kidney/body wt: the percent of kidney weight (g)/body wt (g).

TABLE II Activities of catalase and cGPX in rats treated with DEHP or clofibrate

	Catalase		cGPX	
	Liver (mU/mg)	Kidney (mU/mg)	Liver (U/mg)	Kidney (mU/mg)
Exp. 1				
Control ($n=6$)	527 ± 35	197 ± 12	1.27 ± 0.19	826 ± 56
DEHP ($n=6$)	742 ± 74	172 ± 27	1.06 ± 0.13	818 ± 86
	$p<0.001$	ns	ns	ns
Exp. 2				
Control ($n=6$)	587 ± 74	200 ± 35	1.22 ± 0.07	840 ± 108
Clofibrate ($n=6$)	847 ± 46	204 ± 37	1.17 ± 0.12	846 ± 74
	$p<0.001$	ns	ns	ns

Data are mean ± SD. ns: not significant.

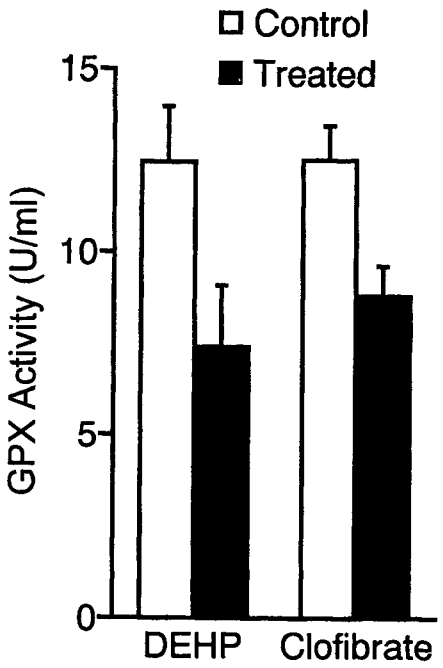

FIGURE 1 Effect of peroxisome proliferators on serum GPX activity. Data are expressed as U/ml ($n=6$). The brackets indicate SD. The enzyme activity of GPX was assayed in rat serum. The assay was performed in duplicate and one μl of rat serum was applied to assay mixture. The GPX activity is markedly decreased in the sera of both DEHP- and clofibrate-treated groups (closed bar) compared with respective control groups (open bar).

groups (Figure 1). The serum GPX activity, EC-GPX, in the DEHP- and clofibrate-treated groups was 59.2% and 70.4%, respectively, of that in the respective control groups.

Immunoblot and Northern Blot Analyses for cGPX and EC-GPX in Kidney

To elucidate the molecular mechanism of change in serum EC-GPX level, immunoblot and Northern blot analyses for cGPX and EC-GPX were performed in the kidney of the rats treated with DEHP, which had a greater effect on the serum EC-GPX activity than clofibrate did (Figure 1). The immunoreactivity for cGPX in the kidney homogenate of DEHP-treated group was in a similar intensity to that in the control group (Figure 2). On the other hand, the immunoreactivity for EC-GPX in DEHP-treated kidney was significantly decreased compared to the control kidney (Figure 2).

Figure 3 summarizes the result of Northern blot analysis. The kidney extract gives the mRNA bands for catalase, cGPX, EC-GPX and β-actin at the respective mobility indicated in the Figure 3. For each mRNA, the intensity does not appear to change by the treatment with DEHP. Densitometry ($n=6$ for each mRNA) revealed that the mean relative intensities to that of β-actin band for catalase, cGPX and EC-GPX were not significantly different from each other (data not shown).

Immunostaining for Lipid Peroxide in Kidney

Under light microscopy with routine hematoxylin-eosin staining, no appreciable degenerative

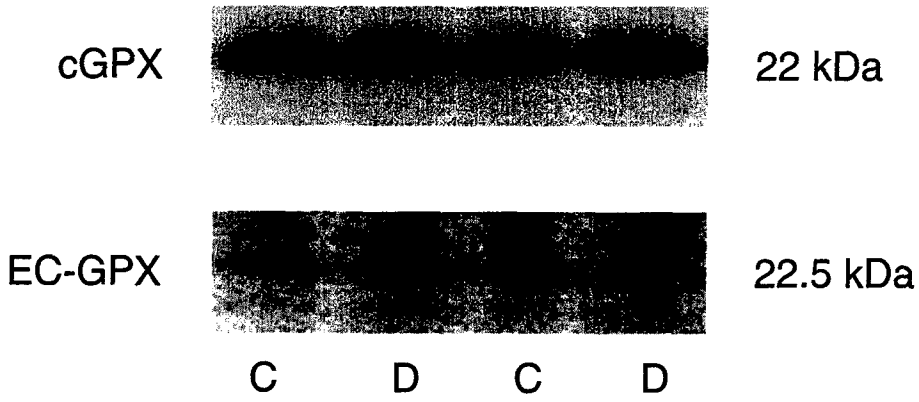

FIGURE 2 The immunoblot analysis for cGPX and EC-GPX in rat kidneys treated with DEHP. Data are the results of a representative experiment from 4 observations. For cGPX, a sample containing 40 μg of protein was loaded on each lane. For autoradiographic detection, the BAS-2000 imaging plate was exposed to the membrane for 1 h. A specific band was detected at the 22 kDa level in each lane. DEHP and control groups give an immunoreactive band with similar intensity (upper panel). For EC-GPX, a sample containing 400 μg of protein was loaded on each lane. The exposure time for autoradiography was 24 h. An immunoreactive band of EC-GPX was detected at the 22.5 kDa level in each lane. The immunoreactive bands were much less intense in the DEHP-treated group than in the control groups (lower panel). Abbreviations: C: control, D: di-2-ethylhexyl phthalate (DEHP)-treated group.

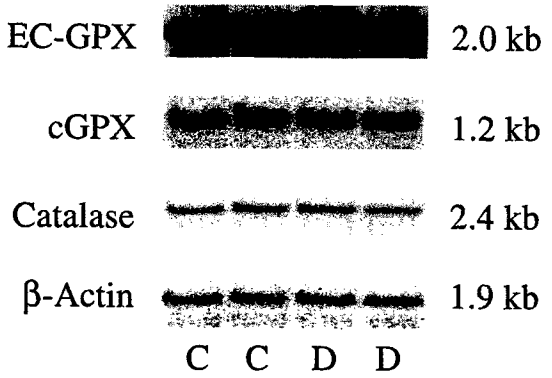

FIGURE 3 Northern blot analysis for EC-GPX, cGPX, catalase and β-actin mRNA in DEHP-treated kidneys. The Northern blot analysis was carried out as described in the Methods section. The sizes of mRNAs were determined on the basis of 18S and 28S rRNA visualized by ethidium bromide staining. Data are the results of a representative experiment from 6 observations. The same filter was used for the 4 blots. The bands of control (C) and DEHP-treated (D) groups appear to be similar in intensity for 4 mRNA studied here.

change was detected in the DEHP-treated kidney tissues (data not shown). The results of immunohistochemical staining for HNE are summarized in Figure 4. In the low power view, the DEHP-treated kidney (Figure 4B) is more intensely stained for HNE than the control kidney (Figure 4A). The high power view (Figure 4C and D) shows that the proximal tubules are stained more intensely than the distal tubules and the glomeruli are stained less intensely than the tubules. Each tissue component of DEHP-treated kidney (Figure 4D) is stained more intensely than the respective component of the control kidney (Figure 4C).

DISCUSSION

In the present study, rats were administered a standard dose of peroxisome proliferators for a relatively short period, so that we investigated an early phase of the drug treatment. The protein level of EC-GPX, but not cGPX, was decreased in the rats treated with the peroxisome proliferators, DEHP and clofibrate. Both DEHP and clofibrate appeared to induce a similar change in EC-GPX and catalase. The mRNA levels for both EC-GPX and cGPX were unaffected by the treatment with DEHP, indicating that the selective decrease in EC-GPX was due to post-transcriptional

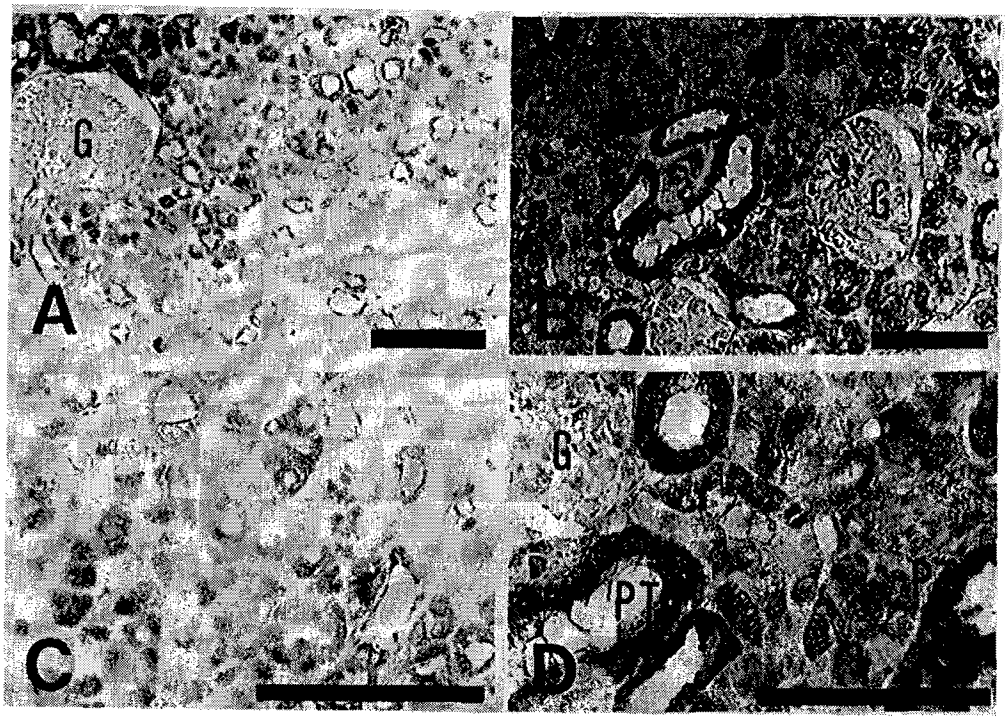

FIGURE 4 Immunohistochemical staining for 4-hydroxy-2-nonenal in DEHP-treated and control kidney. Data are the results of a representative experiment from 4 observations. Formalin-fixed paraffin-sections of rat kidneys obtained from control (A and C) and DEHP-treated group (B and D) are stained for 4-hydroxy-2-nonenal by indirect immunoenzyme method. The tubular epithelial cells in DEHP-treated kidney are more intensely stained than control. Magnifications: A and B, ×100; C and D, ×400. G: glomerulus, PT: proximal tubules. Bar = 100 μm.

regulation. The immunoblot analysis of EC-GPX in the kidney homogenate indicated that EC-GPX protein was decreased within the kidney and before the step of secretion into circulation.

Little is known about the regulatory mechanism of EC-GPX expression. We previously reported that Se deficiency suppressed the expression of both EC-GPX and cGPX in a parallel manner at pre-translational and translational levels.[4] Nafenopin, a peroxisome proliferator, is reported to decrease liver Se level, leading to the suppression of cGPX activity, but not renal Se level.[28] In the present study, the levels of cGPX in the liver and kidney were unaffected. Thus, the present change in EC-GPX did not appear to be due to the alteration of Se level.

Effect of peroxisome proliferator is most marked in rodent hepatocytes, because hepatocytes are most abundant in peroxisomes among all tissues.[7,9,11,29,30] The effect on kidney is less marked.[29,30] Among the kidney tissues, the proximal tubules are most rich in peroxisomes,[7,8] and therefore, most sensitive to the effect of peroxisomal proliferators.

cGPX is widely distributed in kidney of both cortex and medulla.[31–33] On the other hand, EC-GPX is more preferentially expressed in the proximal tubules than in other cellular components of kidney.[1,3] Thus, cGPX occurs in the proximal tubules and also in other kidney tissues that are not affected by peroxisomal proliferators. Even if both cGPX and EC-GPX decrease in a parallel manner in proximal renal tubules, preservation of cGPX in the rest of renal tissues can neutralize the change.

In the present study, the activity of catalase was increased in the liver but not in the kidneys by the treatment with the peroxisome proliferators.

In previous studies, catalase activity in the kidney treated with peroxisome proliferator was variably reported to be increased[34] or unaltered.[30] In such studies, renal peroxisomes were activated by the treatment of peroxisome proliferator, as evidenced by the induction of acyl-CoA oxidase[34] and D-amino acid oxidase[30] more than that of catalase. Such peroxisomal oxidases produce H_2O_2 via direct two-electron reduction of molecular oxygen during oxidation of their specific substrates. Thus, peroxisome proliferator can enhance oxidative stress by an unbalanced induction of H_2O_2-producing system and scavenger.

The peroxisome proliferator significantly modulates renal metabolism in an early phase of treatment. Clofibrate markedly increases the activities of glucose 6-phosphatase, pyruvate carboxylase and phosphoenolpyruvate carboxykinase in kidney cortex and facilitates gluconeogenesis from glutamate, lactate, pyruvate, glycerol and malate in rat kidney cortex within one week of the feeding.[35]

There has been only one previous report demonstrating that the lipid peroxide production is enhanced in kidney by peroxisome proliferator.[12] Dehydroepi-androsterone (DHEA), a peroxisome proliferator, increased the NADPH-dependent lipid peroxidation estimated by the formation of thiobarbituric acid-reactive substances in microsomes of rat liver and kidney cortex. According to this report, the lipid peroxidation by DHEA was an early event, starting from day 2 and reaching maximal level at one week in male rats.[12]

Membrane lipids are one of the major targets of ROS, which initiate free radical chain reaction, resulting in the formation of lipid peroxidation products. Many kinds of aldehydes are generated as a consequence of lipid peroxidation caused by ROS in biological systems.[25] Some of these aldehydes are highly reactive and relatively long-lived than ROS. Among them, HNE is one of the major products derived from ω6-unsaturated fatty acids such as linoleic and arachidonic acids. HNE inhibits cellular synthesis of protein and DNA as the second attack.[36,37] In biological systems, HNE is considered to be the most reliable marker of lipid peroxidation.[25] In the present study, HNE was increased in DEHP-treated kidney. To our knowledge, this is the first histochemical evidence of lipid peroxide increase in kidney treated with peroxisome proliferator, suggesting free radical injury in renal proximal tubules.

A question arises whether increased HNE in the proximal tubules is due to decreased production of EC-GPX. EC-GPX is a secretory-type glycoprotein,[5] and remains in the secretory system even when it is retained in the proximal tubular cells. It does not afford protection against H_2O_2 of peroxisomal origin. Thus, increased HNE in the proximal tubules did not appear to be caused by the decreased EC-GPX. The function of EC-GPX has not yet been well established. EC-GPX has been suggested to reduce submicromolar levels of free fatty acid hydroperoxides and phosphatidylcholine hydroperoxides,[38] because of a high affinity to such substrate.[39]

Chronic DEHP treatment causes renal cysts in rodents and deteriorates renal function estimated by creatinine clearance.[40,41] Renal failure and interstitial nephritis occur after chronic clofibrate treatment in rat.[42] The present study has provided histochemical evidence that peroxisome proliferator induces free radical production from early phase of treatment, leading to protein loss in certain kidney tissue. Continuous peroxisomal activation associated with long-lasting enhanced oxidative stress can result in macro-histological kidney tissue destruction, as was reported previously.[40–42]

In conclusion, the observed decrease in EC-GPX by peroxisome proliferator can be due to protein modification by drug-induced oxidative stress in the renal proximal tubules. These results suggest that peroxisome proliferators induce the imbalance of not only intracellular redox status in renal proximal tubules but also the extracellular antioxidant enzyme defense system *in vivo*. This

new function of DEHP potentially relates to an as yet unknown mechanism of diverse biological hazard caused by this endocrine disrupting substance.

Acknowledgments

This work was supported by Grant-in-Aid #08670866 and #10670714 from the Ministry of Education, Science and Culture of Japan. The authors would like to thank Keiko Kagami for her technical assistance.

References

[1] N. Avissar, E.A. Kerl, S.S. Baker and H.J. Cohen (1994) Extracellular glutathione peroxidase mRNA and protein in human cell lines. *Archives of Biochemistry and Biophysics*, **309**, 239–246.

[2] R.L. Maser, B.S. Magenheimer and J.P. Calvet (1994) Mouse plasma glutathione peroxidase. cDNA sequence analysis and renal proximal tubular expression and secretion. *Journal of Biological Chemistry*, **269**, 27 066–27 073.

[3] S. Yoshimura, K. Watanabe, H. Suemizu, T. Onozawa, J. Mizoguchi, K. Tsuda, H. Hatta and T. Moriuchi (1991) Tissue specific expression of the plasma glutathione peroxidase gene in rat kidney. *Journal of Biochemistry*, **109**, 918–923.

[4] T. Nakane, K. Asayama, K. Kodera, H. Hayashibe, U. Uchida and S. Nakazawa (1998) Effect of selenium deficiency on cellular and extracellular glutathione peroxidase: immunochemical detection and mRNA analysis in rat kidney and serum. *Free Radical Biology and Medicine*, **25**, 504–511.

[5] K. Takahashi, N. Avissar, J. Whitin and H. Cohen (1987) Purification and characterization of human plasma glutathione peroxidase: a selenoglycoprotein distinct from the known cellular enzymes. *Archives of Biochemistry and Biophysics*, **256**, 677–689.

[6] B. Ren, W. Huang, B. Akesson and R. Ladenstein (1997) The crystal structure of seleno-glutathine peroxidase from human plasma at 2.9 Å resolution. *Journal of Molecular Biology*, **268**, 869–885.

[7] I. Singh (1997) Biochemistry of peroxisomes in health and disease. *Molecular and Cellular Biochemistry*, **167**, 1–29.

[8] C. de Duve (1996) The peroxisome in retrospect. *Annals of the New York Academy of Sciences*, **804**, 1–10.

[9] M.S. Rao and J.K. Reddy (1996) Hepatocarcinogenesis of peroxisome proliferators. *Annals of the New York Academy of Sciences*, **804**, 573–587.

[10] P. Bentley, I. Calder, C. Elcombe, P. Grasso, D. Stringer and H.J. Wiegand (1993) Hepatic peroxisome proliferation in rodents and its significance for humans. *Food Chemical and Toxicology*, **31**, 857–907.

[11] P.G. Reddy, M.R. Nemali, M.K. Reddy, M.N. Reddy, P.M. Yuan, S. Yuan, T.G. Laffler, T. Shiroza, H.K. Kuramitsu and N. Usuda (1988) Isolation and sequence determination of a cDNA clone for rat peroxisomal urate oxidase: liver-specific expression in the rat. *Proceedings of the National Academy of Sciences of the United States of America*, **85**, 9081–9085.

[12] J. Swierezynski, P. Bannasch and D. Mayer (1996) Increase of lipid peroxidation in rat liver microsomes by dehydroepiandrosterone feeding. *Biochimica et Biophysica Acta*, **1315**, 193–198.

[13] A. Takagi, K. Sai, T. Umemura, R. Hasegawa and Y. Kurokawa (1990) Relationship between hepatic peroxisome proliferation and 8-hydroxydeoxyguanosine formation in liver DNA of rats following long-term exposure to three peroxisome proliferators; di (2-ethylhexyl) phthalate, aluminium clofibrate and simfibrate. *Cancer Letters*, **53**, 33–38.

[14] C.A. Harris, P. Henttu, M.G. Parker and J.P. Sumpter (1997) The estrogenic activity of phthalate esters *in vivo*. *Environmental Health Perspectives*, **105**, 802–811.

[15] B.J. Davis, R.R. Maronpot and J.J. Heindel (1994) Di-(2-ethylhexyl) phthalate suppresses estradiol and ovulation in cycling rats. *Toxicology and Applied Pharmacology*, **128**, 216–233.

[16] J.A. Thomas and M.J. Thomas (1984) Biological effects of di-(2-ethylhexyl) phthalate and other phthalic acid esters. *Critical Reviews in Toxicology*, **13**, 283–317.

[17] J.C. Corton, C. Bocos, E.S. Moreno, A. Merritt, R.C. Cattley and J.A. Gustafsson (1997) Peroxisome proliferators alter the expression of estrogen-metabolizing enzymes. *Biochimie*, **79**, 151–162.

[18] P. Baudhuin, Y. Beaufay, Li. Rahman, O.Z. Sellinger, R. Wattiaux, P. Jacques and C. de Duve (1964) Tissue fractionation studies. *Biochemical Journal*, **92**, 179–184.

[19] K. Dobashi, K. Pahan, A. Chahal and I. Singh (1997) Modulation of endogenous antioxidant enzymes by nitric oxide in rat C_6 glial cells. *Journal of Neurochemistry*, **68**, 1896–1903.

[20] O.A. Levander, D.P. Deloach, V.C. Morris and P.B. Moser (1983) Platelet glutathione peroxidase activity as an index of selenium status in rats. *Journal of Nutrition*, **113**, 55–63.

[21] M. Bradford (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Chemistry*, **72**, 248–254.

[22] K. Asayama, S. Yokata, K. Dobashi, H. Hayashibe, A. Kawaoi and S. Nakazawa (1994) Purification and immunoelectron microscopic localization of cellular glutathione peroxidase in rat hepatocytes: quantitative analysis by postembedding method. *Histochemistry*, **102**, 213–219.

[23] U.K. Laemmli (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685.

[24] S. Furuta, H. Hayashi, M. Hijikata, S. Miyazawa, T. Osumi and T. Hashimoto (1986) Complete nucleotide sequence of cDNA and deduced amino acid sequence of rat liver catalase. *Proceedings of the National Academy of Sciences of the United States of America*, **83**, 313–317.

[25] S. Toyokuni, N. Miyake, H. Hiai, M. Hagiwara, S. Kawakishi, T. Osawa and K. Uchida (1995) The monoclonal antibody specific for the 4-hydroxy-2-nonenal histidine adduct. *F.E.B.S. Letters*, **359**, 189–191.

[26] S. Toyokuni, X.P. Luo, T. Tanaka, K. Uchida, H. Hiai and D.C. Lehotay (1997) Induction of a wide range of C_{2-12} aldehydes and C_{7-12} acyloins in the kidney of wister rats after treatment with a renal carcinogen, ferric nitrilotriacetate. *Free Radical Biology and Medicine*, **22**, 1019–1027.

[27] K. Dobashi, K. Asayama, H. Hayashibe, N. Uchida, M. Kobayashi, A. Kawaoi and K. Kato (1991) Effect of diabetes mellitus induced by streptozotocin on renal superoxide dismutases in the rat. A radioimmunoassay and immunohistochemical study. *Virchows Archiv B*, **60**, 67–72.

[28] P. Garberg and M. Thullberg (1996) Decreased glutathione peroxidase activity in mice in response to nafenopin is caused by changes in selenium metabolism. *Chemico Biological Interactions*, **99**, 165–177.

[29] F.J. Gonzalez (1997) Recent update on the PPAR alpha-null mouse. *Biochimie*, **79**, 139–144.

[30] F.A. Reubsaet, J.H. Veerkamp, M.L. Bruckwilder, J.M. Trijbels and L.A. Monnens (1991) Peroxisomal oxidases and catalase in liver and kidney homogenates of normal and di(ethylhexyl)phthalate-fed rats. *International Journal of Biochemistry*, **23**, 961–967.

[31] T.D. Oberley, L.W. Oberley, A.F. Slattery and J.H. Elwell (1991) Immunohistochemical localization of glutathione-S-transferase and glutathione peroxidase in adult Syrian hamster tissues and during kidney development. *American Journal of Pathology*, **139**, 355–369.

[32] K. Dobashi, K. Asayama, H. Hayashibe, A. Munim, N. Uchida, A. Kawaoi and S. Nakazawa (1994) Immunohistochemical localization of cellular glutathione peroxidase in adult rat tissues by use of newly prepared polyclonal antibodies. *Yamanashi Medical Journal*, **9**, 69–79.

[33] K. Asayama, K. Dobashi, Y. Kawada, T. Nakane, A. Kawaoi and S. Nakazawa (1996) Immunohistochemical localization and quantitative analysis of cellular glutathione peroxidase in fetal and neonatal rat tissues: fluorescence microscopy image analysis. *Histochemical Journal*, **28**, 63–71.

[34] N.R. Nemali, N. Usuda, M.K. Reddy, K. Oyasu, T. Hashimoto, T. Osumi, M.S. Rao and J.K. Reddy (1988) Comparison of constitutive and inducible levels of expression of peroxisomal β-oxidation and catalase gene in liver and extrahepatic tissues of rat. *Cancer Research*, **48**, 5316–5324.

[35] C.R. Mackerer and J.R. Haettinger (1978) Renal gluconeogenesis in clofibrate-treated rats. *Journal of Pharmacology and Experimantal Theraputics*, **204**, 683–689.

[36] A. Fukuda, T. Osawa, K. Hitomi and K. Uchida (1996) 4-hydroxy-2-nonenal cytotoxicity in renal proximal tubular cells: protein modification and redox alteration. *Archives of Biochemistry and Biophysics*, **333**, 419–426.

[37] H. Bartsch, J. Nair and I. Velic (1997) Etheno-DNA base adducts as tools in human cancer aetiology and chemoprevention. *European Journal of Cancer Prevention*, **6**, 529–534.

[38] R. Mashima, Y. Yamamoto and S. Yoshimura (1998) Reduction of phosphatidylcholine hydroperoxide by apolipoprotein A-I: purification of the hydroperoxide-reducing proteins from human blood plasma. *Journal of Lipid Research*, **39**, 1133–1140.

[39] R.S. Esworthy, F.F. Chu, P. Geiger, A.W. Girotti and J.H. Doroshow (1993) Reactivity of plasma glutathione peroxidase with hydroperoxide substrates and glutathione. *Archives of Biochemistry and Biophysics*, **307**, 29–34.

[40] J.F. Crocker, S.H. Safe and P. Acott (1988) Effects of chronic phthalate exposure on the kidney. *Journal of Toxicology and Environmental Health*, **23**, 433–444.

[41] K.N. Woodward (1990) Phthalate esters, cystic kidney disease in animals and possible effects on human health: a review. *Human and Experimental Toxicology*, **9**, 397–401.

[42] A. Cumming (1980) Acute renal failure and interstitial nephritis after clofibrate treatment. *British Medical Journal*, **281**, 1529–1530.