Hematological Effects of Chlorine Dioxide on *In Vitro* Exposure in Mouse, Rat and Human Blood and on Subchronic Exposure in Mice

Hitoshi Ueno,* Yasuyoshi Sayato, and Katsuhiko Nakamuro

Faculty of Pharmaceutical Sciences, Setsunan University, 45–1, Nagaotoge-cho, Hirakata, Osaka 573–0101, Japan.

(Received October 12, 1999; Accepted December 16, 1999)

Hematological effects of chlorine dioxide (ClO_2) and its metabolites were investigated. *In vitro* exposure of mouse, rat and human blood cells to ClO_2 and the reduction by-product, chlorite (ClO_2^-) resulted in the formation of methemoglobin, a decrease in the activities of glucose-6-phosphate dehydrogenase (G-6-PD) and glutathione peroxidase (GPX) and in the content of reduced glutathione (GSH), and an increase in hydrogen peroxide (H_2O_2) formation and hemolysis. The H_2O_2 formation and hemolysis induced by ClO_2 and ClO_2^- in mouse blood cells were the highest among cells tested, and human blood cells were more resistant to the oxidative stress than rat and mouse blood cells. Both compounds also showed more toxic responses to *E. coli* mutants lacking production of catalase DSH19 (*katEG*), superoxide dismutase DSH56 (*sodAB*) and both of them DSH67 (*katEG sodAB*) than the wild strain DSH7 by *Kat-sod* assay, as a biological detection method for reactive oxygen species, suggesting the production of H_2O_2 and superoxide anion. For subchronic study of ClO_2, mice received drinking water containing 100, 1000, 1500 or 2000 mg/l ClO_2 in the presence of the stabilizer, 1200 mg/l of sodium bicarbonate *ad libitum* for 30, 60 or 90 days. Statistically significant hematological changes were observed in animals exposed to more than 1000 mg/l ClO_2, which showed augmented G-6-PD activity in erythrocytes and increased resistance to hemolysis in hypotonic solution. The results of this study therefore indicate that ClO_2 acutely causes hematotoxicity toward mice by producing reactive oxygen species and by weakening the protection systems to oxidative stress in erythrocytes, although the latter may be induced by long term exposure, while humans appear to be more resistant to this hematotoxicity.

Keywords —— chlorine dioxide, chlorite, hematological effect, *Kat-sod* assay, oxidative stress.

INTRODUCTION

It has been apparent that chlorine disinfection of drinking water containing trace organic compounds such as a naturally occurring humic acids results in the formation of trihalomethanes—principally chloroform.[1,2] The most common trihalomethane (THM), chloroform, has been shown in laboratory animal tests to induce hepatocellular carcinomas and kidney epithelial tumors in mice and rats, respectively.[3] Presently, alternative disinfection methods are still being actively explored as possible substitutes for chlorination of potable water supplies. Chlorine dioxide (ClO_2) disinfection is among the alternatives being considered for adoption because ClO_2 has far less tendency to generate THM during the water treatment.[4] Indeed, ClO_2 has been reported to have some important advantages over chlorine with respect to water quality and stability as a post-disinfectant in the Dutch drinking water system.[5] ClO_2 was recently proven to be effective for medical use, for example for dialysis monitor disinfection,[6] control of *Legionella pneumophila* in hospital water systems,[7] human immunodeficiency virus inactivation on waste disposal,[8] *etc.*

ClO_2 dissolved in water is gradually converted to chlorite (ClO_2^-) and chlorate (ClO_3^-) under neutral conditions. ClO_3^- is further reduced to chloride (Cl^-). It is reported to undergo 48% reduction to ClO_2^-, 22% to ClO_3^- and 28% to Cl^- within 42 h.[9] Studies in rats have revealed that

*To whom correspondence should be addressed: Faculty of Pharmaceutical Sciences, Setsunan University, 45–1, Nagaotoge-cho, Hirakata, Osaka 573–0101, Japan. Tel/Fax: +81-72-866-3123; E-mail: ueno@pharm.setsunan.ac.jp

ClO_2 is also converted to these reduction products in the body following oral administration and ClO_2 and its metabolites are eliminated into urine more rapidly than chlorine.[10] The reported potential health hazards in animals exposed to ClO_2 and its related compounds are the suppression of thyroid activity,[11] reproductive effects[12] and hematological effects including methemoglobinemia.[13,14] *In vitro* toxicological experiments utilizing rat blood treated with ClO_2 and its metabolites have also shown alterations in hematological parameters and induction of oxidative stress.[15] However, no clinically significant effects were detected in human healthy adult volunteers following either acute[16] or chronic[17] administration of ClO_2 and its by-products, and even on glucose-6-phosphate dehydrogenase (G-6-PD) deficient healthy volunteers who are likely to develop hemolytic anemia by oxidative stress.[18] However, the blood from this potential high risk group was shown to undergo methemoglobin formation and a decrease in reduced glutathione (GSH) level by *in vitro* treatment of ClO_2^-.[19] Thus, *in vitro* hematological assays may be more precise for measuring the difference between animals and humans in connection with susceptibility to oxidative stress when ClO_2 is assessed as a possible alternate disinfectant for drinking water supplies. The present study was therefore undertaken to develop *in vitro* hematological effects on mouse, rat and human blood exposed to ClO_2 and its metabolites in comparison with subchronic oral toxicity of ClO_2 in mice.

MATERIALS AND METHODS

Chemicals —— Fresh-prepared alkaline 0.5% ClO_2 solution containing 0.4% sodium bicarbonate ($NaHCO_3$) as a stabilizer was provided from Sukegawa Chemical Co., Ltd. (Kobe, Japan). The concentration of ClO_2 was determined by iodometric method as well as by the DPD method.[20] Sodium chlorite ($NaClO_2$) and sodium chlorate ($NaClO_3$) were purchased from Wako Pure Chemicals and Kanto Chemicals, respectively.

Blood Cell Exposure and Hematological Assays —— Male SPF ddy mice (20—30 g, 5 weeks of age) and male SPF Sprague-Dawley rats (120—180 g, 5 weeks of age) were obtained from Japan SLC, Inc., Hamamatsu. The animals were maintained at 23 ± 1 ℃, approximately 40% relative humidity, and a light/dark cycle of 12 h. The animals had free access to γ-ray-irradiated pellet chow (NMF, Oriental Yeast Co., Tokyo) and sterilized water for a week until a day before sacrifice. Blood was collected in heparinized tubes by cardiac puncture under anesthesia. Human blood was taken from healthy male nonsmoking volunteers with normal G-6-PD activity. Vessels containing blood of each species were incubated at 37 ℃ after the addition of ClO_2, $NaClO_2$ or $NaClO_3$ at final concentrations of 25, 50, 100, 250 or 500 mg/l.

The addition of blood to hypotonic solution was used to determine the osmotic fragility by measuring the concentration of hemoglobin released in the solution by the method of Dacie.[21] Twenty μl of blood sample was added to 2 ml of 0.1, 0.2, 0.3, 0.4, 0.5, 0.55, 0.6, 0.65, 0.75, and 0.9% NaCl solution, mixed gently and then left for 30 min at room temperature. After centrifugation at 500 × *g* for 10 min, the supernatant was read at 545 nm. The osmotic fragility was plotted as percent hemolysis against salt concentration and estimated as percent hemolysis against the maximal salt concentration showing 100% hemolysis in the control. Activities of G-6-PD,[22] glutathione peroxidase (GPX)[23] and GSH reductase,[24] and GSH content[25] in erythrocytes were assayed, respectively. Hydrogen peroxide (H_2O_2) formation and catalase activity were determined based on the complex formation of catalase and 3-amino-1,2,4-triazole in the presence of H_2O_2 according to the method of Heffernan *et al.*[26] Hemoglobin and methemoglobin contents were determined spectrophotometrically.[27]

***Kat-sod* Assay** —— *Kat-sod* assay was carried out with 4 strains of *Escherichia coli* DSH7 (*wild*: F^-, *lacY*, *rpsL*, *thi1*), catalase (CAT)-deficient DSH19 (*katEG*: DSH7 but *his*, *katE1*, *katG17*: Tn10), superoxide dismutase (SOD)-deficient DSH56 (*sodAB*: DSH7 but ϕ (*sodA'-'lacZ*) 49, ϕ (*sodB'-'kan*) IΔ2) and CAT-SOD-deficient DSH67 (*katEGsodAB*: DSH19 but ϕ (*sodA'-'lacZ*) 49, ϕ (*sodB'-'kan*) IΔ2).[28] In brief, each strain was cultured in LB medium at 37 ℃ to the concentration of 1 to 3 × 10^8 cells/ml. Twenty ml of LB-agar medium was poured into each plate, and a 10 mm i.d. hole was opened at the center after congelation. The 4 strains of overnight culture were then streaked radially from the center to make duplicate or triplicate lines for each strain. After adding 100 μl of a solution of the test chemical into each hole, the plates were incubated for 12 h at 37 ℃, and the lengths of growth inhibition were measured. Hydrogen peroxide (0.25 mol/l) and 8-hydroxy quinoline (0.69 mol/l) show specifically cytotoxic responses in DSH19 and DSH67, and DSH56 and DSH67, respec-

tively, and were used as the positive controls. Oxidative cell damage was judged as positive when the mean difference between distance of growth inhibition of the wild strain and the other strains ($n=2$) was estimated to be more than 4-mm.

Subchronic Study Protocol —— Male SPF ddy mice weighing 10—20 g (4 weeks of age) were allowed to acclimate to laboratory conditions as above for a week prior to initiation of exposures and were 5 weeks of age at the start of dosing and were randomly divided into groups of 10 animals each. Drinking water containing various concentrations of ClO_2 and 1200 mg/l $NaHCO_3$ (stabilizer of ClO_2) in freshly autoclave-treated tap water at the following levels: Control (no addition of $NaHCO_3$), 0, 100, 1000, 1500 or 2000 mg/l ClO_2. The animals received the drinking water and pelleted basal diet *ad libitum* for 30, 60 and 90 consecutive days, with the water being changed twice weekly. Animals were observed daily for any overt sign of toxicity. Body weight and consumption of food and water were measured twice weekly.

Blood was collected from the abdominal aorta of animals under pentobarbital anesthesia at the termination of the study and used for hematological assays. Osmotic fragility was assayed in the same manner as the *in vitro* assay. Organ weight measurement and histopathological examinations were also performed at the termination of the study. Serum clinical chemistry measurements were made for aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), G-6-PD, GSH, albumin, total protein, hemoglobin, methemoglobin, serum iron, bilirubin and urea nitrogen (BUN), uric acid, creatine, and creatinine.

Statistical comparisons between control and exposed groups were evaluated by a one-way analysis of variance (ANOVA). Group means were compared with control values using Dunnett's multiple comparison test.[29] The 95% ($p<0.05$) confidence level was chosen as the criterion of significance.

RESULTS AND DISCUSSION

Interspecies Susceptibility to Hematological Parameters

Considerable information has been accumulated on the hematological effects of ClO_2 and its metabolites in particularly animal studies. However, It is still important to investigate dif-

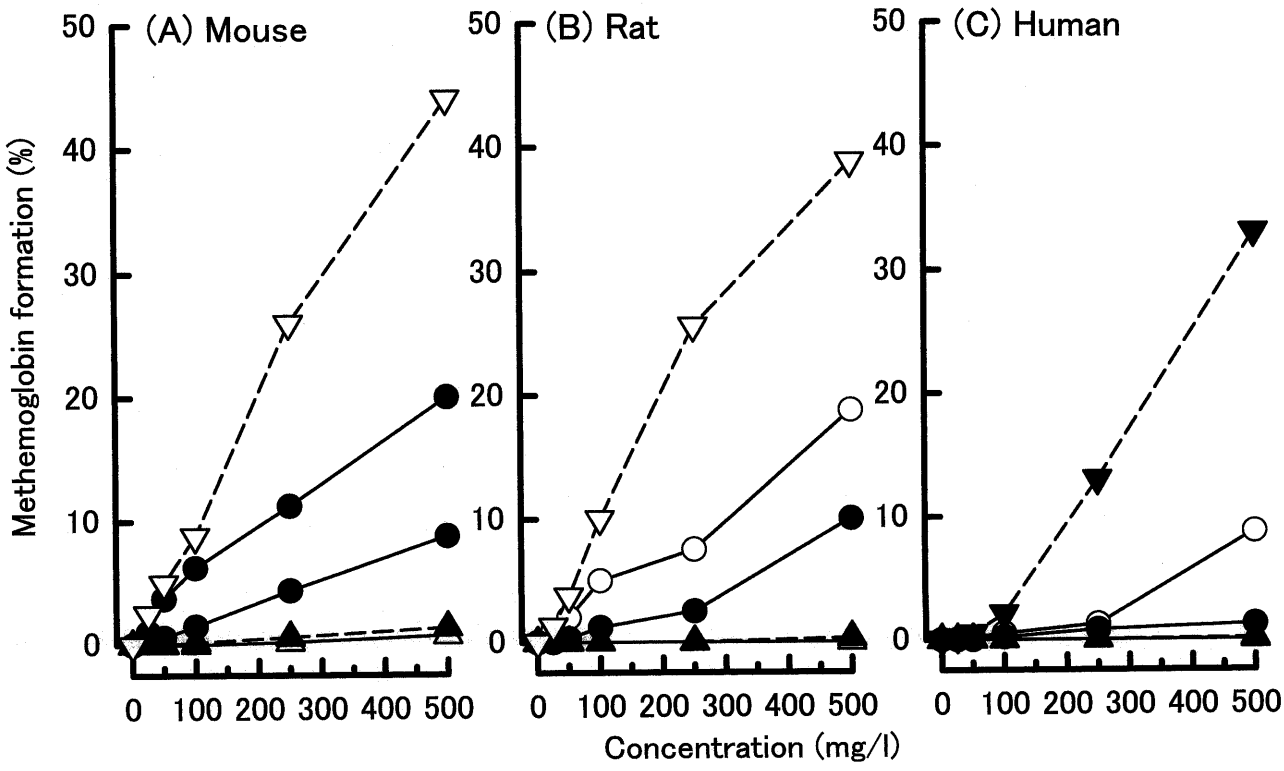

Fig. 1. Methemoglobin Formation by Exposure of Mouse (A), Rat (B) and Human Blood Cells (C) to ClO_2 and its Metabolites

Blood cells were incubated for 1 h at 37 ℃ with various concentrations of ClO_2 (○), $NaClO_2$ (●) or $NaClO_3$ (△). Another disinfectant, NaClO (▲) and the potent methemoglobinemia-inducer, $NaNO_2$ (▽) were also examined for the comparison (dotted line).

ferences between species susceptibility to these oxidative stressors for the assessment of human health effects. In this respect, methemoglobin formation may be one of the best indicators as a measure of the difference in the interspecies susceptibility to oxidative stressors, as described in the Introduction. As shown in Fig. 1, *in vitro* exposure of mouse, rat and human blood cells to oxidative stressors resulted in the formation of methemoglobin with ClO_2 and $NaClO_2$, as well as with $NaNO_2$, a potent methemoglobinemia-inducer. The potential of these agents was observed most significantly in mouse erythrocytes. However, no formation of methemoglobin by $NaClO_3$ was observed even in mouse erythrocytes, and sodium hypochlorite (NaClO) as another disinfectant was also less likely to induce methemoglobinemia. This result indicates that the hematological effects induced by ClO_2 are predominantly due to ClO_2 itself and the one electron-reduction metabolite, ClO_2^-. Further hematological parameters were therefore assayed by comparison of both compounds. Figure 2 shows that both compounds cause a decrease in activities of G-6-PD and GPX and GSH content in mouse and rat blood cells, while these effects were lowest in human blood cells. The H_2O_2 formation and hemolysis by ClO_2 and $NaClO_2$ were also most significant in mouse blood cells, and human blood cells were more resistant to this oxidative stress than rat and mouse (Fig. 3). Both compounds did not affect osmotic fragility (data not shown), although these agents are reported to enhance resistance to hemolysis.[15] As GSH reductase and catalase activities were also not affected by these oxidative stressors (data not shown), the elevation of H_2O_2 level seems to be related to decline of GPX activity and GSH content, and insufficient supplementation of NADPH by the lowered G-6-PD activity. However, the mechanism to generate H_2O_2 by either ClO_2 or ClO_2^- is unclear at the present, although a novel mechanism of hydroxyl radical genera-

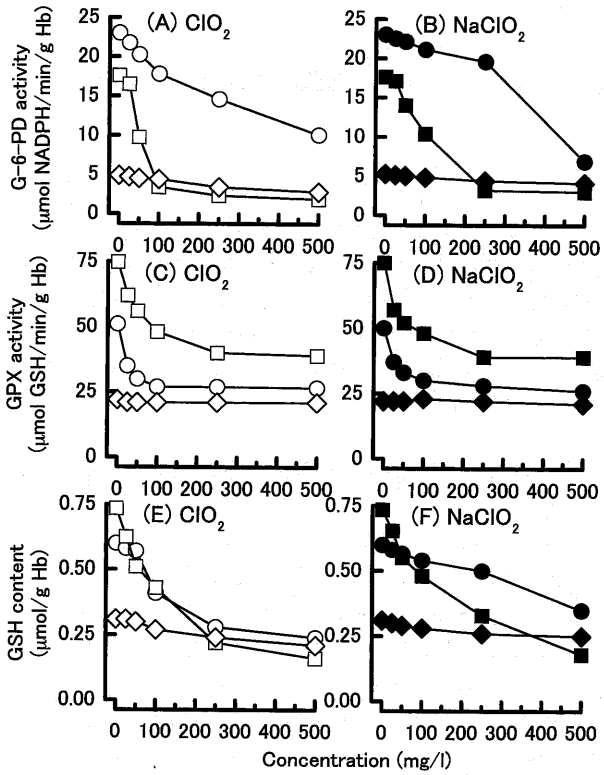

Fig. 2. Effect of ClO_2 and $NaClO_2$ on G-6-PD (A, B) and GPX Activities (C, D), and GSH Content (E, F) in Mouse, Rat and Human Blood Cells

Blood cells from mouse (○, ●), rat (□, ■) and human (◇, ◆) were incubated for 1 h at 37 ℃ with various concentrations of ClO_2 (opened symbol) or $NaClO_2$ (closed symbol).

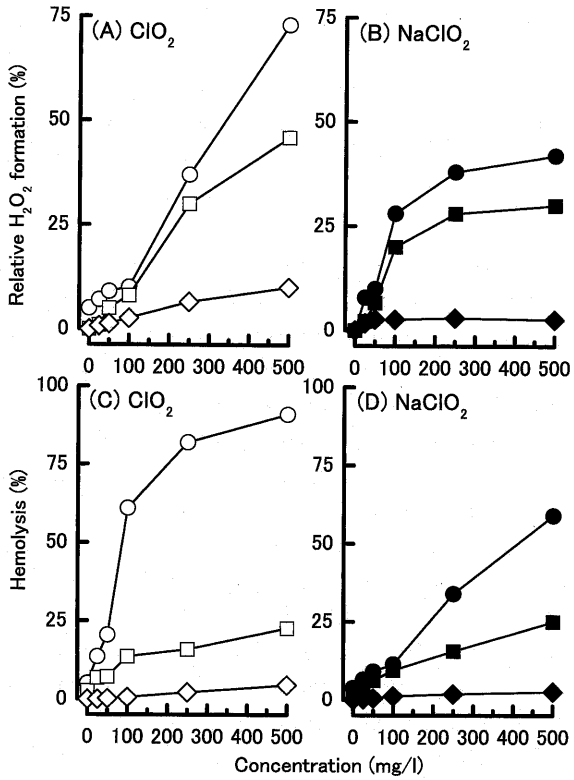

Fig. 3. Hydrogen Peroxide Formation and Hemolysis in Mouse, Rat and Human Blood Cells Exposed to ClO_2 and $NaClO_2$

Blood cells from mouse (○, ●), rat (□, ■) and human (◇, ◆) were incubated for 1 h at 37 ℃ with various concentrations of ClO_2 (opened symbol) or $NaClO_2$ (closed symbol). Relative H_2O_2 formation was estimated as percent inhibition of catalase activity in proportion to H_2O_2 level. Hemolysis represents percent hemoglobin released in isotonic salt solution.

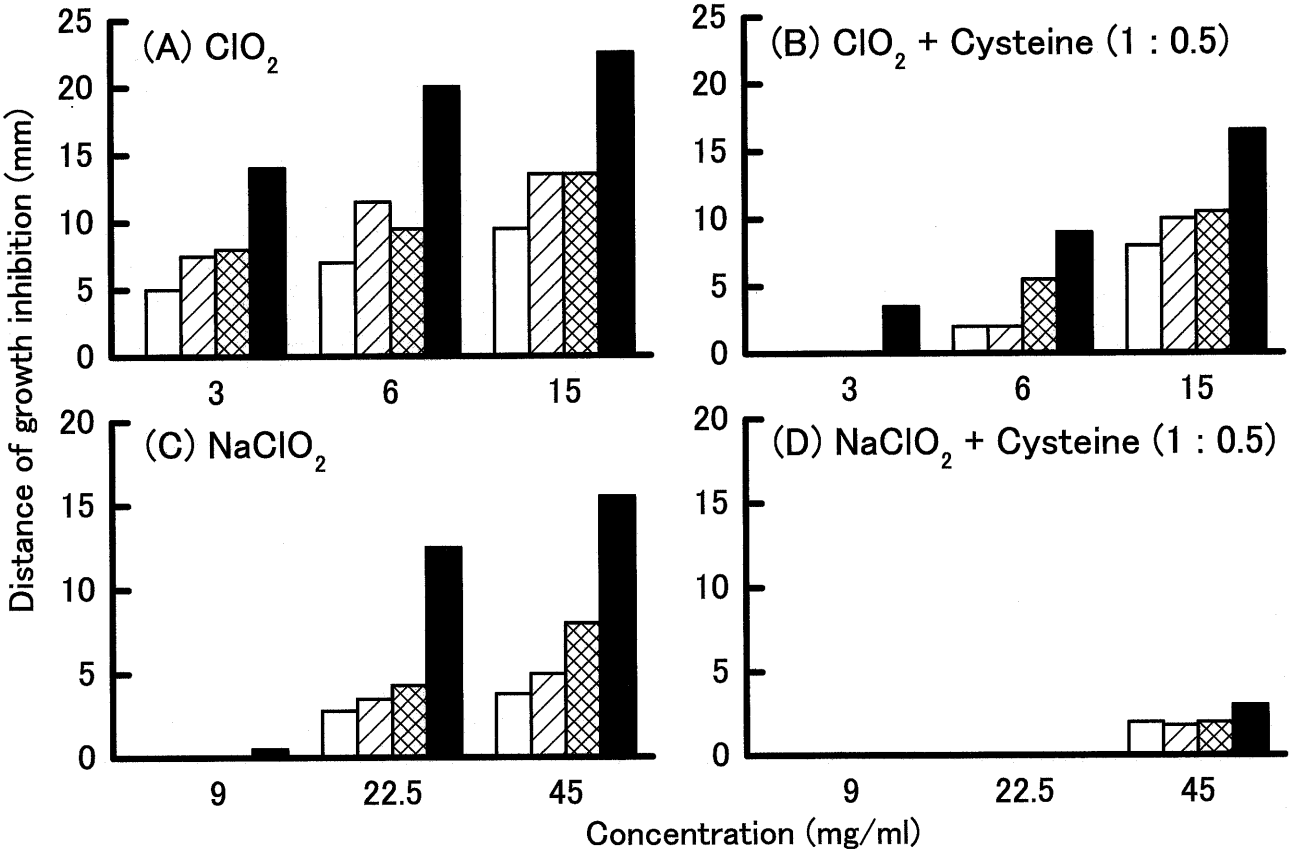

Fig. 4. Oxidative Cell Damage of ClO_2 and $NaClO_2$ with and without Cysteine by *Kat-sod* Assay

□: DSH7 (*Wild*), ▨: DSH19 (*katEG*, catalase-deficient), ▩: DSH56 (*sodAB*, SOD-deficient), ■: DSH67 (*katEGsodAB*, catalase and SOD-deficient).

tion via singlet oxygen by $NaClO_2$ and lactic acid has been proposed.[30] Another possibility for reactive oxygen production is reported to be due to interaction of the oxidant-induced methemoglobin with intracellular reducing agents such as ascorbic acid.[31] The feature of this mechanism is the requirement of heme iron, based on the Fenton-like reaction.

Figure 4 shows the oxidative cytotoxicity of ClO_2 and $NaClO_2$ in oxygen radical scavenger enzyme-deficient *E. coli* mutants by *Kat-sod* assay, as a biological detection method for reactive oxygen production. Both compounds showed more toxic response to *E. coli* mutants lacking producibility of catalase DSH19 (*katEG*), superoxide dismutase DSH56 (*sodAB*) and both of them DSH67 (*katEG sodAB*) than the wild strain DSH7, suggesting the generation of several reactive oxygen species. By the addition of cysteine, cytotoxic responses of both compounds were suppressed in all strains. This result suggests that ClO_2 and ClO_2^- directly generate some oxygen radicals without the requirement of heme iron.

The results of the *in vitro* study therefore indicate that mouse blood is most susceptible to oxidative stress by acute exposure to ClO_2, which produces reactive oxygen species and weakens the antioxidant systems, and human blood was most resistant to it.

Subchronic Hematological Effects of ClO_2 in Mice

As mouse seemed to be more susceptible to the hematotoxicity of ClO_2 following oral administration, a subchronic study of ClO_2 in mice was conducted. Mice received drinking water containing 100, 1000, 1500 or 2000 mg/l ClO_2 in the presence of the stabilizer, 1200 mg/l of sodium bicarbonate *ad libitum* for 30, 60 or 90 days. More than 1000 mg/l ClO_2-exposed groups showed a significant decrease in rate of mean body weight gain, as well as depressed consumption of food and water (data not shown). The average daily intake of ClO_2 estimated from the water consumption was 0.16, 1.00, 1.19 and 0.77 mg/animal/

d for the 100, 1000, 1500 and 2000 mg/l treatment group, respectively. Ten of the 30 mice exposed to 1500 mg/l ClO_2 and 26 of the 30 animals exposed to 2000 mg/l ClO_2 died before the 60 d- and 30 d-terminal euthanasia, respectively. These mice were characterized by a lack of motile activity, dysbasia and hyposthenia with extremely reduced consumption of food and water prior to death. Although darkish red eyes and cyanosis-like spots in the tail were also observed in the surviving animals of the 1500 and 2000 mg/l treatment groups, no apparent changes were seen in the 100 and 1000 mg/l groups. Significant increases in absolute weight of organ and organ-to-body weight ratio were observed in spleen for the 1000 mg/l group at 60 d and 90d and the 1500 mg/l group at 60 d, suggesting hematological effects related to ClO_2 exposure (data not shown). However, histopathological examinations did not reveal exposure-related changes in either the liver, kidneys, spleen, lung, heart, pancreas, stomach, duodenum or testes.

Hematological examinations resulted in no apparent effects related to ClO_2-exposure on AST, ALT, LDH, GSH, albumin, total protein, serum iron, bilirubin, BUN, uric acid, creatine and creatinine. As shown in Table 1, however, a significant increase in methemoglobin was observed in animals exposed to 1000 mg/l ClO_2 for 90 d and 1500 mg/l ClO_2 for 60 d, whereas a decrease in hemoglobin was shown conversely. Contrary to the results of the *in vitro* hematological assays, the exposure to more than 1000 mg /l ClO_2 caused augmentation of G-6-PD activity and a lowering of osmotic fragility in erythrocytes. Osmotic fragility is related to the stability of the erythrocyte membrane and this change by subchronic exposure to ClO_2 indicates an increased resistance to hemolysis in hypotonic solution. The G-6-PD supplies NADPH which is used as a cofactor for reduction of oxidized glutathione by GSH reductase. Exogenous GSH and NADPH also stabilize erythrocyte membrane and protect hemoglobin from release to the hypotonic solution (data not shown). These results therefore indicate that the antioxidant systems in mouse erythrocytes may be induced by long term exposure to ClO_2, although mouse blood was most susceptible to oxidative stress by *in vitro* acute exposure.

In conclusion, these findings demonstrate that although ClO_2 acutely causes hematotoxicity toward mice by producing reactive oxygen species and by weakening the protection systems to oxidative stress in erythrocytes, humans appear to be more resistant to this hematotoxicity. In this subchronic oral toxicity study in mice, the no-observed-adverse effect level (NOAEL) seems to be obtained when at least 100 mg/l ClO_2-containing drinking water was given. Considering the genotoxicity[32] and carcinogenicity,[33] as well as the interspecies susceptibility to oxidative stress and the assumed NOAEL, human health effects from ClO_2 may be

Table 1. Hematological Changes in Mice Received ClO_2-Containing Drinking Water for 30, 60 and 90 Consecutive Days[a]

Parameter measured	Interval on test (d)	Control	$NaHCO_3$	ClO_2 (mg/l)			
				100	1000	1500	2000
Hemoglobin (g/dl)	30	11.34±1.37	13.42±0.26	12.59±1.06	12.04±0.47	10.02±1.02	7.12
	60	12.92±0.50	12.93±0.51	11.52±1.86	9.21±0.85**,++	10.11±0.05**,++	–
	90	13.10±0.72	12.92±1.50	13.03±0.45	11.50±0.80	–	–
Methemoglobin (g/dl)	30	0.17±0.05	0.44±0.37	0.27±0.06	0.47±0.21	0.42±0.13	0.14
	60	0.14±0.03	0.16±0.01	0.17±0.03	0.22±0.09	0.58±0.16*,+	–
	90	0.23±0.12	0.26±0.04	0.34±0.02	0.44±0.04*,+	–	–
Osmotic fragility (%)[b]	30	100.0	100.0	88.7	52.8	46.7	27.5
	60	100.0	100.0	78.2	63.0	49.3	–
	90	100.0	100.0	83.0	57.8	–	–
G-6-PD (units/g Hb)	30	5.94±1.63	6.47±0.89	6.89±0.78	9.55±1.12	13.02±0.43*,+	18.73
	60	5.53±2.08	6.26±0.96	7.59±2.49	10.60±3.04	12.64±1.19*,+	–
	90	7.07±1.36	4.96±1.96	5.83±1.69	13.51±2.83*,++	–	–

a) Data are shown as the mean±S.D. for 3 samples which were made by mixing equally blood from 3—4 mice, with statistically significant differences compared to the control group at $p<0.05$ (*), $p<0.01$ (**), and $NaHCO_3$ group at $p<0.05$ (+), $p<0.01$ (++). – : Not determined for the necrosis.

b) Values represent percent hemolysis of control group.

at least the same as chlorine. As ClO_2 has some important advantages over chlorine with respect to water quality and stability when used for disinfection of drinking water,[5] this disinfectant should be taken into account for application to water oxidation/disinfection.

Acknowledgements The authors thank Dr. Hajime Nishioka (Doshisha University) for generously providing the *E. coli* strains for *Kat-sod* assay, and Susumu Sukegawa for his kind gift of ClO_2. This research was supported in part by Grants-in-aid from the Ministry of Health and Welfare of Japan on Toxicological Evaluation for Control of Chemicals Used in Waterworks.

REFERENCES

1) Rook J.J., *J. Am. Water Works Assoc.*, **68**, 168—172 (1976).
2) U.S. EPA, "Manual Of Treatment Of Techniques For Meeting The Interim Primary Drinking Water Regulations," EPA 600/8-77-005. U.S. EPA Water Supply Research Division, Cincinnati, OH, 1977.
3) NCI, "Report on Carcinogenesis Bioassay of Chloroform," Carcinogenesis Program National Cancer Institute, Bethesda, MD, 1976.
4) Werdehoff K. S., Singer P. C., *J. Am. Water Works Assoc.*, **79**, 107—113 (1987).
5) Wondergem E., van Dijk-Looijaard A.M., *Sci. Total Environ.*, **102**, 101—112 (1991).
6) Palo T.D., Atti M., Bellantuono R., Giordano M., Caringella D.A., *Blood Purif.*, **15**, 188—194 (1997).
7) Walker J.T., Mackerness C.W., Mallon D., Makin T., Williets T., Keevil C.W., *J. Ind. Microbiol.*, **15**, 384—390 (1995).
8) Farr R.W., Walton C., *Infect. Control Hosp. Epidemiol.*, **14**, 527—529 (1993).
9) Miltner, R.J., *AWWA Water Quality Technol. Conf.*, San Diego, CA, Paper No. 2A-5 (1977).
10) Abdel-Rahman M.S., Couri D., Bull R.J., *Environ. Health Perspect.*, **46**, 19—23 (1982).
11) Revis N.W., McCauley P., Bull R., Holdsworth G., *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 1485—1489 (1986).
12) Carlton B.D., Habash D.L., Basaran A.H., George E.L., Smith M.K., *Environ. Res.*, **42**, 238—245 (1987).
13) Couri D., Abdel-Rahman M.S., Bull R.J., *Environ. Health Perspect.*, **46**, 13—17 (1982).
14) Bercz J.P., Jones L., Garner L., Muray D., Ludwig D.A., Boston J., *Environ. Health Perspect.*, **46**, 47—55 (1982).
15) Abdel-Rahman M. S., Couri D., Bull R. J., *J. Am. Coll. Toxicol.*, **3** (4), 269—275 (1984).
16) Lubbers J.R., Bianchine J.R., *J. Environ. Pathol. Toxicol. Oncol.*, **5**, 215—228 (1984).
17) Lubbers J.R., Chauhan S., Miller J.K., Bianchine J.R., *J. Environ. Pathol. Toxicol. Oncol.*, **5**, 229—238 (1984).
18) Lubbers J.R., Chauhan S., Miller J.K., Bianchine J.R., *J. Environ. Pathol. Toxicol. Oncol.*, **5**, 239—242 (1984).
19) Moore G.S., Calabrese E.J., Ho S.C., *J. Environ. Pathol. Toxicol.*, **4**, 465—470 (1980).
20) Greenberg A.E., Clesceri L.S., Eaton A.D., "Standard Methods for the Examination of Water and Wastewater," American Public Health Association, New York, pp. 4-53—4-56, 1992.
21) Dacie J. V., "Laboratory Medicine Hematology, 5th ed.," St. Louis, Mosby, pp. 1020—1021, 1977.
22) Lohr G.W., Waller H.D., "Method of Enzymatic Analysis," Academic Press, NY, p. 636, 1974.
23) Whanger P.D., Weswig P.H., Schmitz J.A., Oldfield J.E., *J. Nutr.*, **107**, 1298—1307 (1977).
24) Schiltz E., Blatterspiel R., Untucht-Grau R., *Eur. J. Biochem.*, **102** (1), 269—278 (1979).
25) Toyo'oka T., Imai K., *J. Chromatogr.*, **282**, 495—500 (1983).
26) Heffernan W. P., Guion C., Bull R. J., *J. Environ. Pathol. Toxicol.*, **2** (6), 1501—1510 (1979).
27) Brown B.P., "Hematology: Principles and Procedures," Lea and Fbiger, p. 185, 1973.
28) Hayashi S., Kuraoka I., Ono T., Yamada K., Hiratsu K., Kimura T., Nishioka H., *The Science and Engineering Review of Doshisha University*, **32** (4), 56—61 (1992) (In Japanese).
29) Dunnett C. W, *Biometrics*, **20**, 482—491 (1964).
30) Bagchi D., Bagchi M., Douglas D. M., Das D. K., *Free Radic. Res. Commun.*, **17** (2), 109—120 (1992).
31) Benatti U., Morelli U., Guida L., De Flora A., *Biochem. Biophys. Res. Commun.*, **111** (3), 980—987 (1983).
32) Meier J. R., Bull R. J., Stober J. A., Cimino M. C., *Environ. Mutagen.*, **7**, 201—211 (1985).
33) Robinson M., Bull R. J., Schamer M., Long R. E., *Environ. Health Perspect.*, **69**, 293—300 (1986).