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Relation of 8-Hydroxydeoxyguanosine Formation in Rat Kidney to Lipid Peroxidation, Glutathione Level and Relative Organ Weight after a Single Administration of Potassium Bromate

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Changes in kidney levels of 8-hydroxydeoxyguanosine (8-OH-dG), lipid peroxidation (LPO), glutathione (GSH) and relative organ weight were examined 6, 24, 48, 72 and 96 h after a single i.p. administration of potassium bromate ($KBrO_3$) at a dose of 70 mg/kg to male F344 rats. The 8-OH-dG level was significantly increased 24 h after the treatment at this dose and thereafter gradually decreased. On the other hand, significant elevation in LPO level was observed from 6 h after the treatment with a continuous increase up to a plateau at 48 h and no subsequent drop. GSH level was significantly raised from 6 to 72 h, and relative kidney weight varied in almost the same manner as the 8-OH-dG level. Investigation of the dose-response relation revealed the 8-OH-dG and LPO levels to be significantly increased from a dose of 40 mg/kg $KBrO_3$ in a dose-dependent manner. The results suggest that enhanced formation of 8-OH-dG in kidney DNA due to $KBrO_3$ is closely related to the increase in LPO levels.

Key words: Potassium bromate — 8-Hydroxydeoxyguanosine — Lipid peroxidation — Glutathione — Kidney

Potassium bromate ($KBrO_3$) is an oxidizing agent which has been used as a food additive mainly for the treatment of wheat flour. This compound, however, was found to have carcinogenic potential, inducing renal cell tumors in male and female F344 rats after oral administration for 2 years.[1–4] Subsequently, promoting action for this compound was also established in a 2-stage rat renal carcinogenesis model.[5] Furthermore, mutagenicity of $KBrO_3$ was demonstrated in the Ames,[6] chromosome aberration[6] and micronucleus[7] tests. It is noteworthy that this mutagenic activity proved most remarkable in the micronucleus test *in vivo*, as compared to the other two tests *in vitro*. These findings suggest that $KBrO_3$ genotoxicity might be enhanced by biological reactions, although it is not known exactly what intermediates and processes are involved. However, it has been found that administration of $KBrO_3$ to rats significantly increases the lipid peroxidation (LPO) level in the kidney.[8] Because $KBrO_3$ has oxidizing properties, it has been suggested that oxygen radical species produced in the kidney might contribute to its renal carcinogenicity and production of LPO.

Recently, the importance of oxidative DNA damage to carcinogenic mechanisms has been stressed.[9] Furthermore, 8-hydroxydeoxyguanosine (8-OH-dG) was identified by Kasai *et al.* as an easily detectable and specific product of oxidative DNA damage.[10] Using this marker, it has already been shown that a significant increase of 8-OH-dG level occurs in kidney DNA after oral administration of $KBrO_3$, whereas no obvious change is found in the liver (non-target organ) DNA.[11] This was the first case where a significant increase of 8-OH-dG level induced by a chemical carcinogen was recognized in an *in vivo* experiment.

In the present study, the mechanism of 8-OH-dG formation was investigated, and the time course and the dose-response relation of changes in the levels of 8-OH-dG, LPO and glutathione (GSH) in the kidney of rats given $KBrO_3$ were simultaneously examined.

MATERIALS AND METHODS

Animals Five-week-old male F344 rats (specific-pathogen-free, Charles River Japan, Inc., Kanagawa) were given F-2 pellet basal diet (Funabashi Farm Co., Chiba) and tap water freely, and were used after 1 week of acclimation.

Chemicals $KBrO_3$ (food additive grade) was purchased from Matsunaga Chemical Industry, Hiroshima. Alkaline phosphatase and nuclease P1 were obtained from Sigma Chemical Co. and Yamasa Shoyu Co. Ltd., Choshi, respectively. All other chemicals used were of specific analytical or HPLC grade.

Treatment of animals with $KBrO_3$ $KBrO_3$ was dissolved in saline and 5 rats in each time/dose group were given a single i.p. administration of $KBrO_3$ or saline alone. In the time-course study, rats received the i.p. injection of $KBrO_3$ at a dose of 70 mg/kg and were killed 6, 24, 48,

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72 and 96 h after the treatment. In the dose-response study, rats were given the i.p. injection of $KBrO_3$ at doses of 0, 20, 40 and 80 mg/kg and killed 48 h after the treatment. The kidneys were immediately excised, weighed and used for measurement of 8-OH-dG, LPO and GSH levels.

Measurement of 8-OH-dG Kidney DNA was immediately isolated by the method of Kasai *et al.*[11] and digested to deoxynucleotides with nuclease P1 and alkaline phosphatase. 8-OH-dG levels (8-OH-dG/10^5 deoxyguanosine) were analyzed by HPLC with an electrochemical detection system (SPD-6A, Shimadzu Co., Ltd., Japan; Coulochem Model-5100A, ESA, USA).

Measurement of LPO level Malondialdehyde level (MDA, nmol/g wet tissue) was assessed as an index of LPO by the method of Uchiyama and Mihara.[12] A 0.1 g portion of kidney was homogenized with 0.9 ml of 1.15% KCl solution and the content of thiobarbituric acid-reactive substances measured.

Measurement of GSH level A 0.1 g portion of kidney was homogenized with 1 ml of 6% trichloroacetic acid and GSH level as non-protein thiol (μmol/g wet tissue) was estimated by the method of Ellman.[13]

Statistical analysis The data were analyzed for significance by using Student's *t* test.

RESULTS

Time-course study After the single i.p. administration of 70 mg/kg of $KBrO_3$, 8-OH-dG level in the kidney was distinctly elevated from 24 h to 96 h. At 24 h after the treatment, the highest level of 8-OH-dG was observed (5.06 ± 0.80, $P < 0.01$ as compared to the control level of 1.42 ± 0.29), with a gradual decrease thereafter, although

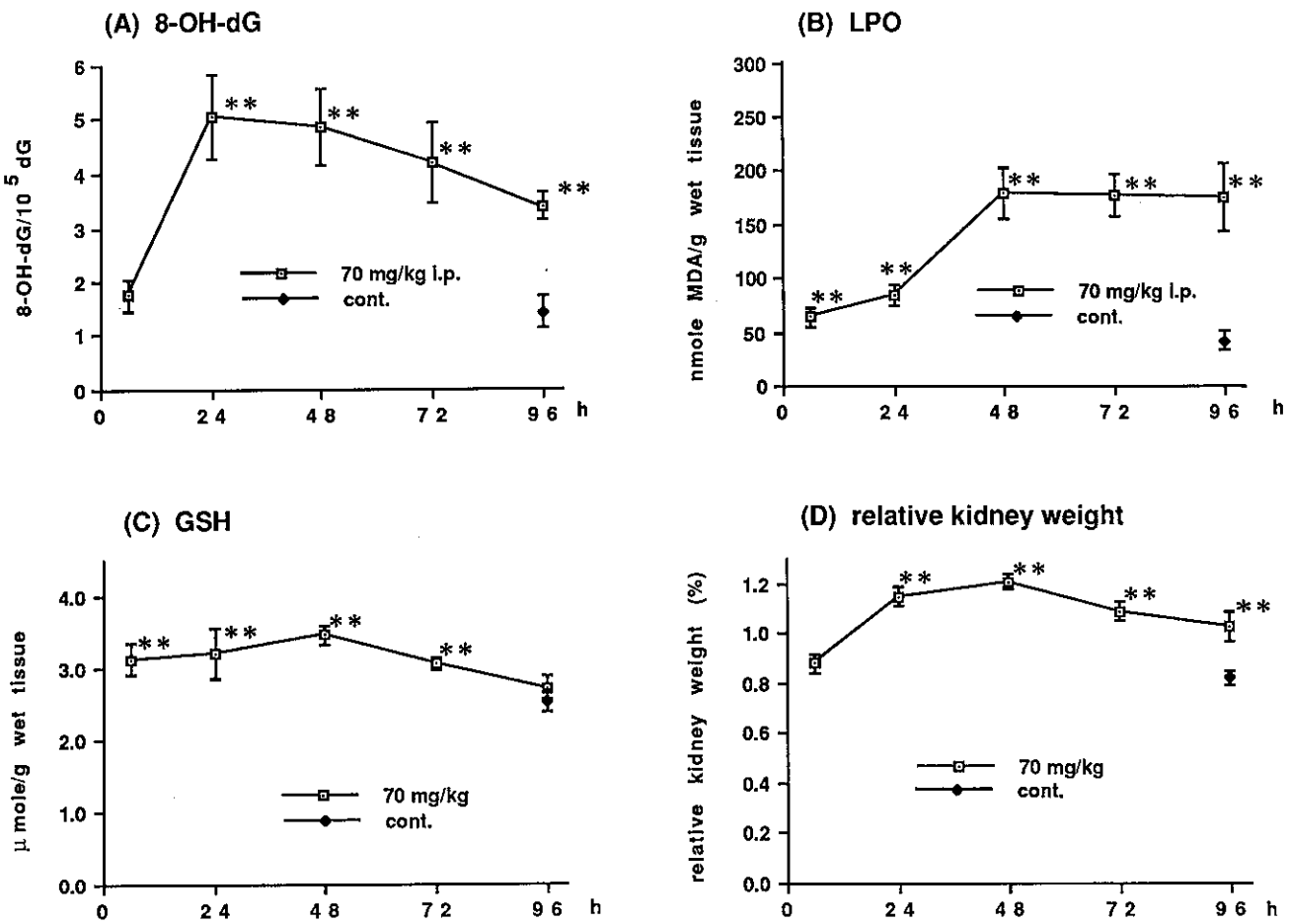

Fig. 1. Changes of (A) 8-OH-dG, (B) LPO, (C) GSH levels in the kidney, and (D) relative kidney weight after a single i.p. administration of 70 mg/kg of $KBrO_3$. Values represent the mean $\pm$ SD of data for five rats. Control animals were treated with saline alone and killed at the 96 h time point. *, ** Significantly different from control group values at * $P < 0.05$ and ** $P < 0.01$.

significant elevation was still evident at 96 h (3.39±0.27, $P<0.01$) (Fig.1 (A)). LPO level was significantly increased from 6 h after the treatment, reaching a plateau by 48 h, when the value was 4.3 times higher (178.8±23.4, $P<0.01$) than the control (41.8±8.5) (Fig. 1(B)). GSH level was significantly elevated from 6 to 72 h; it was highest at 48 h (3.46±0.14, $P<0.01$), then decreased to the control level (2.71±0.16) by 96 h (Fig. 1(C)). Relative kidney weight was significantly increased from 24 to 96 h. The highest value was observed at 48 h (1.21 ±0.03%, $P<0.01$ as compared to the control value of 0.82±0.03). Decrease was evident from 48 h, although it was still high at 96 h ($P<0.01$) ((Fig. 1(D)). With the increase in relative weight, the kidney became swollen with a dark-red appearance, but this change gradually disappeared after 48 h.

Dose-response study Rats were administered various doses of $KBrO_3$ (0 to 80 mg/kg) i.p. and then killed at 48 h, this time point being chosen because of the very high values for all parameters which were observed in the previous time-course study. The 8-OH-dG level was elevated with a dose of 40 mg/kg and above in a dose-dependent manner, the increase being significant at 40 mg/kg (2.79±0.97, $P<0.05$) and at 80 mg/kg (9.82± 1.44, $P<0.01$), as compared to the control value (1.28± 0.45) (Fig. 2(A)). LPO level was also similarly elevated in a dose-dependent manner, with significant increase at 40 mg/kg (54.1±7.7, $P<0.01$) and at 80 mg/kg (162.2 ±32, $P<0.01$), as compared to the control (38.1±6.3) (Fig. 2(B)). With regard to GSH level, a significant increase was observed only at the dose of 80 mg/kg (3.58 ±0.21, $P<0.01$ as compared to the control value of 2.49 ±0.12) (Fig. 2(C)). Relative kidney weight was also significantly increased at the dose of 80 mg/kg (1.30± 0.03%), accompanied with a change of color to dark-red and with swelling. No significant difference from the control level (0.83±0.02) was found for the dose of 20 mg/kg or 40 mg/kg (Fig. 2(D)).

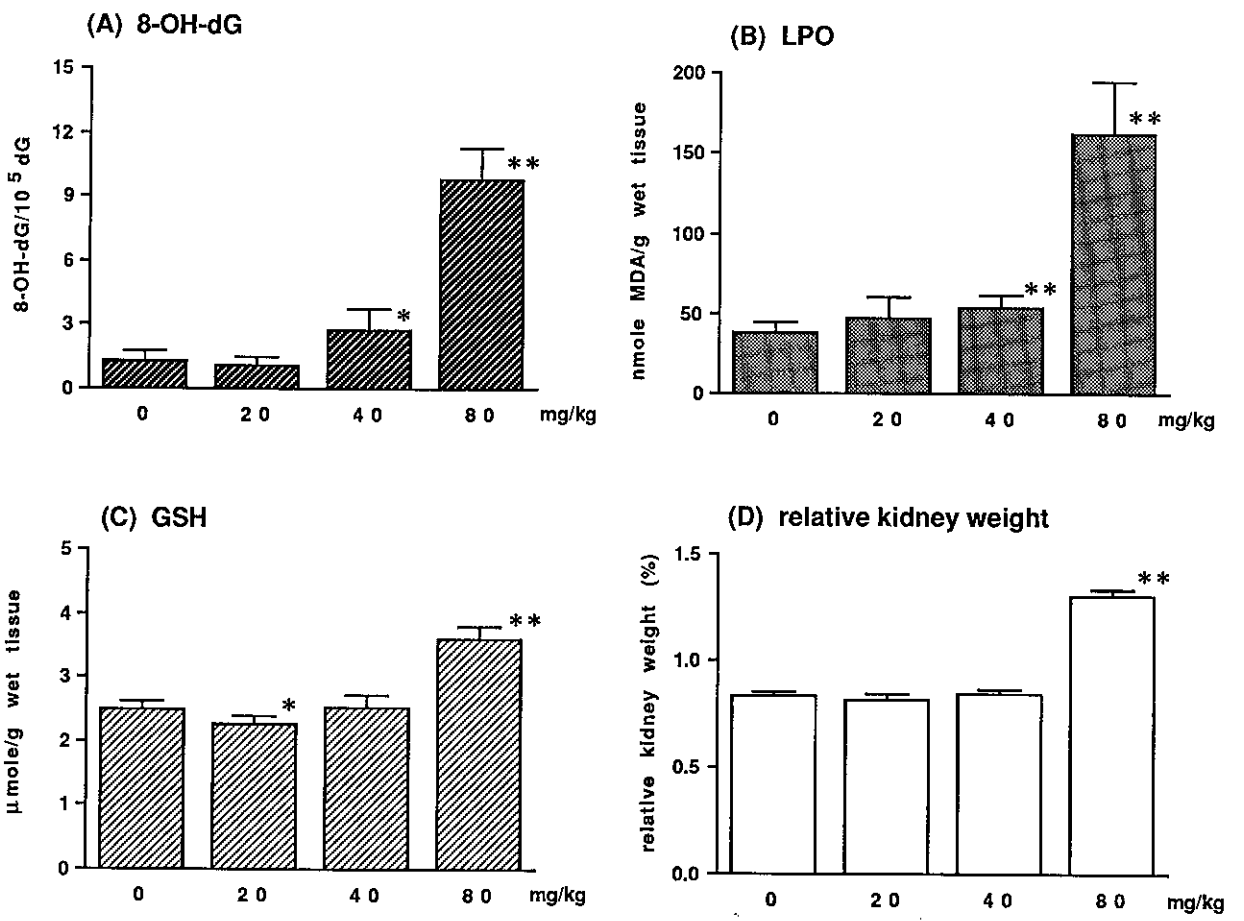

Fig. 2. Changes of (A) 8-OH-dG, (B) LPO, (C) GSH levels in the kidney, and (D) relative kidney weight at 48 h after a single i.p. administration of $KBrO_3$ at doses of 0 to 80 mg/kg. Values represent the mean±SD of data for five rats. *, ** Significantly different from the control group treated with saline alone at * $P<0.05$ and ** $P<0.01$.

DISCUSSION

Recently $KBrO_3$ was reported to induce the production of 8-OH-dG, previously identified as a typical oxidative DNA damage product *in vitro*, in its target organ (kidney) DNA *in vivo*.[11] Subsequently several other carcinogenic chemicals were also found to induce specifically the formation of 8-OH-dG in their target organ DNAs, namely, ferric nitrilotriacetate in the kidney,[14] and di(2-ethylhexyl)phthalate,[15] di(2-ethylhexyl)adipate,[15] ciprofibrate,[16] 2-nitropropane[17] and acetoxime[18] in the liver. Furthermore, Kuchino *et al.* showed misincorporation of nucleotides during the DNA polymerase reaction using synthetic DNAs containing 8-OH-dG as templates,[19] thus demonstrating clear mutagenic potential. From this experimental evidence, 8-OH-dG in DNA is now considered to be a critical factor in oxidative stress-associated carcinogenic mechanisms.

Although an increase of 8-OH-dG level in kidney DNA was observed after p.o. administration of $KBrO_3$ in the previous experiment,[11] we used i.p. administration in this study in order to avoid the delay of the action by intestinal absorption. As a result, $KBrO_3$ showed the same effect by both routes. This was also evident in the micronucleus test, where dose-dependent induction of micronucleated polychromatic erythrocytes was demonstrated with both i.p. and p.o. routes.[20] In contrast to the remarkable effects of $KBrO_3$ in both *in vivo* studies, it has been found that $KBrO_3$ is only very weakly positive in the Ames test, even using the TA102 and TA104 strains, which are particularly sensitive to oxygen radicals, and that 8-OH-dG is not produced by *in vitro* reaction of $KBrO_3$ with DNA.[3] Thus, these findings suggest that the genotoxicity of $KBrO_3$ may be enhanced by or due to biological reactions *in vivo*, such as oxidation of intracellular lipids or proteins, eventually causing DNA oxidation.[21-23]

It was earlier demonstrated that kidney LPO level is increased after i.v. administration of $KBrO_3$, indicating a possible relationship between LPO and oxidative DNA damage. In the present dose-response study, levels of 8-OH-dG and LPO were both elevated in a similar dose-dependent manner. However, the time-course study revealed that the increase of 8-OH-dG level appeared after elevation of LPO. This suggests one possible mechanism for oxidative DNA damage caused by $KBrO_3$ to be as follows. At first, active oxygen species may be produced by $KBrO_3$ directly or as a consequence of the reaction with intracellular molecules which could induce initiation of LPO via the iron-catalyzed Haber-Weiss reaction; the levels of lipid peroxide and intermediate radicals may then be amplified by chain reaction; consequently, the reactive species probably oxidize nuclear DNA, when the LPO reaction occurs in the nuclear membrane.[21-23]

On the other hand, gradual decrease of 8-OH-dG level was observed in the later phase of the time course study while the LPO level remained constant, raising the possibility that the relation is not direct. However, repair of oxidized DNA might take place through the action of DNA glycosylase. This seems likely, because Chung *et al.* have purified a repair enzyme for 8-OH-dG in double-stranded DNA from *E. coli*[24] and have recently found a similar enzyme in mammalian cells (H. Kasai, personal communication).

In our previous study, the production of LPO in kidney and the mortality of rats given $KBrO_3$ could be effectively inhibited by the administration of GSH or cysteine exogenously,[8] demonstrating a preventive role for GSH against $KBrO_3$ toxicity. In the present experiment, a significant increase of GSH level was observed after administration of a high dose of $KBrO_3$, the time-course pattern being correlated with that for 8-OH-dG level. There is thus a possibility that turnover of GSH in the kidney or its transport from the liver may be promoted as a consequence of the *in vivo* protective response against $KBrO_3$-associated oxidative stress.

In the dose-response study, relative kidney weight was increased significantly only with the 80 mg/kg dose, in clear contrast with the significant increase in 8-OH-dG and LPO levels observed at the doses of 40 and 80 mg/kg. Although histology was not performed in this experiment, it is evident from several previous studies that increased kidney weight reflects the nephrotoxicity of $KBrO_3$, especially necrosis of the tubular epithelium.[3] Thus, this might indicate that the activity of $KBrO_3$ regarding formation of 8-OH-dG and LPO is not directly related to its nephrotoxicity.[3] A similar conclusion was drawn from an earlier long-term dose-response study in rats.[25] Thus, renal cell tumors and dysplastic foci were induced in rats given $KBrO_3$ at doses lower than 125 ppm in the drinking water, at which levels the nephropathic changes were only very slight. Therefore, in the case of $KBrO_3$-induced renal carcinogenicity, initiating activity seems to be independent of nephrotoxicity.

In conclusion, the present study suggests that the formation of 8-OH-dG due to $KBrO_3$ might be attributed to increased LPO level. Ongoing investigations are aimed at establishing the physiological function of exogenous GSH, and the effects of GSH depletion on 8-OH-dG and LPO levels of rat kidney given $KBrO_3$, in order to elucidate the protective role of cellular GSH against oxidative stress.

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