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Etiology of bromate-induced cancer and possible modes of action-studies in Japan

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Abstract

Renal cell tumors were significantly increased in male and female rats given potassium bromate at 250 and 500 mg/L in drinking water. In at least one other study renal cell tumors were produced in male rats at 125 mg/L. Among male mice given 750 mg/L of potassium bromate, there were no significant differences in renal cell tumors between treated and control groups after 88 weeks on test. In oxidative DNA damage tests 8-oxodeoxyguanosine (8-oxodG also referred to as 8-OH-dG) was induced in DNA in the male rat kidney in 1 week, and in females after 3 weeks at 500 mg/L, and also in both male and female rats at 250 mg/L, but not at 125 mg/L.

DNA adducts are considered to be an initial step in the carcinogenesis process, however, the administered doses are not always sufficient to cause mutations, possibly due to DNA repair. In the two-step rat renal carcinogenesis model using *N*-ethyl-*N*-hydroxyethylnitrosamine (EHEN) as initiator, promotion activity by potassium bromate was measured using the BrdU labeling index. The promoting activity of bromate in male rats was much greater and extended to doses as low as 60 mg/L in male rats, whereas in females the response was limited to 250 and 500 mg/L. Therefore, it was concluded that the mechanisms contributing to cancer in the male rat were more complex than in the female rat.

The accumulation of $\alpha_{2\mu}$-globulin in the kidneys of male rats exposed to potassium bromate probably accounts for the greater labeling index in the male rat relative to the female rat. Accumulation of $\alpha_{2\mu}$-globulin as a result of treatment with chemicals is unique to the male rat and does contribute to carcinogenic responses. Neither humans nor female rats display this response. Nevertheless, bromate must be considered carcinogenic because of the response of the female rats. The better correlation between 8-oxodG formation and tumor response indicates that dose–response information from the female rat would be much more relevant to human risk assessment. The fact that an elevation of BrdU-LI in the kidney of the female rat is consistent with the possibility that cell proliferation observed in female rats resulted from oxidative stress and/or cytotoxic responses in the kidney. Therefore, oxidative stress is most likely the mechanism of interest for cancer risk in humans.

Keywords: 8-Hydroxydeoxyguanosine; In vivo mutation assay; Cell proliferarion; $\alpha_{2\mu}$-Globulin

1. Carcinogenicity studies

1.1. Carcinogenicity test in rats

Groups of 53 males and 53 females of F344 rats received $KBrO_3$ for 110 weeks at concentrations of

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500 (reduced to 400 ppm at week 60 in the males) and 250 ppm in the drinking water. Incidences of adenomas and adenocarcinomas and their combined incidences as renal cell tumors (RCTs) in the treated rats of both genders were significantly higher than the controls. Tumors of the peritoneum, all diagnosed as mesotheliomas, also occurred at a significantly higher incidence in male rats given 250 or 500 ppm than in the controls (Kurokawa et al., 1983).

1.2. Dose–response study in rats

Groups of 20–24 male F344 rats were given $KBrO_3$ orally at concentrations of 500, 250, 125, 60, 30, 15 and 0 ppm for 104 weeks. Renal adenocarcinomas developed in 3 of the 20 rats given 500 ppm, and the incidences of renal adenomas and RCTs were significantly elevated in rats receiving concentrations of 500, 250 and 125 ppm. The combined incidences of follicular adenomas and adenocarcinomas of the thyroid were significantly increased in rats of the 500 ppm dose group. Incidence of mesotheliomas of the peritoneum in animals receiving 500 ppm was significant (Kurokawa et al., 1986).

1.3. Carcinogenicity test in mice

A total of 27 male mice each in B6C3F1, BDF1 and CDF1 strains were given $KBrO_3$ at a dose of 750 ppm in the drinking water for 88 weeks. Although there were no significant differences in incidences of RCTs between the treated and control groups, one renal adenocarcinoma in a B6C3F1 treated mouse and a total of four renal adenomas in treated mice of three strains were found. Additionally, significant increases in the occurrence of adenomas of the small intestine in CDF1 mice and of adenomas of the liver in B6C3F1 mice were observed (Kurokawa et al., 1990).

2. Initiation and promotion studies in the renal carcinogenesis of rats

2.1. Limited duration study

Groups of 14–20 male F344 rats were given $KBrO_3$ orally at concentration of 500 ppm for 13, 26, 39 or 52 weeks and maintained untreated up to the end of the experiment, week 104. The results revealed that 13 weeks of exposure was necessary to produce increase in the incidence of RCTs (Kurokawa et al., 1987). Further study confirming initiating potential of 13 weeks exposure to $KBrO_3$ at various doses is now on-going based on a two-stage renal carcinogenesis model using trisodium nitrilotriacetate (Na_3NTA) as a promoter.

2.2. Promotion study

A total of 120 male F344 rats were given *N*-ethyl-*N*-hydroxyethylnitrosamine (EHEN) orally at a dose of 500 ppm for 2 weeks and then 0, 15, 30, 60, 125, 250 and 500 ppm $KBrO_3$ orally for the following 24 weeks. The mean numbers of dysplastic foci (DF)/cm^2 were found to be significantly increased in a dose-related manner in rats treated with more than 30 ppm $KBrO_3$ (Kurokawa et al., 1985). A total of 60 female F344 rats were treated with distilled water (DW) or 0.05% EHEN for the first 2 weeks with subsequent administration of DW or $KBrO_3$ at a dose of 500 ppm in the drinking water for 30 weeks. The mean number of atypical tubules, atypical hyperplasias and renal cell tumors per rat in animals treated with $KBrO_3$ after EHEN initiation were significantly higher than those in animals receiving DW after EHEN initiation (Umemura et al., 1995).

3. Mode of action in the renal carcinogenesis

3.1. Initiation activity

3.1.1. Oxidative DNA damage

Five male and female rats in each group were administered $KBrO_3$ at a concentration of 500 ppm in the drinking water for 1, 2, 3, 4 and 13 weeks, and then 8-hydroxydeoxyguanosine (8-OH-dG) levels in kidney nuclear DNA were measured using HPLC-ECD system. 8-OH-dG levels were increased at 1, 2, 3, 4 and 13 weeks in kidney DNA of male rats chronically exposed to $KBrO_3$ at a carcinogenic dose, significant increase also being observed from 3 weeks up to the end of the experiment for females (Umemura et al., 1998). Five male and female rats in each group were administered $KBrO_3$ at concentrations of 0, 15, 30, 60, 125, 250 and 500 ppm in the drinking water for 4 weeks, and then thiobarbituric acid-reactive substances (TBARS) levels together with 8-OH-dG levels in the kidney were measured. While $KBrO_3$ in the drinking water did not cause elevation of TBARS in kidneys of either sex at any doses examined, 8-OH-dG levels in male and female rats exposed to $KBrO_3$ were increased at concentrations of 250 ppm and above in a clearly dose-dependent manner (Umemura et al., 2004).

3.1.2. In vivo mutagenicity test

Five male *gpt* delta rats in each group were given $KBrO_3$ at concentrations of 0 and 500 ppm in the drink-

ing water for 12 weeks and then *gpt* mutation (*gpt* assay) representing point mutations and red/gam mutation (Spi^- assay) representing deletion mutation were examined. Both of *gpt* and Spi^- mutation frequencies (MFs) in the kidney of treated rats were significantly higher than those in the control, especially Spi^- MF in rats given $KBrO_3$ was almost five-fold higher as compared to the control value. Mutation spectra analysis revealed that G:C to A:T transition and A:T to C:G transversion were predominant in observed gpt mutants (to be submitted). Five male gpt delta rats in each group were treated with $KBrO_3$ at concentrations of 0, 60, 125, 250 and 500 ppm in the drinking water for 13 weeks. While MFs of *gpt* were increased dose-dependently from 250 ppm in spite of the values being not statistically significant, Spi^- MF of rats given $KBrO_3$ at a dose of 500 ppm was significantly higher than the control.

3.2. Promotion activity

3.2.1. Cell proliferation

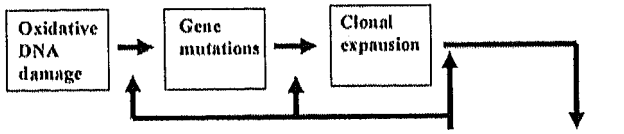
Five male and female rats in each group were administered $KBrO_3$ at a concentration of 500 ppm in the drinking water for 1, 2, 3, 4 and 13 weeks, and then bromodeoxyuridine labeling index (BrdU-LI) in three kinds of tubules (proximal convoluted, PCT; proximal straight, PST and distal, DT) of the kidney was counted by γ-GT/BrdU double staining. Significantly increased BrdU-LI was found in the PCT of treated males throughout the experimental period compared with the relevant controls, significant elevation of the BrdU-LI being observed after 13 weeks in the females (Umemura et al., 1998). Five male and female rats in each group were administered $KBrO_3$ at concentrations of 0, 15, 30, 60, 125, 250 and 500 ppm in the drinking water for 4 weeks, and then BrdU-LI was examined as in the above study. BrdU-LIs of PCT in the males were elevated in a dose-dependent manner, with significant increases at 30 ppm and above. In the females, although there were no changes up to 125 ppm, dose-dependent increase was subsequently observed at 250 ppm and above with statistically significance (Umemura et al., 2004).

3.2.2. $\alpha_{2\mu}$-Globulin accumulation

Based on the previous data showing hyaline droplets observation (Umemura et al., 1993) and $\alpha_{2\mu}$-globulin deposition by immunohistochemistry (Umemura et al., 1998) in kidney of male rats chronically exposed to $KBrO_3$, five male and female rats in each group were administered $KBrO_3$ at concentrations of 0, 15, 30, 60, 125, 250 and 500 ppm in the drinking water for 4 weeks, and then $\alpha_{2\mu}$-globulin contents in kidneys were measured using a commercially available ELISA kit. Only in the males, increase was evident at 30 ppm and above in a dose-dependent fashion, the elevation being statistically significant at 125 ppm and above (Umemura et al., 2004).

4. Summary for the overall

The overall data were summarized in Fig. 1. Carcinogenicity studies demonstrated significantly elevated incidences of renal cell tumors in male and female rats given $KBrO_3$ at 250 and 500 ppm in the drinking water. A further dose–response study using only male rats showed



		8-OH-dG	*In vivo* mutagenicity	α2u-globulin	Cell proliferation	Carcino-genicity
Male	500 ppm	+	+	+	+	+
	250 ppm	+	±	+	+	+
	125 ppm	—	—	+	+	+ ?
	60 ppm	—	—	±	+	—
	30 ppm	—	—	±	±	—
	15 ppm	—	—	—	—	—
Female	500 ppm	+	N.D.	—	+	+
	250 ppm	+	N.D.	—	N.D.	+
	125 ppm	—	N.D.	—	N.D.	N.D.

Fig. 1. Summary for the overall data and possible modes of action.

125 ppm to also be a carcinogenic dose. However, since another group showed that a dose of 200 ppm failed to induce renal tumors in male rats (Wolf et al., 1998), it seems equivocal whether 125 ppm has a carcinogenic potential. Increases of 8-OH-dG formation in kidney DNA of male and female rats given $KBrO_3$ at 250 and 500 ppm, but not at 125 ppm and below were observed. Although DNA adducts have been considered to be an initial step in the carcinogenesis, the existence of their repair enzymes, e.g. OGG1 for 8-OH-dG indicates that the dose inducing DNA adducts formation is not always enough to cause mutations. The present results revealing that elevation of MFs in in vivo mutation assay was evident at 500 ppm, but not 250 ppm and below might involve the rationale. In spite of the initiation activity at 250 ppm and below remaining uncertain, the data of the mutagenicity test at least can explain the fact that 13 weeks exposure at 500 ppm was sufficient for exerting the initiation activity. It has been accepted that promotion activity plays an important role all the way in carcinogenesis. The present data showing that 250 ppm was a carcinogenic dose in absence of the mutagenicity potential may suggest participation of the promoting effects in addition to the difference of the duration of exposure between the mutation assay and the carcinogenicity test. In the two-stage rat renal carcinogenesis model using EHEN as an initiator, promoting activity of $KBrO_3$ was apparent in both sexes of rats. In particular, in males, a dose of 30 ppm was able to exert promoting action, the same dose causing increase of BrdU-LI in the PCT. $\alpha_{2\mu}$-Globulin accumulation in the kidney of male rats exposed to $KBrO_3$ was also observed in a dose-dependent manner at 30 ppm and above, even though the increases at 30 and 60 ppm were not statistically significant. In consideration of the fact that $\alpha_{2\mu}$-globulin accumulation occur at PCT, it is highly probable that $KBrO_3$-induced cell proliferation in PCT and subsequent tumor-promoting activity observed in males might imply the accumulation of the male rat-specific urinary protein. Nevertheless, the finding that BrdU-LI in PCT of females at doses of 250 and 500 ppm were elevated indicates an involvement of some other mechanism. Considering that $KBrO_3$ might be reduced to form more reactive species at PCT (Murata et al., 2001), the good correlation between the doses inducing 8-OH-dG formation and elevation of BrdU-LI allow us to speculate that the cell proliferation observed in female rats might result from oxidative stress. For risk assessment of $KBrO_3$ in the human situation, it is essential to focus on oxidative stress and to eliminate $\alpha_{2\mu}$-globulin-mediated effects. We propose that more than 250 ppm of $KBrO_3$ in the drinking water is able to exert carcinogenic activities in the kidney of rats of both genders by use of the generated oxidative stress.

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