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Molecular biomarkers of oxidative stress associated with bromate carcinogenicity☆

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Abstract

Potassium bromate ($KBrO_3$) is a chemical oxidizing agent found in drinking water as a disinfection byproduct of surface water ozonation. Chronic exposures to $KBrO_3$ cause renal cell tumors in rats, hamsters and mice and thyroid and testicular mesothelial tumors in rats. Experimental evidence indicates that bromate mediates toxicological effects via the induction of oxidative stress. To investigate the contribution of oxidative stress in $KBrO_3$-induced cancer, male F344 rats were administered $KBrO_3$ in their drinking water at multiple concentrations for 2–100 weeks. Gene expression analyses were performed on kidney, thyroid and mesothelial cell RNA. Families of mRNA transcripts differentially expressed with respect to bromate treatment included multiple cancer, cell death, ion transport and oxidative stress genes. Multiple glutathione metabolism genes were up-regulated in kidney following carcinogenic (400 mg/L) but not non-carcinogenic (20 mg/L) bromate exposures. 8-Oxodeoxyguanosine glycosylase (*Ogg*1) mRNA was up-regulated in response to bromate treatment in kidney but not thyroid. A dramatic decrease in global gene expression changes was observed following 1 mg/L compared to 20 mg/L bromate exposures. In a separate study oxygen-18 (^{18}O) labeled $KBrO_3$ was administered to male rats by oral gavage and tissues were analyzed for ^{18}O deposition. Tissue enrichment of ^{18}O was observed at 5 and 24 h post-$KBr^{18}O_3$ exposure with the highest enrichment occurring in the liver followed by the kidney, thyroid and testes. The kidney dose response observed was biphasic showing similar statistical increases in ^{18}O deposition between 0.25 and 50 mg/L (equivalent dose) $KBr^{18}O_3$ followed by a much greater increase above 50 mg/L. These results suggest that carcinogenic doses of potassium bromate require attainment of a threshold at which oxidation of tissues occurs and that gene expression profiles may be predictive of these physiological changes in renal homeostasis.

Keywords: Potassium bromate; Oxidative stress; Gene expression; Tissue oxidation; Oxygen-18; Glutathione metabolism; Threshold; Risk assessment; Molecular biomarkers

☆ The research described in this article has been reviewed by the Health and Environmental Effects Research Laboratory, United States Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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1. Introduction

Potassium bromate ($KBrO_3$) is a chemical oxidizing agent that has been used extensively in the food and cosmetic industries. Potassium bromate is also found in drinking water as a disinfection byproduct of surface water ozonation. Acute human exposures to potassium bromate have occurred primarily by accidental or purposeful ingestion of $KBrO_3$ solutions from permanent hair-wave kits (IARC, 1986). Nephrotoxicity and neurotoxicity are the primary responses to $KBrO_3$ with death occurring from solutions containing as little as 12 g $KBrO_3$. Chronic exposures to $KBrO_3$ cause renal cell carcinomas in rats, hamsters and mice and thyroid and mesothelioma tumors in rats (Kurokawa et al., 1990; DeAngelo et al., 1998). Due to its cross species carcinogenicity, bromate is considered a probable human carcinogen.

Most studies investigating the mechanism of potassium bromate-induced carcinogenicity have focused on DNA damage due to oxidative stress (Chipman et al., 1998; Umemura et al., 1998; Murata et al., 2001). Potassium bromate induces point mutations in bacteria and chromosomal aberrations in mammalian cells (Ishidate et al., 1984). It has been suggested that the base modification, 8-oxodeoxyguanosine (8-oxodG) is prominently involved in generating the mutations (Ballmaier and Epe, 1995). In vivo investigations using transgenic mice lacking the 8-oxodeoxyguanosine glycosylase (*Ogg*1) DNA repair enzyme and containing a retrievable *gpt* gene demonstrated a three-fold enhancement of kidney mutations induced by $KBrO_3$ compared to vehicle (Nishimura, 2002). Together, these studies support the hypothesis that $KBrO_3$ is damaging DNA through oxidative stress and that this leads to increases in mutation frequency.

More recent studies investigating the mechanism of action of bromate have characterized gene expression changes associated with bromate exposure in rat mesothelial cells in vitro (Crosby et al., 2000). The expression level of many of these genes was altered by as much as 50-fold in response to bromate treatment. These biomarker genes included p21/Waf1, p38 kinase, cyclin D1, heme oxygenase, HSP70 and GADD45 which regulate important cellular responses to chemical insults including DNA damage recognition, cell cycle control and oxidant scavenging. Many of these genes inducible by p53 protein accumulation respond to changes in the redox state of the cell also supporting an oxidative stress mode of action. However, to further examine gene expression changes important in the cancer process, an in vivo correlate is necessary to provide information regarding adaptation to chronic exposure and in identifying transcript changes in transformed renal cells.

Finally, susceptibility to bromate carcinogenicity does not appear to be dependent solely on the magnitude of oxidative stress induced in the target organ (Kurokawa et al., 1987). Levels of renal lipid peroxidation did not correlate with tumor susceptibility across rodent species and strain suggesting that mechanisms other than lipid peroxidation may also be important in bromate carcinogenicity. In an effort to further characterize these and other potential mechanism(s) of bromate action in these studies, we have examined global gene expression changes in the rat kidney following chronic exposures to potassium bromate and oxygen-18 (^{18}O) deposition from labeled potassium bromate ($KBr^{18}O_3$) as a dosimeter of oxidative stress in target and non-target organs. These studies provide a more comprehensive framework for understanding the interaction of the oxidative stress response with other renal responses that may be involved in bromate carcinogenicity.

2. Materials and methods

2.1. In vivo studies

Male F344 rats, approximately 6–8 weeks of age, were obtained from Charles River Laboratories and held for 1 week in quarantine. Rats were administered potassium bromate ($KBrO_3$) in their drinking water for 2–100 weeks. In a separate study, F344 rats were administered a single gavage dose (2.5 μg–25 mg/kg) of oxygen-18 (^{18}O) labeled potassium bromate and necropsied 1, 5, or 24 h after dosing. At necropsy organs and/or tumor tissues were collected fresh for biochemical analyses, frozen in liquid nitrogen for gene expression analysis, placed in dry ice for ^{18}O analyses, or placed in neutral buffered formalin for histopathology. All aspects of these studies were conducted in compliance with the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and approved by the Institutional Animal Care and Use Committee (IACUC). $KBrO_3$ was purchased from Sigma Chemical Company (St. Louis, MO).

2.2. Histopathology

Fixed tissues were processed by routine methods for paraffin embedment, sectioned at 5 μm, stained with hematoxylin and eosin, and examined by light microscopy.

2.3. Microarray analyses

Rat kidney, thyroid, and mesothelial cell RNA was prepared for microarray and/or RT-PCR analyses. Kidney gene expression analysis was performed according to the Affymetrix recommended protocol using Affymetrix Rat Genome 230A GeneChips®. Briefly, total RNA from three to