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The study using wild-type and *Ogg1* knockout mice exposed to potassium bromate shows no tumor induction despite an extensive accumulation of 8-hydroxyguanine in kidney DNA

Tsuyoshi Arai [a], Vincent P. Kelly [a,b,c,1], Osamu Minowa [b], Tetsuo Noda [b], Susumu Nishimura [a,c,*]

[a] *Tsukuba Research Institute, Banyu Pharmaceutical Co. Ltd., Okubo 3, Tsukuba, Ibaraki 300-2611, Japan*
[b] *The Cancer Institute, Japanese Foundation for Cancer Research, Ariake, Koto-ku, Tokyo 135-8550 Japan*
[c] *University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305-8577, Japan*

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Abstract

In order to assess the effect of potassium bromate ($KBrO_3$) on the induction of tumor formation, a 1-year carcinogenesis study was performed using *Ogg1* knockout mice (*Ogg1*$^{-/-}$) and wild-type mice (*Ogg1*$^{+/+}$). The mice were chronically exposed to $KBrO_3$ by putting it in the drinking water for 29 weeks, at 2 g/l for the first 18 weeks, and then at 1 g/l for another 11 weeks. After termination of treatment the mice were kept for an additional 23 weeks. The amount of 8-hydroxydeoxyguanosine (8-OH-dG) in kidney DNA after 29 weeks of $KBrO_3$ exposure reached 500 8-OH-dG/10^6 dG, almost 250-fold that of untreated wild-type mice. During the course of study the mice appeared normal, although a decrease of body weight gain in both *Ogg1*$^{-/-}$ and *Ogg1*$^{+/+}$ mice exposed to $KBrO_3$, and some kidney malfunction in $KBrO_3$ treated *Ogg1*$^{-/-}$ mice was observed.

Surprisingly, when *Ogg1*$^{-/-}$ and *Ogg1*$^{+/+}$ mice were sacrificed at 52 weeks, no tumor formation could be found in kidney or other organs such as lung, liver, spleen, thymus, stomach and intestine. Microscopic examination also showed the absence of precancerous foci in all tissues of both *Ogg1*$^{-/-}$ and *Ogg1*$^{+/+}$ mice. A possible explanation is presented to reconcile these results with those of others which showed an increased incidence of tumor formation in untreated *Ogg1*$^{-/-}$ mice.

Keywords: Potassium bromate ($KBrO_3$); *Ogg1* knockout mice; 8-Hydroxyguanine (8-OH-G); 8-hydroxydeoxyguanosine (8-OH-dG); Chronic exposure; Carcinogenesis

1. Introduction

In 1983, we discovered the oxidative DNA lesion 8-hydroxyguanine (8-OH-G) (Kasai and Nishimura, 1983, 1984). 8-Hydroxyguanine is also named later as 7,8-dihydro-8-oxoguanine, abbreviated as 8-oxoguanine. It is formed by agents that generate oxygen radicals not only in vitro but also in vivo (Kasai et al., 1986). Over

* Corresponding author at: University of Tsukuba, Tennodai 1-1-1, Tsukuba, Ibaraki 305-8577, Japan. Tel.: +81 29 853 2008; fax: +81 29 853 5810.
E-mail address: nishimura@hq.tsukuba.ac.ip (S. Nishimura).
[1] Present address: School of Biochemistry and Immunology, Trinity College, Dublin 2, Ireland.

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the past 20 years, studies on the biological significance of 8-OH-G have established that it is an important effecter of DNA mutagenesis and/or carcinogenesis (Kasai and Nishimura, 1991; Nishimura, 1999, 2002). Although 8-OH-G in DNA induces mostly G → T transversions other mutations have also been demonstrated to occur (Nishimura, 2002).

There are three genes whose protein products are involved in the prevention and repair of 8-OH-G in DNA. In mammalian species, these consist of the mammalian homologues of the bacterial MutM (MMH/OGG), MutY (MYH) and MutT (MTH) proteins. OGG1 is a glycosylase/apurinic, apyrimidnic lyase (AP) that hydrolyzes the glycosidic bond of 8-hydroxydeoxyguanosine (8-OH-dG) to release 8-OH-G, and then processes the residual abasic site by cleaving the phosphodiester bond (Abrutani et al., 1997). MTH is an 8-OH-dGTPase that degrades 8-OH-dGTP in the nucleotide pool, thereby preventing its incorporation into DNA (Sakumi et al., 1993). MYH is a monofunctional glycosylase which removes an A base when mispaired to 8-OH-G (Slupska et al., 1996). Recently, it has been reported that *Ogg1* deficient mice have an increased incidence of tumor formation (Sakumi et al., 2003). However, the tumor occurrence in *Ogg1* deficient mice was not strikingly dissimilar to that of control wild-type mice, though the difference was statistically significant.

In 1986, we showed that a single oral administration of the oxidizing agent and renal carcinogen $KBrO_3$ to rats, specifically induced 8-OH-G formation in kidney DNA but not liver DNA, indicating that formation of 8-OH-G is target organ specific (Kasai et al., 1987). Recently we reported a substantial increase of 8-OH-G in kidney DNA from $Ogg1^{-/-}$ mice when treated with $KBrO_3$ given in the drinking water, and that the level of accumulated 8-OH-G did not decline even after ceasing $KBrO_3$ exposure (Arai et al., 2002). The amounts of 8-OH-G in kidney DNA of $KBrO_3$ treated $Ogg1^{-/-}$ mice were approximately 150 times that found in non-treated $Ogg1^{-/-}$ or $Ogg1^{+/+}$ mice. Furthermore, we showed that DNA mutation rates were higher in $KBrO_3$ treated $Ogg1^{-/-}$ mice compared to non-treated $Ogg1^{+/+}$ mice. Since the mice in these studies were kept for only 12 weeks after administration of $KBrO_3$, an assessment on tumor induction was not possible. Therefore, in the present study, $Ogg1^{-/-}$ mice as well as $Ogg1^{+/+}$ mice were administered $KBrO_3$ for a much longer period of time, almost one year, to ascertain whether $KBrO_3$ treatment of $Ogg1^{-/-}$ mice can enhance tumor formation.

2. Materials and methods

2.1. Mice

The generation of *Ogg1* knockout mice by gene targeting in embryonic stem cells has been described previously (Arai et al., 2003; Minowa et al., 2000). The $Ogg1^{+/-}$ mice (F1 hybrid of 129sv and C57BL/6J) were crossed with C57BL/6J mice. The offspring were mated to obtain $Ogg1^{+/+}$ and $Ogg1^{-/-}$. Pups were genotyped by PCR analysis of DNA isolated from tail tips. A combination of the primer pair 5′-CCATCCTGGTGGCCCTGTATCTGCA-3′ and 5′-CTCACTGGAGTGGCGTGCTGGCAGA-3′ identified the wild-type allele as a 318 bp fragment, whereas the primer pair 5′-AGTGGCGTGCTGGCAGATCAAGTA-3′ and 5′-GTGGTTCCTGGGATTTGGACTCAGG-3′ distinguished the mutant allele as a 394 bp product. Seven-to eight-week-old mice were used for the experiment.

2.2. Treatment of mice with $KBrO_3$

$KBrO_3$ was given to mice in the drinking water at a concentration of 2 g/l for the first 18 weeks, and at 1 g/l for another 11 weeks. The mice had free access water throughout the experiment. Control mice were given distilled water. The mice were fed a standard laboratory diet (NIH-07, CLEA Japan) and maintained under SPF conditions. Body weights were recorded twice per week and water consumption was measured three times per week. The carcinogenesis study was terminated at 52 weeks from the beginning of administration. The tissues from kidney, liver, lung, spleen, thymus, stomach and intestine were observed macroscopically and microscopically in order to check tumor formation. All animal experiments were conducted in accordance with the institutional guideline at Central Institute for Experimental Animals.

2.3. Measurement of blood biochemical markers

Blood was collected from the inferior vena cava at the completion of the experiment and plasma was stored at −80 °C until the measurement of blood chemistry. Plasma total cholesterol, total protein, blood urea nitrogen, creatinine and inorganic phosphate levels were measured with a 7070 automatic analyzer (Hitachi) using commercially available kits [LTCII (Kyowa), HATP (Wako), L-type UN(Wako) Creatinine-PAPII (Roche) and IP (Daiichi)]. Plasma calcium, sodium, potassium and chloride ion levels were measured with synchron EL-ISE (Beckman).

2.4. Measurement of 8-OH-dG

From each mouse, one whole kidney was used for the extraction of high molecular weight genomic DNA. The DNA extraction method, conversion to deoxynucleosides by nuclease P1, subsequent phosphatase treatment, and measurement of 8-OH-dG by HPLC coupled electrochemical detection were