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The Utility of Earthworms as a Monitoring Organism for Soil Pollution

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ABSTRACT

To assess risks to human health from soil contamination, bio-monitoring systems are required. Earthworms are promising candidates as bio-monitoring organisms for soil contamination. However, there have been few studies concerning the utility of earthworms as bio-monitoring organisms. 8-Oxoguanine (8-oxo-Gua), a relatively abundant form of oxidative DNA damage, plays a critical role in carcinogenesis. In our previous study, we found that the levels of 8-oxo-Gua in DNA were increased in cadmium (Cd)-exposed earthworms, suggesting that the analysis of 8-oxo-Gua generated in the DNA of earthworms may be useful for monitoring metal polluted soil. In this review article, we discuss the utility of earthworms as a bio-monitoring organism for soil pollution, with reference to our recent study.

Keywords: bio-monitoring, DNA repair, earthworm, heavy metal, metallothionein, oxidative DNA damage, 8-oxoguanine
Abbreviations: MT, metallothionein; **8-oxo-Gua**, 8-oxoguanine; **OGG1**, 8-oxoguanine DNA glycosylase 1; **ROS**, reactive oxygen species

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INTRODUCTION

In 1881, Charles Darwin described the central role of the earthworm in soil formation, quality, and fertility (Stürzenbaum *et al*. 2001). Over 100 years later, we are facing the danger of soil contamination and are considering the use of earthworms as bio-monitoring organisms. However, despite their possible utility, very few studies concerning earthworms as bio-monitors have been performed. To overcome the dangers of soil contamination, we need information about utility organisms in soil and their use. In this context, since the earthworm is the most promising organism for soil bio-monitoring, we should characterize its properties more thoroughly.

One of the most interesting issues about soil pollutants is their effect on the human genome. It is likely that DNA damaging agents for humans also damage the DNA of organisms in soil, although there are many different biological properties. Recently, we analyzed 8-oxoguanine (8-oxo-Gua), a type of DNA damage produced by reactive oxygen species (ROS), generated in earthworm DNA, by using HPLC equipped with an electrochemical detector (Nakashima *et al*. 2008). In the study, we found that cadmium chloride ($CdCl_2$) increased 8-oxo-Gua generation, but nickel chloride ($NiCl_2$) did not. Analyses of the heavy metal accumulation in the earthworm body by atomic absorption spectrometry revealed that $CdCl_2$ accumulated in the earthworm's body, but $NiCl_2$ did not. Taken together, these findings suggested that the utility of earthworms as a bio-monitor requires target chemical pollution.

Among soil pollutants, heavy metals in soil and sediments are widespread across the globe and have a direct impact on aquatic systems and water quality (Saint-Laurent *et al*. 2010). High concentrations of these contaminants are considered to affect wildlife, and to generate human tumors, and other deformities (Murdock 2005). Therefore, bio-monitoring systems for soil pollutants, especially for heavy metals, should be established (Calis *et al*. 2011; Sardo *et al*. 2011; Wen *et al*. 2011). In this short review, we discuss the utility of earthworms for bio-monitoring, with focusing on metallothioneins (MTs).

8-OXOGUANINE

8-Oxoguanine (7, 8-dihydro-8-oxoguanine, 8-oxo-Gua) is a mutagenic lesion formed spontaneously in the genomic DNA of aerobic organisms (**Fig. 1A**) and by the actions of exogenous factors, such as ionizing radiation, chemical pollutants, heavy metals, food, and bacteria. Although 8-oxo-Gua is not necessarily the most abundant form of oxidative DNA damage, it has been the most extensively studied, because it is quite easily measured by HPLC with electro-

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chemical detection in laboratories (Floyd *et al*. 1986; Marnett 2000). Since 8-oxo-Gua was discovered and reported in 1984 (Kasai and Nishimura 1984), this form of DNA damage and its repair systems have been studied vigorously. 8-Oxo-Gua induces GC-to-TA transversion type point mutations (Cheng *et al*. 1992), and thus it is believed to play a key role in gene stability (Bravard *et al*. 2009). In fact, molecular epidemiologic case-control studies indicated that a polymorphism of 8-oxoguanine DNA glycosylase 1 (OGG1), the 8-oxo-Gua repair enzyme (**Fig. 1B**), was associated with a higher risk of developing several types of human cancers, such as lung (Hung *et al*. 2005; Kohno *et al*. 2006), gastric (Farinati *et al*. 2008), prostate (Chen *et al*. 2003), and bladder (Kakehi *et al*. 2010) cancers.

We previously reported that some environmental pollutants, including heavy metals and cigarette smoke, increased 8-oxo-Gua generation. For example, $CdCl_2$ increased 8-oxo-Gua generation in the DNA of reduced glutathione (GSH)-depleted rat liver (Hirano *et al*. 1997). Arsenic compounds, such as arsenic trioxide, sodium arsenite, and sodium hydrogen arsenate, also induced 8-oxo-Gua generation in cultured human lung carcinoma cells (Mei *et al*. 2002) and cultured mouse liver cells (Hirano *et al*. 2006). Cigarette smoking induced an increase in 8-oxo-Gua in human leukocytes (Asami *et al*. 1996) and a central site of the human lung (Asami *et al*. 1997). Moreover, diesel exhaust particles induced 8-oxo-Gua generation in rat lung (Tsurudome *et al*. 1999).

Based on these findings, we concluded that human DNA generally suffers from environmental pollutants, which increase the risk of gene mutations. To defend our genome against mutagenic environmental pollutants, we should continue to identify the environmental factors that induce DNA damage. Thus, bio-monitoring systems for environmental pollutants should be established.

HEAVY METALS AND 8-OXOGUANINE / 8-OXO-GUA REPAIR SYSTEM

As stated above, we have studied the relationship between environmental pollutants and 8-oxo-Gua generation. To maintain the integrity of the mammalian genome, DNA repair systems act by removing DNA damage (**Fig. 1B**), reducing the mutation frequency of cancer-related genes, minimizing replication errors, and resolving deleterious rearrangements arising via aberrant recombination (Hoeijmakers *et al*. 2001). As 8-oxo-Gua is believed to induce point mutations in nuclear DNA, the relationship between environmental factors, such as chemical agents, irradiation, and food, and 8-oxo-Gua generation and its repair capacity has been investigated. In this context, studies of the effects of heavy metals on DNA repair systems have provided interesting and useful information.

The association of heavy metals with 8-oxo-Gua repair systems has been extensively studied. In 1997, we first described an association between Cd exposure and the inhibition of 8-oxo-Gua excision repair activity in rat testes (Hirano *et al*. 1997). After the cloning of mammalian OGG1 in 1996 (Auffret van der Kemp *et al*. 1996; Nash *et al*. 1996), the relationship between heavy metals and OGG1 expression was reported. It was demonstrated that Cd exposure down-regulated OGG1 expression in rat lung and alveolar epithelial cells (Potts *et al*. 2003). Youn *et al*. (2005) suggested that Cd attenuated the removal of γ-ray-induced 8-oxo-Gua adducts, which in turn increased the mutation frequency, and that this effect might, at least in part, result from the suppression of hOGG1 transcription via the inactivation of the Spl transcription factor, as a result of Cd treatment (Youn *et al*. 2005). These inhibitory effects of Cd on OGG1 activity are similar to the inhibition of 8-oxo-dGTPase activity induced by Cd treatment, which led to the accumulation of 8-oxo-Gua in DNA (Bialkowski *et al*. 1999). Although it is likely that Cd exposure might broadly disturb the 8-oxo-Gua repair system, the exact mechanism of the inhibition remains unclear. In addition to Cd, other

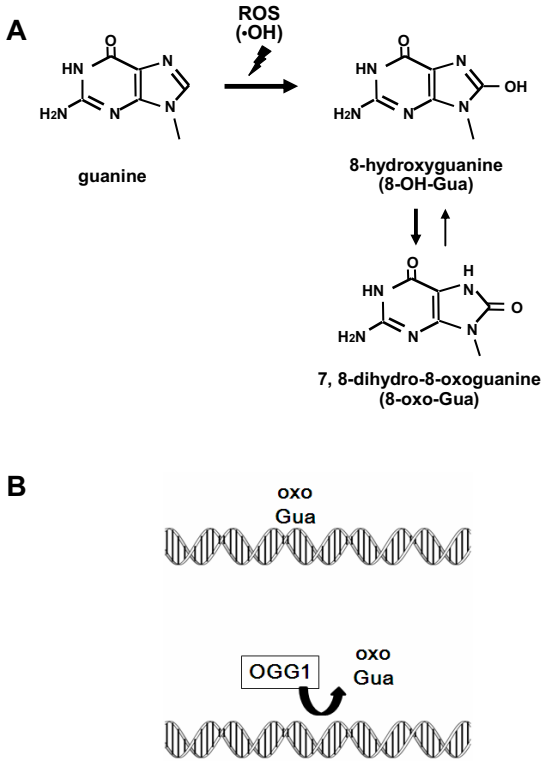

Fig. 1 (**A**) Structure of 8-oxo-Gua. 8-Oxo-Gua is generated by the attack of a hydroxyl radical (•OH) on guanine at the C-8 position. (**B**) Schema of OGG1 action. OGG1 removes 8-oxo-Gua from DNA by its glycosylase activity.

metals, such as Mn, As, Cr, and Pb, also displayed similar effects on OGG1 activity or OGG1 expression (Hirano 2008).

As described above, many kinds of heavy metals are capable of inhibiting OGG1 activity. It is likely that the inhibitory action of heavy metals occurs by a common mechanism. Several possible mechanisms have been proposed, such as the effects on various transcription factors functioning cooperatively in a complex network, involving both protein-DNA and protein-protein interactions. Several putative sites for transcription factor binding have been identified in the human OGG1 promoter (Dhenaut *et al*. 2000). Recently, we presented another mechanism for the inhibitory action of heavy metals. We reported that some chemicals and metals, such as etoposide, mitomycin C and arsenite, respectively, inhibited OGG1 activity by cleaving OGG1 (Hirano *et al*. 2004; Hirano *et al*. 2006). However, the detailed mechanism remains unclear.

EARTHWORM METALLOTHIONEINS

As we mentioned above, earthworms are possibly useful as bio-monitoring organisms for chemical pollutants in soil, especially for heavy metals. When we discuss heavy metals in a living organism, we should consider the metal-binding proteins, termed metallothioneins (MTs) (Homa *et al*. 2010; Maity *et al*. 2011). MTs are cysteine-rich cationic proteins involved in metal detoxification. They exist in many kinds of organisms, including humans (Pulido *et al*. 1966), rats (Wiśniewska *et al*. 1970), mouse (Nordberg *et al*. 1975), fish (Bouquegneau *et al*. 1975), and others. In 1980, Suzuki *et al*. reported the cadmium-binding ability of the earthworm MT protein (Suzuki *et al*. 1980). There are two isoforms of MT (MT-1 and MT-2) in the earthworm (**Fig. 2**) (Stürzenbaum *et al*. 1998; Stürzenbaum *et al*. 2001; Stürzenbaum *et al*. 2004). Analyses indicated that only MT-2 is involved in cadmium binding (Stürzenbaum *et al*. 2001).

In terms of the relationship between MT expression and oxidative DNA damage, we observed an increased tendency (but not a statistically significant difference) of 8-oxo-Gua

MT-1: MADASNTQCCGFDACPRRGAACACTNCRCLKSECSPNCRKLCCADSQGKCGNAGCKCGAACKCAAGACASGCKKGCCGD
MT-2: MADAFNTQCCGNKTCPREGSTCACSKCRCPKDDCAPNCKKLCCADAQ----CGNASCSCGAACKCAAGSCASGCKKGCCGD

Fig. 2 The amino acid sequences of MT-1 and MT-2 of the earthworm, *Lumbricus rubellus*. Letters in boxes indicate common amino acids between these two isoforms. The DDBJ / GenBankTM / EBI Data Bank accession numbers of MT-1 and MT-2 are AJ005822 and AJ005823, respectively.

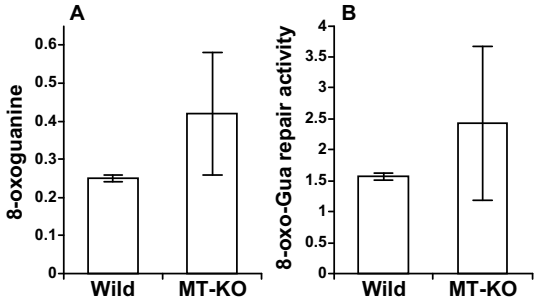

Fig. 3 (**A**) The levels of 8-oxo-Gua generated in mouse liver DNA (wild type and MT-knockout). The indicated values of 8-oxo-Gua are the number of residues per 10^5 Gua. (**B**) 8-Oxo-Gua repair activity of mouse liver. Repair activities were analyzed by an endonuclease nicking assay, according to the method of Hirano *et al*. (1995).

Fig. 4 Photograph of *E. fetida* in a 20-liter stainless steel tank.

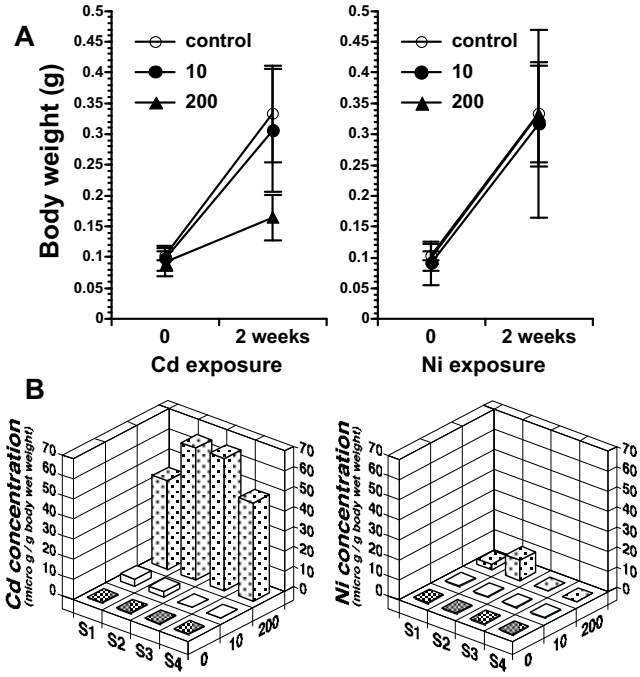

Fig. 5 (**A**) *E. fetida* were weighed under wet conditions in the 2-week experiment. Each data point represents the mean of six *E. fetida*. The treatment of *E. fetida* with 200 μg Cd/g soil resulted in body weight loss, suggesting Cd-induced growth inhibition. On the other hand, no growth inhibition was observed in *E. fetida* with 10 and 200 μg Ni/g soil. (**B**) Heavy metal accumulation in *E. fetida* in the 2-week experiment. *E. fetida* were cut into four rough segments: S1 ~ S4. S1: head region, S2: anterior body region, S3: posterior body region, S4: tail region. Each data point represents the mean of three individuals. Heavy metal concentrations were measured by atomic absorption spectrometry, and are expressed as μg per g body wet weight. Figure reproduced from **Nakashima T, Okada T, Asahi J, Yamashita A, Kawai K, Kasai H, Matsuno K, Gamou S, Hirano T** (2008) 8-Hydroxydeoxyguanosine generated in the earthworm *Eisenia fetida* grown in metal-containing soil. *Mutation Research* **654**, 138-144, ©2008, with kind permission from Elsevier Science Ltd., license number: 2630630193768.

generation in Cd-treated MT-knockout mouse liver (**Fig. 3**, unpublished data). Although no study has been reported so far, it is likely that MTs play a key role in the metal-induced oxidative DNA damage generated in the earthworm. Therefore, when we use the earthworm as a bio-monitoring organism, analyses of MT functions should be considered, such as gene expression and metal binding.

8-OXOGUANINE GENERATION IN EARTHWORMS

Among the many kinds of organisms living in soil, earthworms are quite familiar creatures. Earthworms are considered to be useful for the evaluation of metal contamination, because several studies indicated significant positive correlations between the metal concentrations in the earthworm and the soil Cd, Cu, Pb and Zn concentrations (Morgan and Morgan 1988).

We recently analyzed the 8-oxo-Gua accumulated in the DNA of *Eisenia fetida* (*E. fetida*) exposed to metals, to determine if a method using earthworms as a bio-monitor is appropriate for the assessment of soil mutagenicity (Nakashima *et al*. 2008). In the study, *E. fetida* were kept in a 20 liter stainless steel tank at an ambient temperature of 24°C, using mold with skim milk as a food source, until metal exposure (**Fig. 4**). Three to six individuals were kept in a 600 mL glass container containing 50 g of soil with or without metal (Cd or nickel (Ni)). They were exposed to 10 or 200 μg metal/g soil for 1, 2, and 3 weeks or 10 μg metal/g soil for 3 months. As a result, we detected a high level of Cd accumulation in *E. fetida*. On the other hand, no Ni accumulation was observed. Some of the data are shown in **Fig. 5**.

The 8-oxo-Gua levels in the DNA of *E. fetida* treated with Cd for 3 months were significantly higher than those in the control *E. fetida* or Ni-treated *E. fetida*. In addition, we observed positive 8-oxo-Gua staining in the seminal vesicles of the *E. fetida* treated with 10 μg of Cd for 3 months. The seminal vesicles are considered to be MT-poor organs. Therefore, it seems reasonable to speculate that a lower level of MT expression is involved in Cd-induced DNA damage accumulation.

CONCLUSIONS

Although chemical products are produced every day, few methods for their detection and monitoring have been established. Earthworms are very familiar organisms in soil, and they may be suitable as bio-monitors for soil pollution. Although only small amounts of data concerning the utility of earthworms as a bio-monitoring organism have been presented so far, the system using earthworms for soil pollutants looks promising. Therefore, we should continue to

study and reinforce the findings on the utility of earthworms as a bio-monitoring organism.

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