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

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 (CdCl2) increased 8-oxo-Gua generation, but nickel chloride (NiCl2) did not. Analyses of the heavy metal accumulation in the earthworm body by atomic absorption spectrometry revealed that CdCl2 accumulated in the earthworm's body, but NiCl2 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-
chemical detection in laboratories (Floyd et al. 1986; Mar nett 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-oxo-Gua 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 Cd exposure might broadly disturb the 8-oxo-Gua repair system, the exact mechanism of the inhibition remains unclear. In addition to Cd, other factors, 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. 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 functioning cooperatively in a complex network, involving both protein-DNA and protein-protein interactions. They exist in many kinds of organisms, including humans (Puilde et al. 1966), rats (Wiemerska et al. 1970), mouse (Nordberg 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 generation in the DNA of Cd-exposed earthworms. These findings suggest that Cd exposure might broadly disturb the 8-oxo-Gua repair system, the exact mechanism of the inhibition remains unclear.

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 re-arrangements 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 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-oxodGTPase 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 factors, such as Mn, As, Cr, and Pb, also displayed similar effects on OGG1 activity or OGG1 expression (Hirano 2008).
Earthworms and 8-oxoguanine. Hirano and Tamae

8-OXOGLUANINE GENERATION IN EARTHWORMS

Among the many kinds of organisms living in soil, earthworms are quite familiar creatures. Earthworms are among the many kinds of organisms living in soil, earthworms are quite familiar creatures. Earthworms are Among the many kinds of organisms living in soil, earthworms are quite familiar creatures. Earthworms are

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 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

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 658, 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.

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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