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Is gastrointestinal microbiota relevant for endogenous mercury methylation in terrestrial animals?

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ABSTRACT

The active role of gastrointestinal microbiota in mercury (Hg) methylation has been investigated in different terrestrial organisms from insects or annelids to rats and mammals, including the human beings. Some findings reveal the animal digestive tracts as new potential niches for Hg methylation especially in terrestrial invertebrates. However, contradictory results have been reported so far and there is still a long way to fully understand how important the MeHg production in this habitat could be, as well as its implications on the toxicity and biomagnification of MeHg within terrestrial food chains. It is important to know what has been studied in the past and discuss the previous results according to the new perspectives opened in this field. Therefore, the aim of this work is to review the present state of knowledge about the potential capability of gastrointestinal microbiota in Hg methylation with special emphasis in terrestrial animals and to propose new approaches profiting the new and powerful molecular and analytical tools.

1. Introduction

Mercury (Hg) is a global pollutant with different chemical forms such as elemental mercury (Hg^0), inorganic mercury (IHg, mainly Hg^{2+}) or organic mercury (mainly monomethylmercury, MeHg). The toxicity of Hg strongly depends on its chemical species and MeHg is particularly worrying because it is an important neurotoxicant. Moreover, it can be bioaccumulated and biomagnified in the aquatic ecosystems leading to warning levels in fish for human consumption (Driscoll et al., 2013; UNEP, 2013). Fish ingestion is the main route of exposure to Hg for the general human population. The problem is even worse because the highly toxic MeHg can be produced by methylation of IHg in the environment by different biotic and abiotic processes (Río Segade et al., 2010). Nowadays, the most accepted hypothesis is that MeHg is produced in sediments mainly by anaerobic microorganisms and then it is bioaccumulated preferently due to its higher liposolubility (Benoit et al., 2003; Gilmour et al., 2011). However, it is assumed that different pathways can exist.

A very important achievement in this field has been the recent identification of the genes in bacteria required for the methylation of Hg, because they linked a specific gene cluster (*hgcAB*) to Hg methylation (Parks et al., 2013; Smith et al., 2015). This gene ortholog was present in confirmed methylators but no present in non-methylators. Gilmour et al. (2013) confirmed that possessing *hgcAB* predicts Hg methylation capability and they demonstrated that a number of species other than sulfate- (SRB) and iron- (FeRB) reducing bacteria, including methanogens, and syntrophic, acetogenic, and fermentative *Firmicutes*, can methylate Hg. These species expand our known niches for MeHg production including anaerobic and methanogenic habitats such as the animal digestive tract and open new perspectives that should be further investigated. At this point, there are some previous ancient and recent works with different species (from invertebrates to humans) and experimental approaches where the methylation by gut bacteria was tested with contradictory results in some cases (Diaz-Bone and Van de Wiele, 2010; Hill, 1995). Recently, Podar et al. (2015) found that *hcgAB* was absent in the studied gastrointestinal microbiomes of mammals (datasets corresponding to humans, bovine, sheep, swine, mouse, dog, wallaby, giant panda and reindeer) and birds (datasets corresponding to broiler chicken and hoatzin), which suggests a low risk of endogenous MeHg production in terrestrial vertebrates. However, *hcgAB* was identified in the gut microbiome of arthropods (cockroach, beetle and termite species), which can explain some routes of MeHg exposure in the terrestrial food webs (Podar et al., 2015).

The role of gastrointestinal microbiota in Hg methylation in terrestrial animals is far from being understood and properly approached. Therefore, the aim of this review is to know the present

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state of knowledge about this topic and to propose new approaches based on the application of the new molecular and analytical tools that are currently available.

2. Mercury metabolism studies in vertebrates

The role of the gastrointestinal microbiota in the potential transformation of IHg into MeHg has been widely investigated in terrestrial vertebrates (especially in human and rats) since several decades ago. The traditional approach has been *in vivo* or *in vitro* studies and the main works about this subject are summarized in Table 1. Factors affecting Hg methylation/demethylation pathways such as pH, redox potential and the presence of Hg methylators are described in Fig. 1 that represents the potential compartments of Hg transformation in the digestive tract of monogastric and ruminants.

2.1. Human intestine

Once that the ability of bacteria to methylate IHg was discovered, one of the first object of study investigated was the intestine of human and rats. Thus, Rowland et al. (1975a) tested the intestinal bacteria capability using pure culture of bacteria (*Escherichia coli*, streptococci, staphylococci, lactobacilli, bacteroide and bifidobacteria) and yeast obtained from human faeces. The incubation with $^{203}Hg^{2+}$ took place during 44 days at 37 °C under aerobic or anaerobic conditions depending on the strain investigated. Most strains of the aerobic bacteria could synthesize MeHg but in contrast obligate anaerobes and lactobacilli could not do it. The active role of bacteria obtained from the human intestine in Hg methylation was a very important finding. However, the information cannot fully trusted because the methods either to detect microbes or to determine their strains, species or physiology were very rudimentary by that time.

In another old study with human faecal suspensions, Edwards and McBride demonstrated in 1975 that the human intestine has the potential to transform IHg to MeHg. Isotopically marked $^{203}Hg^{2+}$ was spiked to a suspension obtained with freshly voided human faeces and the incubation took place at 37 °C under anaerobic conditions. The generation of MeHg was found in the incubations with $^{203}Hg^{2+}$ and the amount of MeHg was proportional to the amount of IHg spiked. It was a very fast process with a maximum in the first 48 h of incubation followed also by a fast decrease. The MeHg decrease was also further investigated by spiking $^{14}CH_3Hg$ under similar conditions. The isotopically marked MeHg disappeared at a constant rate during the 7 days of the experiment. Therefore, the demethylation by intestine bacteria was also demonstrated, however this experimental design does not allow to discriminate between the chemical or biological pathways responsible for MeHg generation and/or degradation in the anaerobic faecal specimens.

There are several limitations in these very old studies. Firstly, in these experiments the use of control experiments with the same spikes and incubation conditions but using sterilized culture media without faecal or bacterial strains addition are not described. Another drawback it is the high spiked concentration (5 mg/L in Rowland et al. and 0.2–20 mg/L in Edward and McBride), which are several orders of magnitude higher than naturally occurring concentrations in the intestine and may induce erroneous metabolic responses. It is also worth mentioning that all these studies have used the analytical methods available by that

Table 1.
In vitro and *in vivo* studies investigating mercury methylation by gastrointestinal microbiota in terrestrial organisms.

Species	Experimental conditions	References
Vertebrates		
Humans	*In vitro* inoculation of staphylococci, streptococci, bacteroides, bifidobacterias and *E. coli* isolated from human faeces and extraction of MeHg by method of Westöo	Rowland et al. (1975a)
	In vitro incubation of a suspension of freshly voided human faeces spiked with isotopically marked $^{203}Hg^{2+}$ under anaerobic conditions and extraction of MeHg by method of Westöo	Edwards and McBride (1975)
	In vitro incubation of Na_2S solution simulating sulfur-rich large intestinal fluid and supplemented cells with cinnabar extracts	Zhou et al. (2010)
	In vitro inoculation of prepared human intestinal bacteria into a general anaerobic medium broth containing IHg in form of cinnabar, HgS and $HgCl_2$	Zhou et al. (2011)
Rats	*In vivo* study using rats (with jejunal blind loops and non-surgically treated) fed with IHg (1 mg of $HgCl_2$ per day)	Abdulla et al. (1973)
	In vitro incubation of suspensions of caecal contents spiked with $HgCl_2$ or suspensions of caecal or small intestinal contents from male Wistar rats spiked with isotopically marked $^{203}HgCl_2$	Rowland et al. (1975b)
	In vitro incubation of suspensions of caecal or small-intestinal contents from Wistar rats spiked with mercuric chloride labeled with ^{203}Hg under aerobic or anaerobic conditions	Rowland et al. (1977)
	In vitro incubation of intestinal loops (intestinal segments of the gastrointestinal tract from duodenum to caecum) with sterile $HgCl_2$ solutions	Ludwicki (1989)
	In vitro incubation of intestinal contents spiked with $HgCl_2$, MeHg and phenylmercury acetate	Ludwicki (1990)
Cow and sheep rumen	*In vitro* incubations of strained rumen fluid obtained from cows and sheep with radioactive Hg compounds ($^{203}Hg^{2+}$ and $CH_3{}^{203}Hg^{+}$)	Kozak and Forsberg (1979)
Invertebrates		
Termite (*Mastotermes darwiniensis*)	*In vivo* study using *Desulfovibrio intestinalis* isolated from the gut of termite *M. darwiniensis* fed with saw dust of spruce containing different concentrations of IHg	Limper et al. (2008)
Isopod (*Porcellio scaber*)	*In vivo* study using isopod *P. scaber* fed with $^{203}Hg^{2+}$ or $CH_3{}^{203}Hg^{+}$ dosed food	Jereb et al. (2003)
	In vivo study with isopod *P. scaber* fed with hazelnut leaves spiked with $^{203}Hg^{2+}$	Nolde et al. (2005)
Earthworms	*In vitro* incubation of earthworms *Eisenia foetida* with high elemental Hg (Hg^0) concentrations in organic acid solutions	Hinton and Veiga (2008)
	In vitro incubation of earthworms *Lumbricus terrestris* in non-polluted Hg soils freshly spiked with $HgCl_2$ and in Hg-polluted soils with and without spike of $HgCl_2$	Rodríguez-Álvarez et al. (2014)
	In vitro incubation of gut inhabiting bacteria of the earthworm *Lumbricus terrestris* treated with $HgCl_2$ or CH_3HgCl in sterile and non-sterile soils	Rieder et al. (2013)
	In vitro incubation of intestinal microbiota of the earthworm *Eisenia foetida* and intestinal sulfate reducing-bacteria in pure cultures with $HgCl_2$ in sterile and non-sterile soils	Kaschak et al. (2014)

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