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Hormetic effect and mechanism of imidazolium-based ionic liquids on the nematode *Caenorhabditis elegans*

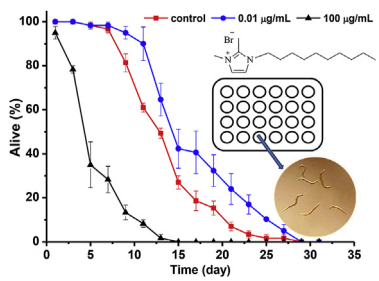
Chun-Jie Zhu, Yong Peng, Zhong-Hua Tong*, Li-Ya Lu, Yin-Hua Cui, Han-Qing Yu

CAS Key Laboratory of Urban Pollutant Conversion, Department of Chemistry, University of Science & Technology of China, Hefei, 230026, China

HIGHLIGHTS

- Ionic liquids showed hormetic effects on the lifespan of *Caenorhabditis elegans*.
- Ionic liquids prolonged the lifespan of *C. elegans* by removing the ROS.
- *sod-5* and *daf-16* genes play an important role in the lifespan extension.
- The hormetic effect caused by ionic liquids might be an over-compensation response.

GRAPHICAL ABSTRACT



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ABSTRACT

In the present study, we used *Caenorhabditis elegans* assay system to investigate in hormetic effects of imidazolium-based bromide Ionic Liquids (ILs) and explored the possible underlying mechanism. Firstly, *C. elegans* was treated with ILs with different alkyl chain lengths at different concentrations. We found that exposure to ILs at 0.01 mg/L extended the mean lifespan of *C. elegans* and the ILs with longer alkyl chain showed more obvious effects. To investigate the possible mechanism, the nematodes were exposed to the three ILs at 0.01 mg/L for 2, 5, 7, 9 and 11 days. The levels of reactive oxygen species (ROS) in *C. elegans* increased significantly when treated for 2 days and then declined gradually compared to those of respective controls as time went on. After exposure for 11 days, the ROS levels and liposuscin accumulation were significantly lower in the treated groups than those of control group. Meanwhile, the expression of aging-related genes *sod-5* and *daf-16* were both massively up-regulated for the three ILs examined. Our results show that low concentration of ILs exert hormetic effect on *C. elegans*. ROS generation and expression of aging-related genes may play important roles in the IL-induced hormetic effect on *C. elegans*.

1. Introduction

Ionic liquids (ILs) have been considered as an eco-friendly alternative to traditional organic solvents due to its unique properties of excellent solubility, well chemical and thermal stability, negligible vapor pressure and high electrical conductivity (Niu et al., 2014; Villanueva et al., 2015). Nevertheless, some researchers have found the poor biodegradability of ILs (Neumann et al., 2010; Pham et al., 2010), raising concerns on their environmental effects. Several studies have shown that some ILs have certain toxicity to aquatic organisms and cells. Mikkola et al. (2015) found that the amphiphilic biomass-dissolving ionic liquids were toxic to human corneal epithelial cells and bacteria. Du et al. (2014) reported that the ILs induced DNA damage and oxidative stress in

* Corresponding author.
E-mail address: zhtong@ustc.edu.cn (Z.-H. Tong).

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zabrafish. Previous studies mainly focused on their lethal toxicities and explored the toxicity mechanism at lethal or sub-lethal concentration (Ventura et al., 2012; Wu et al., 2013). Recently, several studies reported that ILs have hormetic (stimulating) effects on the growth and metabolic activities of bacteria (Nancharaiah and Francis, 2015; Zhang et al., 2013a).

Hormesis is an adaptive response characterized by a low dose stimulation and high dose inhibition, which is proposed to enrich current risk assessment theory (Calabrese, 2002; Mattson, 2008). This response is expected to become a better alternative to predict and extrapolate the toxicity of harmful chemicals than traditional models (Calabrese, 2004). Whereas, studies on the hormetic effect of ILs are mainly focused on the hormetic response in bacteria and microalgae. Several imidazolium ionic liquids have been demonstrated to stimulate the growth of microalgae (Cho et al., 2007). Time-dependent hormetic effects of ILs on *Vibrio qinghaiensis* sp. were observed by Wang et al. (2011) and Zhang et al. (2013b), and such stimulatory effect was demonstrated as a result of physical blocking of AMP molecule by the imidazolium ring of IL (Chen et al., 2015). Nancharaiah and Francis (2015) have found remarkable hormesis of 1-ethyl-3-methylimidazolium acetate on the growth of both Gram positive and Gram negative bacteria, which might be mediated by the buffering capacity of the acetate anion.

Hormetic response exposed to oxidative stress has also been observed in *Caenorhabditis elegans* (Heidler et al., 2010; Zhao and Wang, 2012). While our previous study has demonstrated the ROS induction in *C. elegans* by high concentration of ILs (Wu et al., 2013), we presume that low-concentration ILs may have hormetic effect on the worms. With more than 40 percent of its genome homologous to that of humans, *C. elegans* has been one of the most widely used model animals to study animal development and to assess chemical toxicities (*C. elegans* Sequencing Consortium, 1998). As the first multicellular organism which genome was completely sequenced, it is also an ideal model organism for mechanistic study. Therefore, the main objective of this study was to study the hormetic effect induced by ILs and to explore the underlying mechanism, using *C. elegans* as a model animal. We measured the levels of reactive oxygen species (ROS) and lipofuscin accumulation which have intimate connections with disease development and aging acceleration (Du et al., 2013; Ureshino et al., 2014). Quantitative real-time polymerase chain reaction (RT-PCR) was used to determine the expression of aging-related genes *sod-5* and *daf-16* in *C. elegans*. Our results may provide useful information for understanding IL-induced hormesis mechanism in animals.

2. Materials and methods

2.1. Ionic liquids and reagents

The imidazolium-based bromide ILs used in this study were 1-octyl-2-methyl-3-methyl-imidazolium bromine ([OMMIM]Br), 1-decyl-2-methyl-3-methyl-imidazolium bromine ([DMMIM]Br), and 1-dodecyl-2-methyl-3-methyl-imidazolium bromine ([DoMMIM]Br). They were obtained from Shanghai Cheng Jie Chemical Co., China with purities more than 99%. 5-fluoro-2′deoxyuridine (FUDR) (99%) was purchased from Sigma-Aldrich (St. Louis, MO). Other reagents were analytical grade and purchased from various companies.

2.2. Nematode maintenance and synchronization

The Bristol N2 (wild type) obtained from Caenorhabditis Genetics Center (Minneapolis, USA) were used in this study. The worms were grown in Petri dishes on nematode growth medium (NGM) with *Escherichia coli* OP50 strain as a food source. The nematode culture was age-synchronized by sodium hydroxide/hypochloride treatment as described previously (Brenner, 1974) and grown at 20 °C until L4 larval stage.

2.3. Lifespan assay

In the lifespan assay, 20 ± 1 worms at L4 stage were transferred to wells in 24-well costar plates containing the indicated concentration (0.01, 0.1, 1, 10, 100 mg/L) of ionic liquids in S medium (Sulston and Hodgkin, 1988) and supplemented with 120 μM FUDR to prevent progeny production and bacterial contamination.

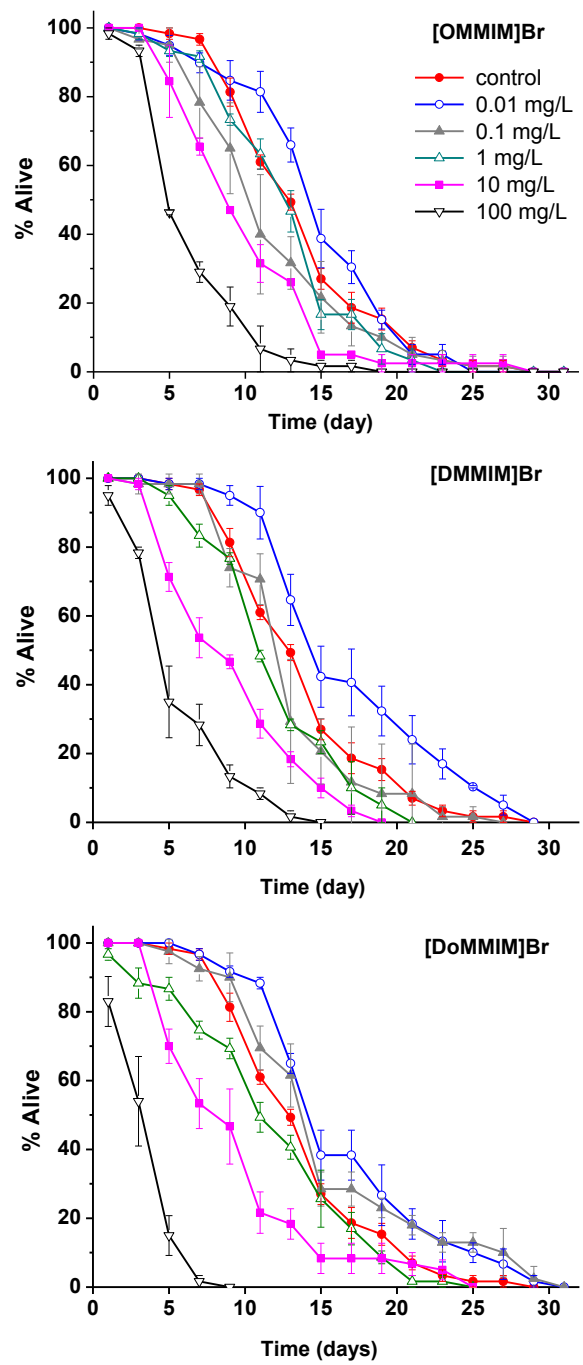

Fig. 1. The lifespan curves of *C. elegans* exposed to different concentrations of ILs: [OMMIM]Br, [DMMIM]Br, [DoMMIM]Br. Twenty worms were examined per treatment. Data are expressed as mean ± SE, n = 3.