Environmental Toxicology and Pharmacology 41 (2016) 32–38

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Acute exposure of mercury chloride stimulates the tissue regeneration program and reactive oxygen species production in the *Drosophila* midgut

Zhi Chen*,[1], Xiaochun Wu[1], Hongjie Luo, Lingling Zhao, Xin Ji, Xianfeng Qiao, Yaping Jin, Wei Liu*

Key Laboratory of Animal Biotechnology, The Ministry of Agriculture, College of Veterinary Medicine, Northwest A&F University, Yangling 712100, Shaanxi, China

ARTICLE INFO

Article history:
Received 7 September 2015
Received in revised form
13 November 2015
Accepted 16 November 2015
Available online 22 November 2015

Keywords:
Drosophila
Mercury chloride
Midgut
ROS
Vitamin E

ABSTRACT

We used *Drosophila* as an animal model to study the digestive tract in response to the exposure of inorganic mercury ($HgCl_2$). We found that after oral administration, mercury was mainly sequestered within the midgut. This resulted in increased cell death, which in turn stimulated the tissue regeneration program, including accelerated proliferation and differentiation of the intestinal stem cells (ISCs). We further demonstrated that these injuries correlate closely with the excessive production of the reactive oxygen species (ROS), as vitamin E, an antioxidant reagent, efficiently suppressed the $HgCl_2$-induced phenotypes of midgut and improved the viability. We propose that the *Drosophila* midgut could serve as a suitable model to study the treatment of acute hydrargyrism on the digestive systems.

1. Introduction

Mercury chloride ($HgCl_2$) is a heavy metal compound that has been widely used in agriculture and medicine. It is also a dangerous environmental pollutant harming various animal tissues, especially the gastrointestine, kidney, liver and neuron. Its toxicity is mainly attributed to the ability of mercury to bind and inhibit certain antioxidant factors, which results in the overproduction of the deleterious ROS (Carvalho et al., 2008; Clarkson and Magos, 2006; Hansen et al., 2006; Stohs and Bagchi, 1995; Tchounwou et al., 2012).

Ingestion is a common way for mercury exposure. Normally, the gastrointestinal epithelium serves as the primary barrier to prevent the absorption of many kinds of toxins into the body. The gastrointestinal epithelia constantly suffer from cell loss induced by various physical, chemical or bacterial stressors. As a defense mechanism, organisms have developed the stem cell-mediated regeneration program, which is critical to maintain the tissue's structural and functional integrity (Scoville et al., 2008). To date, many studies have focused on the impairments of the gastrointestinal system brought by mercury exposure; few works have been done to examine the responses of the tissue regeneration system to the wounds.

The *Drosophila* midgut, the functional equivalent to the mammalian intestine, is an ideal model system to study stem cell homeostasis and tissue regeneration (Apidianakis and Rahme, 2011; Pitsouli et al., 2009). It is essentially a monolayer epithelial tube, which is composed of four major cell types: the intestinal stem cells (ISCs) marked by Delta (Dl), the transient enteroblasts (EBs) identified by *Suppressor of Hairless* [Su(H)], the secretary enteroendocrine cells (EEs) marked by Prospero (Pros) and the polyploid enterocytes (ECs). ISC is the only cell type in the midgut that can proliferate and often directly contacts with the basement membrane. Following one round of asymmetric cell division, each ISC produces two different daughters: one renewed ISC and one immature EB, collectively referred to as the intestinal progenitor cells. The transient EB no longer divides but differentiates into either an EC or an EE depending on the strength of Notch activity. Much like its mammalian counterpart, the *Drosophila* midgut epithelium is also a rapidly self-renewing tissue with a high turnover rate. Various toxins can induce suicide of the epithelial cells, which in turn secrete protective signals to the environment, such as the cytokine unpaired-family proteins, to initiate the ISC-mediated repairing

* Corresponding authors.
E-mail addresses: xiaolongzaishi@sina.com (Z. Chen), wliucn150@yahoo.com (W. Liu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.etap.2015.11.009
1382-6689/

program (Buchon et al., 2009; Jiang et al., 2009; Li et al., 2013; Tian et al., 2015; Zhou et al., 2015).

In this work, we used *Drosophila* midgut as a model to investigate the responses of the digestive tract to the injuries after oral administration of $HgCl_2$. Our data revealed that the ingested $HgCl_2$ was mostly retained within the midgut. As a consequence, ROS was locally overproduced and the apoptotic epithelial cells were increased. This stimulated the tissue regeneration through accelerated proliferation and differentiation of the ISCs. Surprisingly, cell death and ROS were barely detectable in the brain, a region that was believed to be especially vulnerable to the mercury toxin. In addition, we demonstrated that suppression of the ROS by anti-oxidant vitamin E efficiently alleviated the tissue damages and promoted the survival rate. Our work demonstrated that the *Drosophila* midgut could serve as an ideal model to study the mechanism of tissue recovery from mercury exposure and also to develop novel treatments of hydrargyrism.

2. Materials and methods

2.1. Drosophila stocks and culture maintenance

We used the following flies, which have been described previously (Liu et al., 2010): w^{1118}; *esg-Gal4*, *UAS-GFP*; *Su(H)GBE-lacZ*; *hsp-FLP*; *tubulin-Gal80*, *FRT40A*; *actin-Gal4*, *UAS-GFP*. Flies were cultured on standard corn meal at 25 °C, 60% humidity.

2.2. Feeding experiment

3-Day-old adult flies were starved and dehydrated in empty vials for 2 h. After that, 50 flies were transferred to a new vial containing a piece of 3 cm × 2 cm filter paper saturated with the feeding medium. The filter papers were changed twice a day. Both $HgCl_2$ and vitamin E (Sigma Aldrich) was diluted with 5% sucrose (Inamdar et al., 2010; Jimenez-Del-Rio et al., 2008). The final concentration of vitamin E in the feeding medium was 1.5 mM and it was administrated the same way as $HgCl_2$. To check the protective role of vitamin E on mercury toxin, flies were fed with a mixture of $HgCl_2$ and vitamin E.

2.3. Mercury quantification assay

Briefly, flies (both genders) that aged for 2–3 days were fed with 400 μM of $HgCl_2$ for 3 days. After that, they were transferred to new vials supplied with 5% sucrose and cultured for 12 h to excrete the residual $HgCl_2$-medium within the lumen of the digestive tract. Flies were extensively washed by double distilled water (ddH_2O) to remove the contaminants on the body surface. To prepare the midgut samples, 300 flies were dissected. The connecting structures, including cardia, malpighian tube and hindgut were removed. The adults (300) or midguts were dehydrated at 60 °C for 5 h. After weighing, each sample was put into a 50 ml conical flask. Then, 0.5 ml of ddH_2O, 5 ml of HNO_3 and 2.5 ml of H_2SO_4 were sequentially added. The samples were heated at 140 °C for 30 min, cooled and diluted with 50 ml of ddH_2O. The amount of mercury was measured using the cold atomic absorption spectrometry.

2.4. MARCM clone analysis

Female flies with the genotype of *hsFLP*; *tubulin-Gal80*, *FRT40A/FRT40A*; *act-Gal4*, *UAS-GFP/+* were generated by standard crosses. To induce mitotic recombination, 4-day-old flies were heat-shocked for 60 min in a 37 °C water bath, and allowed to recover for 24 hours at 25 °C supplied with standard corn meal. Subsequently, flies were starved in empty vials for 2 h, fed with appropriate medium for 3 days. Midguts were dissected on the fourth day after clone induction.

2.5. Immunostaining and image capture

Midguts were dissected in 1× phosphate-buffered saline (PBS) and fixed for 30 min in 4% paraformaldehyde. After that, samples were permeabilized by soaking in PBST (0.1% Triton X-100) for 10 min and washed for three times in PBS. Incubation with the primary antibody was performed overnight at 4 °C, and for 2 h for the secondary antibodies at room temperature. The following primary antibodies were used: mouse anti-Delta (1:50, DSHB); mouse monoclonal anti-Prospero (1:50, DSHB); rabbit polyclonal anti-GFP (1:500, Invitrogen); rabbit anti-phospho-histone H3 (PH3, 1:1000, CST); and mouse anti-LacZ (1:500, Promega). Fluorescent secondary antibodies used were obtained from Invitrogen (1:500). Images were captured using the Nikon A1R-si confocal microscope system.

2.6. TUNEL assay and measurement of ROS

Midguts and brains were dissected and the TdT-mediated dUTP nick end labeling (TUNEL) staining was performed following the manufacturer's instructions (Millipore, S7165). ROS was monitored by 2′,7′-dichloro-dihydro-fluorescein diacetate (DCF-DA) staining using the ROS detection kit (Beyotime, S0033).

2.7. Quantification analysis

The fluorescence-staining cells, including those of esgGFP+, Dl+, Su(H)GBE-lacZ+ and TUNEL+, were counted in an area (100 μm × 100 μm) of the same designated region of the posterior midguts. The DCF-DA fluorescence signal within an area (150 μm × 150 μm) was measured using the Image J program. All quantification data are shown as mean ± SD. Statistical significance was measured by Student's *t*-test. *P* values of <0.05 = * were considered significant.

3. Results

3.1. Acute exposure of $HgCl_2$ causes dose dependent lethality in Drosophila

It has been reported before that ingestion of excess $HgCl_2$ killed flies in a dosage dependent manner (Paula et al., 2012). To examine the consequences of *Drosophila* midgut upon mercury exposure, we first performed a similar feeding experiment in order to set up an appropriate dose of $HgCl_2$ for the subsequent experiments. Adult flies aged for 3 days were fed with different doses of $HgCl_2$. Acute lethality was observed at high concentrations (1000 and 4000 μM), of which more than 50% of flies died within 2–3 days (Fig. 1A). At concentrations (40 and 100 μM), about 10% died during the first three days. At 400 μM, this number increased up to 20% (Fig. 1A). We then used 400 μM in the following $HgCl_2$ treatment.

3.2. Ingestion of $HgCl_2$ induces cell death of the midgut epithelium

Midgut is the major region of food digestion and nutrients absorption in *Drosophila*. The luminal epithelium serves as the original surface for toxin exposure. As an initial step to check the possible damages to midgut, we first examined cell viability using the TUNEL assay. After feeding with $HgCl_2$ for 3 days, the number of dying cells on the gut epithelia was markedly increased (Fig. 1B and C). Thus, acute ingestion of $HgCl_2$ can kill the cells of the *Drosophila* midgut epithelium.