ENVIRONMENTAL TOXICOLOGY AND PHARMACOLOGY 39 (2015) 659–667

Available online at www.sciencedirect.com

ScienceDirect

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

Malathion-induced testicular toxicity is associated with spermatogenic apoptosis and alterations in testicular enzymes and hormone levels in male Wistar rats

Xiao Geng[a,b], *Hua Shao*[a,*], *Zhihu Zhang*[a], *Jack C. Ng*[c,d], *Cheng Peng*[c,d]

[a] *Shandong Academy of Occupational Health and Occupational Medicine, Jinan 250062, Shandong Province, PR China*

[b] *School of Medical and Life Sciences, Shandong Academy of Medical Sciences, Jinan University, Jinan, PR China*

[c] *The University of Queensland, National Research Centre for Environmental Toxicology – Entox, 39 Kessels Road, Coopers Plains, Brisbane 4108, Australia*

[d] *Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC-CARE), Adelaide 4095, Australia*

ARTICLE INFO

Article history:
Received 17 November 2014
Received in revised form
11 January 2015
Accepted 17 January 2015
Available online 25 January 2015

Keywords:
Malathion
Reproductive effects
Histopathological changes
Testicular enzymes
Apoptosis
Reproductive hormones

ABSTRACT

Malathion has a broad range of toxicities while its reproductive effects have not been fully elucidated. In this study, we treated animals with malathion by gavage at doses of 0, 33.75, 54, and 108 mg/kg for 60 days and evaluated the alterations in histology, biochemistry and serology. Malathion caused the reduction in the sperm counts and motility. The reduced body and testis weights were coupled with mild to severe degenerative changes in seminiferous tubules. We found malathion at 54 mg/kg increased spermatogenic apoptosis rate which was confirmed by changes in protein expression of Bax and Bcl-2. The activities of testicular enzymes including ACP, LDH and γ-GT were significantly altered with the reduced level of reproductive hormones such as LH, FSH and T. These results indicate that malathion can elicit deleterious effects on reproductive system of rats. The abnormal levels of hormones and apoptotic proteins induced by malathion may play important roles.

1. Introduction

Malathion is an organophosphorus (OP) pesticide widely used for controlling pests in live stock and agricultural and garden plants. Residents near the farms and farmworkers may be exposed to organophosphate pesticides due to their common use in agriculture and households (Quirós-Alcalá et al., 2011; Ojha and Srivastava, 2014). The main exposure routes include ingestions through contaminated food and drinking

* *Corresponding author at*: Shandong Academy of Occupational Health and Occupational Medicine, 18877 Jingshi Road, Jinan, Shandong 250062, PR China. Tel.: +86 531 82595888.
E-mail address: chinashaohua5888@163.com (H. Shao).
URL: http://www.sdzfy.cn (H. Shao).

http://dx.doi.org/10.1016/j.etap.2015.01.010
1382-6689/

water (Flores-Garcia et al., 2011; Sapbamrer and Hongsibsong, 2014), inhalation during production, handling and application of insecticides (Machera et al., 2003), and dermal contact with contaminated soil and plants and from accidental spills (Tuomainen et al., 2002). Acute exposure to OP insecticides and subsequent OP poisoning with muscle dysfunction can be a cause of deaths in humans due to mitochondrial dysfunction (Karami-Mohajeri et al., 2014). Long term exposure to OP pesticides has been proved to impose higher risks of various chronic diseases (Mostafalou and Abdollahi, 2013).

Malathion is widely used because of its relatively low acute toxicity compared to other OP insecticides. Extensive studies have been conducted for assessing the potential health effects of malathion in a variety of biological models from amphibians to mammalians. It has been reported that malathion induces toxicity through the inhibition of acetylcholinesterase (AchE) and subsequent activation of cholinergic receptors (Lasram et al., 2008). Animal study suggested that inhibition of enzymes leads to age-dependent neurologically disorders neurological and behavior disorders (Vidair, 2004). Moreover, many OP pesticides including malathion have been identified as endocrine disruptors (ED) which can interfere with hormone levels through binding to and activating estrogen, androgen and other hormone receptors (McKinlay et al., 2008; Mnif et al., 2011). Malathion was found to inhibit catecholamine secretion and bind to thyroid hormone receptors (Ishihara et al., 2003). Other general toxicities of malathion at the cellular and organ level have also been identified by *in vitro* methods (Jira et al., 2012).

In addition, malathion has been reported to affect male reproductive system and spermatogenesis in animals (Choudhary et al., 2008). Treatment with malathion decreased the body weight of earthworms' and the spermatic viability in spermatheca (Espinoza-Navarro and Bustos-Obregón, 2005). Studies have suggested that endocrine disruptor pesticides can interfere reproductive and sexual development (McKinlay et al., 2008; Mnif et al., 2011). Malathion impaired steroidogenesis, induced apoptosis in germ cells with proliferation of the seminiferous epithelium (Penna-Videau et al., 2012). A recent study showed malathion induced lower testosterone level, inhibited acetylcholinesterase, and decreased reproductive performance in male mice (Slimen et al., 2014). Exposure to malathion at the pubertal age led to alteration of semen parameters (Slimen et al., 2014). Furthermore, malathion can affect late stages of spermatogenic cells maturation in mice causing damaged DNA and reduced chromatin in spermatogonia and spermatids (Ojha and Srivastava, 2014). However, the full spectrum of the reproductive effects of malathion has not been fully elucidated. This study aimed to investigate the toxicity of malathion on testes in rats. We exposed the rats with different concentrations of malathion for 60 days and then investigated the activities of testicular enzymes, examined the histological changes in testis and sperm quality, and measured the hormone levels including luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone (T) using radioimmunoassay (RIA). We further assessed the expression levels of genes related to reproduction.

2. Materials and methods

2.1. Chemicals

Malathion (95% pure) was obtained from WanDuoFu Chemical (Shandong, China). Malathion was dissolved in distilled water (solubility in water: 145 mg/L at 25 °C) before use. RIA (Radioimmunoassay) kits for luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone (T), and assay kits for testicular enzymes including acid phosphatase (ACP), lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), γ-glutamyl transpeptidase (γ-GT) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). TdT-mediated dUTP digoxigenin nick end labeling (TUNEL) kit was purchased from Roche R&D Centre (China) Ltd. (Shanghai, China). Bax and Bcl-2 anti-rat monoclonal antibody was purchased from Santa Cruz Biotechnology (Gene Company Ltd., Beijing, China) and immunohistochemistry strept avidin–biotin complex (SABC) kit was purchased from Wuhan Boster Biological Technology Ltd. (Wuhan, China). All other chemicals were of analytical grade and obtained from local commercial sources.

2.2. Animals and experimental design and treatment

Male Wistar rats of SPF (Specific Pathogen Free) quality weighing between 80 and 100 g were purchased from Laboratory Animal Centre of Shandong University (Shandong, China). All animals were housed in an animal room with the temperature (20 ± 2 °C) and relative humidity (55 ± 5%). Lighting period was maintained at 12 h/12 h light and dark cycle. Food (manufactured by Laboratory Animal Centre of Shandong Province, Shandong, China) and tap water were provided *ad libitum*. After 1 week of acclimatization, 40 rats were randomly divided into four groups including three exposure groups and a control group of 10 animals each. In the exposure groups, the rats were exposed to malathion by oral gavage at dosages of 33.75, 54, and 108 mg/kg daily for 60 days. Selection of dosage range was based on our pilot study for 2 weeks in which the LD_{50} of malathion is 1080 mg/kg. The control rats were administered with an equivalent volume of distilled water in the same manner. Throughout the experimental period, all animals were observed at least once daily for clinical signs of toxicity related to malathion exposure. Animals were sacrificed 60 days post dosing. Handling of animals strictly followed the ethical guidelines (Couto, 2011) in accordance with the institutional Animal Ethics Committee approval.

2.3. Physiological assessment

Body weights of rats were recorded weekly during the study and upon animal euthanasia. Then the testes were collected and weighed.

2.4. Sperm evaluation

At necropsy, the left epididymis of each rat was collected, and the caudal epididymis was used to prepare sperm suspension for measuring sperm counts, motility, and dysmorphology