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Pubertal exposure to saisentong: Effects on thyroid and hepatic enzyme activity in juvenile female rats

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Abstract

Saisentong, as a thiadiazole fungicide, is widely used in China. The structure of saisentong shows a closer relationship to N, N-methylene-bis (2-amino-1, 3, 4-thiadiazole) (Bis-A-TDA), which is a teratogen. A few studies have shown that some of the thiadiazole fungicides act as endocrine disruptors via disturbance in thyroid hormone homeostasis. Little is known about the effect of pubertal exposure to saisentong on the development in pubertal female rats. Based on the protocol of the 20-Day Pubertal Female Assay, we attempt to estimate the possible effect of exposure to saisentong on thyroid hormone and hepatic enzyme activity in female rats. Postnatal days (PND) 22 old SD rats were administered with saisentong daily by oral gavage at doses of 0, 5, 10 or 15 mg/kg/day for 20 days. After treatment, the rats were sacrificed for blood collection; the reproductive organs, liver, pituitary, adrenal and thyroid gland were harvested. The results indicated that saisentong administration increased thyroid weight and thyroid stimulating hormone (TSH) concentrations, and induced hepatic uridine diphosphate glucuronyl transferase (UDPGT) activities in the highest-dose group, although not statistically significant.

The high dose caused a decrease in weight at vaginal opening (VO), but the age at VO was unaffected by saisentong in all treatment groups. No histological changes were observed in uterus and ovaries. These data and changes demonstrate that saisentong is a potential thyroid disrupter in female rat following exposure during development, but does not affect the development of pubertal female rats.

Keywords: Saisentong; Pubertal development; Thyroid hormone; Hepatic enzyme activity

Introduction

Finding novel green compounds with high activity and low toxicity is a development status and trend of future pesticide. Due to thiadiazole compound's excellent bioactivities, especially to bacterial blight, which is one of the most destructive diseases of rice worldwide (Guo et al., 2005), and various chemical structures, it is becoming a new research hotspot for more and more scientists. Thiadiazole contains the five-membered diunsaturated ring structure composed of two nitrogen atoms and one sulfur atom (Fig. 1). With the introduction of thiadiazole, many compounds display broad-spectrum bioactivities. Thiadiazole and its

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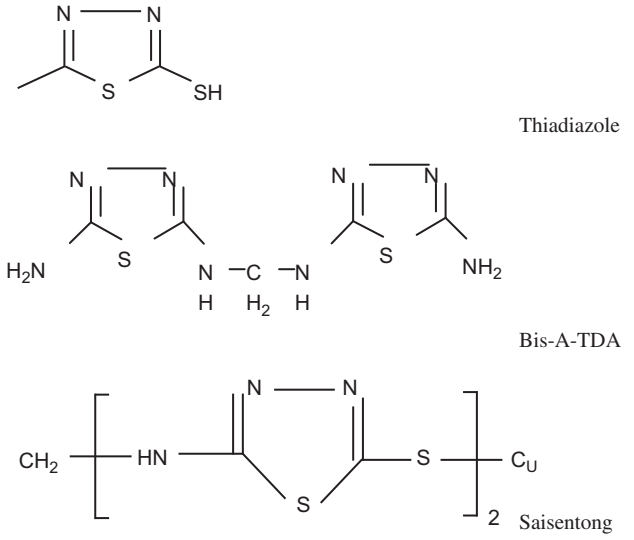

Fig. 1. Structures of thiadiazole, Bis-A-TDA and Saisentong.

derivatives are used for biological activities such as antiviral, antibacterial, antifungal and antituberculous activities. Saisentong [N, N-methylene-bis (2-amino-5-sulfhydryl-1, 3, 4-thiadiazole) copper], which belongs to the sort of thiadiazole fungicide, is a Chinese-created systemic fungicide that has been used to control rice diseases *Xanthomonas oryzae pv. Oryzae* and *Xanthomonas oryzoe pvoryzicola*, (Xing and He, 2007).Since saisentong has a broad spectrum of activity and low toxicity, it was used widely in southern China.

Indeed, the structure of saisentong shows a closer relationship to N, N-methylene-bis (2-amino-1, 3, 4-thiadiazole) (Bis-A-TDA), which is a teratogen (Xu and Gao, 1991; Gu and Qian, 1991). Meanwhile, in our previous study, we found bismerthiazol, another thiadiazole fungicide, to be likely a thyroid disrupter in female rat following exposure during development (Zhang et al., 2008). Nevertheless, little is known about saisentong and its possible effect on thyroid hormone disruption, hepatic enzymes activity and development in pubertal female rats.

Pubertal female rat assay, as one of the screening batteries for identifying thyroid toxicants, is used to quantify the effects of chemicals on pubertal development and thyroid function in the intact peripubertal female rat. Two essential endpoints that are routinely used for identifying compounds that alter thyroid function are thyroid hormone measurements and histopathology of the thyroid gland (O'Connor et al., 1999). In addition, since thyroid hormone concentrations can be considered as an indicator of thyroid toxicants and thyroid gland histopathology has been judged by Duke University Workshop to be the most sensitive parameter for the detection of compounds that adversely affect thyroid function, these two endpoints may be the most useful criteria for identifying thyroid toxicants. Furthermore, the pubertal female assay also examines the endpoints associated with the development of female sex organs and secondary sexual characteristics (Kim et al., 2002), and the vaginal opening (VO) can be used as an indicator of pubertal development.

In the present study, we used the pubertal female rat assay to detect the effect of saisentong on pubertal development, thyroid hormone disruption and hepatic enzyme activity in the intact juvenile female rat. We have examined the effect of saisentong on several thyroid endpoints (serum hormone concentrations, thyroid gland weight and thyroid gland histopathology), development endpoints (the age and weight of VO in the juvenile female rat and reproductive organs) and hepatic enzyme activity about 4-nitrophenol uridinediphosphate-glucuronosyltransferase (UDPGT), which could help us understand whether pubertal exposure to saisentong acts as endocrine disrupters in female rats.

Materials and methods

Animals

Sprague–Dawley rats were purchased from SINO-BRITISH SIPPR/BK Laboratory Animal Ltd. in Shanghai under specific pathogen-free (SPF) condition, bred in the animal room of the National Shenyang Center for Drug Safety and Evaluation Research (GLP). Juvenile female rats were derived from individually housed pregnant females that were bred in-house. All dams were pregnant for the first time and timed to deliver on the same day. Dams delivered their pups naturally. Any litters with fewer than 8 total litter (including both males and females) and any litters not delivered by gestation day (GD) 23 were excluded from the study. To maximize uniformity in growth rates, the litters were standardized to 8 pups per litter in postnatal days (PND) 4. Body weights are monitored weekly and any unthrifty litters or runty pups were excluded from the study. Before being placed on study, dams with litters of 21-day-old female rats were housed together in clear polycarbonate cages. All animals were maintained in a well-ventilated room at a temperature of $22\pm2\,^{\circ}C$ and a relative humidity of $55\pm10\%$, with a 12 h light/12 h dark cycle. Food and tap water were provided ad libitum. The sterilized feed was purchased from Qianming Animal Feed Factory, at Yuhong district in Shenyang. All animals, including the control group used in this experiment, were handled in an accredited Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) facility. On PND 22, the animals were allocated to different treatment groups in accordance with their body weight (b.w.).