Toxicology Letters 180 (2008) 38–45

Contents lists available at ScienceDirect

Toxicology Letters

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

Mechanism of trifluralin-induced thyroid tumors in rats

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ARTICLE INFO

Article history:
Received 15 April 2008
Received in revised form 27 May 2008
Accepted 27 May 2008
Available online 6 June 2008

Keywords:
Trifluralin
Dinitroanilines
Thyroxine (T4)
Triiodothyronine (T3)
Thyroid stimulating hormone (TSH)
Thyroid tumor in rats

ABSTRACT

Trifluralin, an herbicide, has been reported to cause a significant increase in thyroid follicular cell tumors in male Fischer 344 rats. This study was designed to determine the mechanism of thyroid hyperactivity after trifluralin exposure. A group of 15 male Fischer 344 rats were exposed to trifluralin-fortified (6500 ppm) diet for 2 weeks. The time weighted average daily intake of trifluralin was 441 ± 77 mg/kg/day. Ten rats of the group were sacrificed and the sera analyzed for T3, T4, and TSH levels. The livers were also analyzed for selected T4-specific *UGT* gene expression and total UGT enzyme activity. In the trifluralin treated rats, the serum T3 and T4 levels decreased by 17% and 90%, respectively and TSH increased by 37% more than the control rats. Trifluralin-induced total hepatic UGT enzymes (2.4-fold) and mRNA expression of selected hepatic UGT isozymes (UGT1A1, 1.4-fold; UGT1A6, 6.4-fold; UGT2B1, 3.7-fold). For the remaining 5 rats in the group, bile was collected for 2 h and analyzed for free and conjugated T3 and T4. The total amount of T4 in bile more than doubled in trifluralin treated rats. Trifluralin treatment increased bile flow, caused a 3.2-fold increase in biliary elimination of conjugated T4 and 63% increase in conjugated T3. Based on these data, the decrease in total serum T3 and T4 levels in the trifluralin treated rats was due to enhanced peripheral metabolism and an increase in bile flow that results in a compensatory increase in TSH synthesis and secretion. The increased levels of TSH with chronic exposure to trifluralin would exert a continuous stimulation of the thyroid gland leading to cellular hypertrophy and proliferation predisposing to the development of follicular cell tumors in rats.

1. Introduction

Trifluralin is one of the 2,6-dinitroaniline herbicides used to control many germinating annual grass and broadleaf weeds. The dinitroaniline herbicides prevent cells in roots and shoots from dividing and developing (Grover et al., 1997). Some of the other members of the 2,6-dinitroaniline herbicide family include benfluralin, ethalfluralin, oryzalin and pendimethalin. Trifluralin has been reported to cause a significant increase in thyroid follicular cell tumors in male Fischer 344 rats only at the highest dietary dose of 6500 ppm in a 2-year chronic study (Emmerson et al., 1980). Other dinitroaniline herbicides have also been reported to cause thyroid tumors in rats (USEPA, 1997, 2003, 2004). There are no specific studies investigating the mechanism by which trifluralin causes these thyroid cell tumors.

Thyroid gland secretes thyroid hormones (TH) thyroxine (T4) and triiodothyronine (T3) in response to thyroid stimulating hormone (TSH) from the anterior pituitary gland, as illustrated in Fig. 1. Thyroid hormones bind strongly, but not covalently, to serum protein and are critical in regulating normal growth, development and metabolism. Humans, primates and dogs possess a high-affinity TH-binding protein, thyroxine-binding globulin (TBG). Rats lack TBG; however, both humans and rats possess low affinity carrier proteins for TH, thyroxine-binding prealbumin (transthyretin) and albumin (Hill et al., 1989, 1998). Binding affinity of TH to albumin and transthyretin is 3 and 5 orders of magnitude lower than TBG, respectively (McClain, 1992). The lack of TBG makes rats more susceptible to TH removal from blood through metabolism and excretion, which is evident from the shorter serum half-lives of T4 and T3 in rats. The serum half-lives of T4 and T3 in rats are 0.5–1 day and 0.25 day, respectively; whereas in humans, the half-lives are 5–9 days and 1 day, respectively (Choksi et al., 2003; Hill et al., 1998; McClain, 1992). This results in higher stimulation of the thyroid gland by TSH in rats, which is evident from the relatively small follicles in rat thyroid glands, often surrounded by cuboidal epithelium (McClain, 1992; Hill et al., 1998). Whereas, thyroid follicular cell in humans are large and less active with abundant colloid surrounded

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0378-4274/$ – see front matter © 2008 Published by Elsevier Ireland Ltd.
doi:10.1016/j.toxlet.2008.05.019

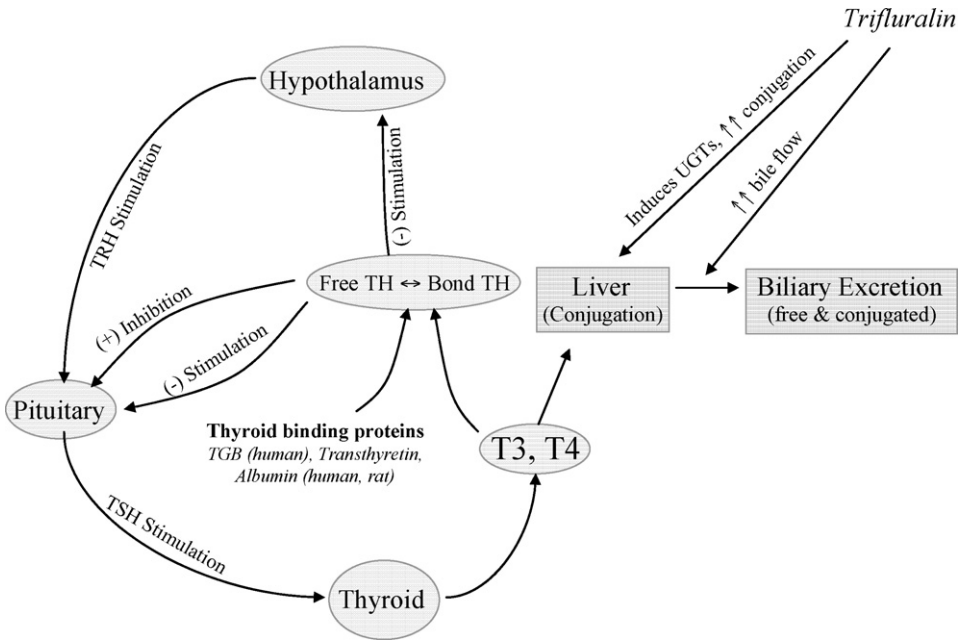

Fig. 1. Possible trifluralin-induced perturbation in thyroid hormones in the hypothalamic-pituitary-thyroid axis in rats that can lead to thyroid follicular cell tumors. Abbreviations: TRH, thyrotropin-releasing hormone; TSH, thyroid stimulating hormone; T3, triiodothyronine; T4, thyroxine; TH, thyroid hormones; TGB, thyroxine-binding globulin; UGT, uridinediphosphate glucuronosyltransferase.

by relatively flattened epithelium (McClain, 1992; Hill et al., 1998). Consequently, thyroid gland in rats is chronically stimulated and thus, a slight perturbation in TH levels may lead to higher than basal TSH levels causing the gland to increase its growth (McClain, 1992; Hill et al., 1998). Chemicals causing thyroid tumors in rats through this mechanism are considered less relevant to humans as their thyroid follicular cells are less susceptible to TSH stimulation and are able to better modulate responses to changing TH levels than rats (Boobis et al., 2006; Cohen et al., 2004; Dellarco et al., 2006; Hill et al., 1998; Miyawaki et al., 2003).

One of the major mechanisms of perturbation in TH is the enhanced clearance of T4 from the body after conjugation via uridinediphosphate glucuronosyltransferases (UGTs) (Barter and Klaassen, 1992; Vansell and Klassen, 2002). Therefore, any chemical that induces UGTs in liver may lead to an increase in the metabolism and excretion of TH. In humans, this increased metabolism and excretion would initially be compensated for by the reservoir of TH bound to TBG. However, in rats, a decrease in serum T4 would lead to an increased production of TSH by the pituitary gland due to the lack of TBG causing hyper-stimulation of thyroid gland, if sustained, may lead to hyperplasia.

This study was designed to determine the mechanism of trifluralin-induced thyroid hyperactivity in rats after trifluralin exposure to the most susceptible gender (male: Chen, 1984; Hill et al., 1998) and strain (Fischer 344: NCI, 1978) of rats. Only one dose of trifluralin (6500 ppm) was used, as the dose-response of trifluralin-induced thyroid tumors in male rats has been well established (Emmerson et al., 1980). Phenobarbital (PB) was used as positive control due to its ability to perturb TH.

2. Materials and methods

2.1. Chemicals

Trifluralin (2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl)benzenamine) was obtained from Dow AgroSciences, LLC (Indianapolis, IN). Phenobarbital (5-ethyl-5-phenylbarbituric acid) and β-glucuronidase from *Helix pomatia*, type HP-2, were purchased from Sigma–Aldrich (St. Louis, MO). Purity of trifluralin and phenobarbital was 97.1% and 99.0%, respectively. Methyl cellulose ether was obtained from The Dow Chemical Co. (Midland, MI). All other chemicals used were of purest grade available and obtained from standard sources. Control bile obtained from SD rats was purchased from Taconic Farms, Inc. (Germantown, NY).

2.2. Dose preparation and analysis

Desired concentration of trifluralin in the diet was achieved by serially diluting concentrated trifluralin-fortified diet with the ground feed. The PB dose suspension was prepared in 0.5% methyl cellulose ether in water (w/w). Concentration, homogeneity and stability of the test materials in fortified samples (feed and suspension) were determined in aliquots after extraction in acetonitrile and analyses by HPLC/UV. The HPLC system consisted of Agilent HP 1100 equipped with YMC ODS AQ; 2 mm × 50 mm column, mobile phase was water with 0.1% acetic acid (90%) and acetonitrile with 0.1% acetic acid (10%); analytes were detected at 254 nm.

2.3. Animals (care and dosing)

The Institutional Animal Care and Use Committee of The Dow Chemical Company approved the experimental protocols. Animal care and treatment were conducted in accordance with the established guidelines. The study followed good laboratory practice guidelines.

Young adult male Fischer 344 rats (~10 weeks old) were purchased from Charles River Laboratories Inc. (Raleigh, NC) and allowed to acclimatize to the animal facility for 1 week prior to their use in the study. Animals were housed one per cage in stainless steel cages, in rooms designed to maintain adequate conditions (22 ± 3 °C, 40–70% relative humidity, 12 h light/dark photocycle, air exchange 12–15 times/h). The stainless steel cages had wire-mesh floors suspended above catch pans. Cages contained hanging feeders and pressure activated nipple-type watering systems. Animals were provided with LabDiet® Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, MO) in pelleted form during acclimation. Feed and municipal water were provided *ad libitum*.

After acclimatization, rats were randomly selected for dosing. Groups of 15 rats were exposed to trifluralin (6500 ppm) in diet or orally gavaged with PB (75 mg/kg/day, positive control) for 2 weeks. A group of 15 non-exposed rats served as negative control. Rats were provided with ground feed during the 2 weeks of dosing. The dose of trifluralin (6500 ppm or 0.65% in diet) used in this study was the highest dose used by Emmerson et al. (1980) which increased the incidence of follicular cell tumors of the thyroid gland in male Fischer 344 rats in 2-year dietary study. This dose was thought to be appropriate to determine the possible early mechanistic changes in the hypothalamic-pituitary-thyroid axis that may lead to the formation of follicular cell tumors of thyroid. Oral gavage of PB was based on a previously published study where 2 weeks of PB exposure around this dose was shown to induce UGT causing increase in thyroid weight and perturbation in TH (T3, T4, TSH) (O'Connor et al., 2002).