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Mechanistic analysis of metabolomics patterns in rat plasma during administration of direct thyroid hormone synthesis inhibitors or compounds increasing thyroid hormone clearance

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HIGHLIGHTS

- Male and female rats treated with reference compounds producing thyroid toxicity.
- Patterns of common metabolite changes of thyroid effects were established.
- Metabolites separating indirect and direct thyroid effects were identified.
- Tox patterns for direct and indirect thyroid effects are different in male and female rats.
- Biochemical explanation of MoA relevant metabolites is provided.

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ABSTRACT

For identification of toxicological modes of action (MoAs) a database (MetaMap®Tox) was established containing plasma metabolome consisting of approximately 300 endogenous metabolites. Each five male and female Wistar rats per groups were treated with >500 reference compounds over a period of 28 days. More than 120 specific toxicity patterns of common metabolite changes associated with unique MoAs were established.

To establish patterns predictive effects on the thyroid, animals have been treated with reference compounds directly acting on the thyroid hormone formation (such as methimazole, ethylenethiourea) as well as liver enzyme inducers leading to an increased excretion of thyroid hormones and therewith to a secondary response of the thyroid (such as aroclor 1254 and boscalid). Here we present the plasma metabolite changes which form the patterns for direct and indirect effects on the thyroid. It is possible to identify metabolites which are commonly regulated irrespective of an indirect or direct effect on the thyroid as well as groups of metabolites separating both MoAs. By putting the metabolite regulations in the context of affected pathways helps to identify thyroid hormone inhibiting MoAs even when the hormone levels are not consistently changed. E.g., direct thyroid hormone synthesis inhibitors affect some enzymes in the urea cycle, increase the ω-oxidation of fatty acids and decrease glutamate and oxoproline levels, whereas indirect thyroid hormone inhibiting compounds interact with the lipid mediated and liver metabolism.

1. Introduction

BASF SE and metanomics GmbH have established characteristic profiles of endogenous metabolites in rat plasma correlated to toxicological effects of multiple modes of action (MoAs) with a single repeated dose study. For that purpose, a comprehensive database (MetaMap®Tox) has been built up. About 300 metabolites are measured in plasma samples of Wistar rats after 4 weeks repeated administration of about 500 pharmaceutical, chemical and agrochemical compounds for which the toxicity profile is well known. Sets of common metabolite level changes (metabolite patterns) were arranged to characterize their toxicological MoAs.

The thyroid gland is one of the largest of the endocrine tissues. Most thyroid-toxicological events are associated with the follicular cells that are responsible for synthesis, storage and secretion of the thyroid hormones thyroxine (T4) and 3,5,3′-triiodothyronine (T3).

The hypothalamus secretes thyrotrophin releasing hormone (TRH). This hormone stimulates the thyrotropic cells within the

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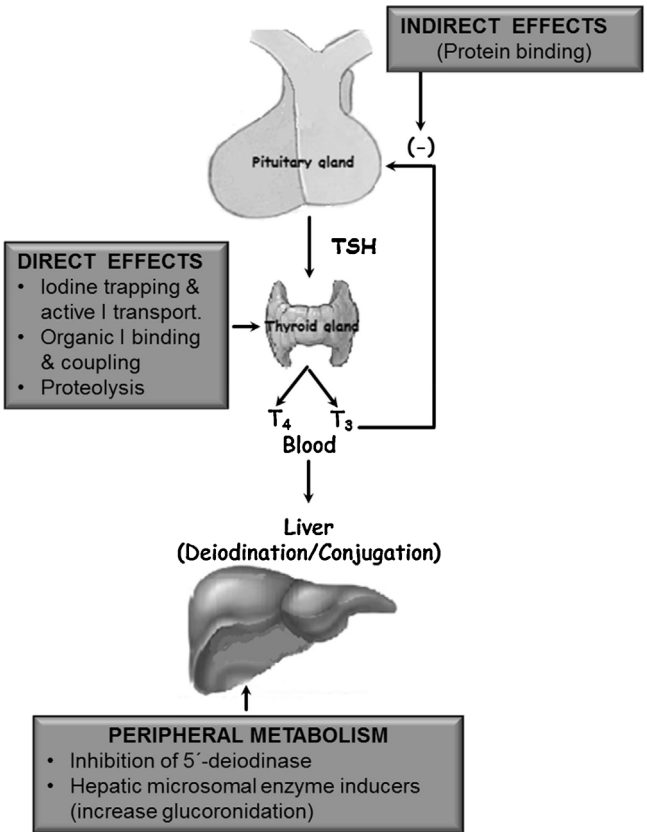

Fig. 1. Schematic representation of the possible sites of interaction of compounds that causes hypothyroidism.
Adapted from Davies (1993).

pituitary gland to release thyroid stimulating hormone (TSH). The activity of the thyroid follicular cells is regulated by the concentration of TSH, acting on the thyroid gland to stimulate the synthesis and release of T3 and T4. In the circulation, both are protein bound (in humans to a high-affinity protein called thyroxine binding globuline (TBG) and in rodents to a nonspecific transport and low affinity protein, albumin (prealbumin and postalbumin), Dohler et al., 1979) and less than 1% of these hormones circulate as free-T3 and free-T4 (Davies, 1993). The inhibitory effects of thyroid hormones and the stimulatory action of TRH (via the hypothalamic–hypophyseal portal system) regulate TSH production in order to maintain optimal thyroid hormone levels in plasma and reflect a feed-back control interrelationship between the secretion and plasma concentration of the thyroid hormones, on one hand, and the secretion of thyrotropin releasing hormone, on the other.

It is worth noting that only free hormones are physiologically active and T3, mainly produced by the deiodination of T4, is about four fold more biologically active on target tissue nuclear receptors than T4. The liver conjugates T3 and T4 by a process of glucuronidation and sulphation, promoting their excretion via bile into the small intestine (Coelho-Palermo and van Ravenzwaay B., 2007; Cunha and van Ravenzwaay B., 2005; Davies, 1993). Thus, concentrations of circulating thyroid hormones depend not only on the rate of secretion from the thyroid gland but also on how fast the hormones are cleared.

Several compounds induce hypothyroidism by different MoAs, these are illustrated in Fig. 1. Despite these differences in toxicological MoAs, their consequences are quite similar. The most common change is thyroid follicular cell hyperplasia and hypertrophy mediated by an increased release of TSH from the anterior pituitary in response to reduced circulating levels of thyroid hormone. There is also evidence that sustained thyroid follicular-cell hypertrophy and hyperplasia can lead to tumor formation in rats (Hood et al., 1999; Klaassen and Hood, 2001; Meek et al., 2003). Additionally, it has been suggested that induction of T3 glucuronidation, rather than T4 glucuronidation, mediates increases in serum TSH of rats treated with microsomal enzyme inducers (Klaassen and Hood, 2001; McClain, 1989). By contrast, thioamides such as methimazole and 6-propyl-2-thiouracil (PTU) inhibit the enzyme thyroid peroxidase (TPO) in the thyroid, reducing the synthesis of T3 and T4, thereby blocking uptake of iodotyrosines from the colloid. They also block iodine release from peripheral hormones (Braverman and Cooper, 2012).

In general, direct mechanisms on thyroid hormone synthesis comprise the inhibition of iodide uptake by the thyroid gland, organification defects from inhibiting TPO catalyzed reactions, the inhibition of thyroid hormone secretion through an excess of iodide and thyroid cytotoxicity. Otherwise, indirect mechanisms are the inhibition of TSH synthesis, competition for thyroid hormone binding proteins or inhibition of T4 deiodination and changes in thyroid hormone conjugation (such as the induction of UDP-glucoronosyltransferase).

Distinguishing between direct and indirect effects in the thyroid is of great significance for the regulatory assessment of thyroid-related findings. To date, the perchlorate discharge assay has been used for this purpose (Coelho-Palermo and van Ravenzwaay B., 2007). Within the framework of metabolomics, compounds known to induce thyroid toxicity through direct and indirect mechanisms were investigated for their common profile changes. For the first case the pattern is referred to as the “thyroid toxicity direct pattern” the reference compounds were ethylenethiourea (ETU), 6-propyl-2-thiouracil (PTU) and methimazole. For the “thyroid toxicity indirect pattern” the selected reference compounds were pendimethalin, fipronil, boscalid and aroclor 1254.

The aim of this publication is the mechanistic analysis of the regulation of metabolites in the aforementioned patterns.

2. Materials and methods

2.1. Animals and maintenance conditions

Wistar rats (CrI:WI(Han)) were supplied by Charles River, Germany at an age of 59–67 days at the beginning of the studies and underwent an acclimatization period of 1 week. The animals were singly housed in standard cages (floor area 800 cm^2), supplied by Becker & Co., Castrop-Rauxel, Germany. The animals were maintained in an air-conditioned room at a temperature of 20–24 °C, a relative humidity of 30–70%, and a 12 h light/12 h dark cycle. Before the animals' arrival, the room was completely disinfected using a disinfector (“AUTEX”, fully automatic, formalin-ammonia-based terminal disinfector, supplied by Dr. Gruß KG, Neuss, Germany). During the study, the floor and walls were cleaned weekly with a solution of 0.1% Incidin® (supplied by Henkel, Düsseldorf, Germany) in water. Ground Kliba mouse/rat maintenance diet was supplied by Provimi Kliba SA, Kaiseraugst, Switzerland. The diet and drinking water were available *ad libitum* (except immediately before blood sampling) and regularly assayed for chemical contaminants and the presence of microorganisms.

2.2. Treatment of animals with compounds

The studies were performed according to the German Animal Welfare legislation 23 177-07/G 08-3-001. The laboratory is AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care) certified.

The compounds were administered via either the feed or via gavage to each group (five rats per sex) in individual tests and the doses were chosen based on BASF internal studies or literature data and reflected the 28 days maximum-tolerated dose (MTD) (Table 1).

2.3. Blood sampling

Between 7:30 and 10:30 h, blood samples were taken from the retrobulbar sinus in all rats under isoflurane anesthesia (1.0 ml K-EDTA blood on study days 7, 14 and 28) after a fasting period of 16–20 h. The blood samples were centrifuged (10 °C, 2000 × *g*, 10 min) and EDTA plasma was separated. The EDTA plasma