Developing a quantitative AOP for liver-mediated thyroid modulation after prenatal exposure to a xenobiotic compound in rats

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INTRODUCTION

Xenobiotic-induced hepatic metabolism causes elevation of phase I and phase II enzymes, which in turn may cause enhanced clearance of thyroid hormones (e.g. Shelby & Klaassen 2006). In humans, the thyroid hormone is needed for orderly development during the first trimester of pregnancy, when the foetus is entirely dependent on the maternal transfer of triiodothyronine (T3) and thyroxine (T4). A decrease in maternal circulating T4 during the first trimester may well result in irreversible mental and psychomotor impairments (Morreale, 2001). Knowledge of the quantitative relationships between the various parameters in the liver/thyroid interaction (adverse outcome) pathway (AOP) after prenatal exposure is crucial for determining points of departure for adverse effects. This project therefore aims to monitor relevant maternal and offspring liver and thyroid parameters after prenatal exposure to a model liver enzyme inducer in rats using BMD modelling in order to define quantitative relationships early in the AOP. Developmental neurotoxicity arising from hepatic elimination of thyroid hormones will be described.

METHODOLOGY

Pregnenolone-16α-carbonitrile (PCN), a model pregnane-X-receptor agonist and UGT1A isozyme inducer, was administered in corn oil to time-mated female rats (Wistar Han) from gestational day (GD) 6 to GD 20 by means of oral gavage. The study consisted of 9 groups: one vehicle control and 8 dose levels (0.1, 0.3, 1, 3, 10, 30, 100, and 300 mg/kg bw/day). The control group consisted of 30 dams, while eight dose groups consisted of 15 dams each. Upon completion of dosing on GD 20 pregnant rats, each treatment group was evenly divided into two cohorts. One cohort was terminated at GD21 and maternal and foetal parameters were assessed. The other cohort was allowed to deliver and was terminated at weaning, and dam and pup parameters were assessed. General parameters included food consumption, body weight, organ weights and histopathology. The blood, livers, thyroids and brains were collected and analysed for thyroid hormones, liver metabolic enzymes, deiodinases, and gene expression levels of relevant metabolic enzymes as appropriate. Dose-response analyses were performed using PROAST software (Slob, 2002).

RESULTS

Pregnancy rate, body weight and food consumption were unaffected throughout the study. Dam liver and thyroid weight were reduced at GD21 in correlation with the dose, and this effect had disappeared at weaning. In general, responding parameters showed full sigmoid dose-response curves with effects appearing at the mid-dose range and plateauing at the higher dose levels. Litter size and pup body weight gain until weaning were unaffected by treatment. At GD21, liver T3 concentrations decreased in the dams while liver T4 concentrations increased, accompanied by up-regulation of relevant Cyp enzymes, glucuronidases, sulphatases, and D3 activity. Serum T3 and T4 decreased at GD21, with no effect on TSH. These parameters had all returned to control values at weaning. Foetal brains at GD21 and pup blood and brains at weaning did not show any changes in thyroid hormone concentrations.

DISCUSSION

The data confirm that PCN acts as a strong phase II liver enzyme inducer in adult rats, followed by secondary modulation of the thyroid axis. After cessation of treatment at GD20, all parameters measured returned to control levels at weaning, indicating reversibility of the effect. The exposure duration prescribed in the OECD 414 prenatal developmental toxicity study protocol, also applied in this study, does not fully cover the brain developmental period in the rats, which continues until adulthood. Therefore, the interpretation of possible brain developmental effects of thyroid hormone modulation (also if mediated by liver metabolic changes) observed in this study protocol encounters limitations in the light of the hazard and risk assessment. Furthermore, effects on offspring brain development in this study cannot as yet be excluded and this requires further analysis. To that end, foetal and pup brains from this study have been collected and stored. Endocrine modulation within normal homeostatic ranges is unlikely to cause adverse developmental effects. The challenge lies in determining the thresholds of hormone level changes beyond which adversity may occur.