Reproductive Toxicology 46 (2014) 85–90

Contents lists available at ScienceDirect

Reproductive Toxicology

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

Effects of furan on male rat reproduction parameters in a 90-day gavage study

Gerard M. Cooke*, Marnie Taylor, Christine Bourque, Ivan Curran, Susan Gurofsky, Santokh Gill

Toxicology Research Division, Sir Frederick G. Banting Research Centre, 2202D1 Tunney's Pasture, Ottawa, ON, Canada K1A 0L2

ARTICLE INFO

Article history:
Received 2 August 2013
Received in revised form 30 January 2014
Accepted 15 February 2014
Available online 12 March 2014

Keywords:
Furan
Gavage
Male reproduction
Testosterone
LH
Steroidogenesis

ABSTRACT

Furan is produced in foods during processing and preservation techniques that involve heat treatment. Previously, we reported that furan-exposed rats exhibited dose-dependent gross and histological changes in liver which correlated with changes in liver serum enzymes ALT, AST and ALP. Here we report the effects of furan on the male reproductive system. There were no histological or weight changes in the reproductive organs. Serum testosterone levels were increased in a dose-dependent manner whereas serum LH was decreased. There were no changes in 17-OHase, 3β-HSD and 17β-HSD activities or serum FSH. Furan did not alter mRNA expression levels for the LH receptor or Tspo but in contrast, mRNA levels of StAR were increased in all doses of furan. The mRNA for the cholesterol side-chain cleavage enzyme (Cyp11a1) was increased by furan at the high dose, as was the level of intratesticular testosterone. We conclude that subchronic furan exposure affects testicular steroidogenesis.

1. Introduction

Furan is a volatile and lipophilic compound which is formed unintentionally through thermal treatment of food [1–4]. Earlier studies have shown that furan is a hepatotoxin and causes cancer in rodents [5,6]. A major source of furan for humans is coffee but appreciable levels are found in canned foodstuffs, jarred baby foods and infant formula [7]. Although identified as a flavour component in food in 1979, dietary exposure assessments for furan were first reported in 2004 when the Food and Drug Administration (FDA) published the results of a survey of furan in canned and jarred foods that undergo heat treatment [8]. For infants and young children, concern has been expressed about potential dietary exposure to furan from the consumption of baby foods sold in jars or cans [9–11]. Recent assessments of human furan intake show ranges between 30 and 590 ng/kg/day for adults but infants, toddlers and children all range from below 100 ng/kg/day to 200–300 ng/kg/day. While coffee consumption probably accounts for the furan in adults, children are not usually drinking coffee and so their exposure is probably due to the consumption of baby food and formula or other sources such as apple juice [7,9–14]. The long-term effects of furan on the health of children is unknown but considering that children may be exposed to particularly high doses of furan via diet, we have investigated the subchronic effects of furan on the male reproductive system of rats [6]. The data from this study was used by JECFA for a risk assessment of furan [3].

Currently, there is little information about toxicological effects of furan on the reproductive system in males. Recently, Karacaoğlu and Selmanoğlu [15] conducted studies on the effects of furan on the male reproductive tract using doses higher than those used in this study [6]. Their results showed histological changes in the testis, a decrease in serum luteinizing hormone (LH) and a decrease in serum testosterone levels. In order to corroborate these results, we evaluated the reproductive neuroendocrine status in male rats by measuring serum FSH, LH, serum and intratesticular testosterone levels. In addition, we examined the activities of testicular steroidogenic enzymes involved in the conversion of pregnenolone to testosterone and the expression levels of the LH

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; ANOVA, one-way analysis of variance; AST, aspartate transaminase; Cyp11a1, cholesterol side-chain cleavage enzyme; DHT, dihydrotestosterone; FSH, follicle stimulating hormone; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; LH, luteinizing hormone; LHR, LH receptor; 17-OHase, 17-hydroxylase, C17,20-lyase; StAR, steroidogenic acute regulatory protein; Tspo, translocator protein.

* Corresponding author. Tel.: +1 613 957 0990; fax: +1 613 941 6959.
E-mail address: Gerard.Cooke@hc-sc.gc.ca (G.M. Cooke).

http://dx.doi.org/10.1016/j.reprotox.2014.02.003
0890-6238/

receptor, steroidogenic acute regulatory (StAR) and the translocator (Tspo) proteins, and Cyp11a1 which converts cholesterol into pregnenolone in mitochondria [16–20].

2. Materials and methods

2.1. Reagents

TRIS, nicotinamide cofactors (NADPH and NAD+), and β-mercaptoethanol were purchased from Sigma–Aldrich (St. Louis, MO). Unlabelled steroids were obtained from Steraloids Inc. (Newport, RI), labelled steroids [4-^{14}C]androstenedione, [4-^{14}C]dehydroepiandrosterone, and [4-^{14}C]progesterone, were purchased from American Radiolabelled Chemicals Inc. (St. Louis, MO) and [1,2,6,7-^{3}H]testosterone from Dupont NEN (Boston, MA). Organic solvents, including acetone, chloroform, ethyl acetate, hexane, and methanol, were obtained from EM Science Merck KgaA (Darmstadt, Germany). Plastic coated Whatman™ PE SIL G silica gel chromatography plates were obtained from Chromatographic Specialties (Montreal, QC). Scintillation fluid was purchased from Anachemia (Montreal, QC) and Tween 80 from Fisher (Montreal, QC).

2.2. Animal experiments

Test compound and dosing solutions were prepared and handled as described previously [5,6]. Animal handling and treatment procedures complied with the Guidelines of the Canadian Council on Animal Care and were approved by the Health Canada Ottawa Animal Care Committee. In brief, 5- to 6-week-old male *Fischer* F344 rats were obtained from Charles River Laboratories Inc. (St.-Constant, QC) and acclimatized for a period of twelve days before studies began. Groups of 12 male rats were treated by gavage over a 90-day period, 5 days a week [5,6], such that they received doses of furan equivalent to 0.0, 0.03, 0.12, 0.5, 2.0 or 8.0 mg/kg bw/day. At the end of the study, animals were killed by exsanguination via the abdominal aorta under isoflurane anaesthesia and were necropsied. Serum and tissues were weighed and collected [6]. Testes for histological examination were fixed in Bouin's fixative [6].

2.3. Serum testosterone assay

Serum testosterone assays were conducted for male rats in duplicate using a commercial ELISA kit, (IBL Minneapolis, USA). The cross reactivity of dihydrotestosterone (DHT) is quoted as 0.8%. The inter- and intra-assay coefficients of variation (CV%) for serum T were 3.3% ($n = 3$) and 7.1% ($n = 3$), respectively. Normal expected ranges for serum testosterone in male rats are 2.0–6.9 ng/ml.

2.4. Intratesticular testosterone

The intratesticular testosterone was measured as previously described [21]. Briefly, testes were thawed, decapsulated and approximately 100–200 mg testis tissue were weighed accurately and homogenized with a Pro250 Polytron homogenizer (Pro Scientific, Monroe, CT) in 50 mM TRIS buffer containing 0.25 M sucrose, 25 mM KCl, 5 mM $MgCl_2$ and 7 mM mercaptoethanol (pH 7.4). Aliquots of testis homogenate were spiked with tritiated testosterone (approximately 85,000 CPM) and a portion retained for recovery calculations. The remaining homogenate was extracted twice with hexane (2 ml), vortexed, centrifuged at $800 \times g$ for 10 min and the organic phase was collected. Extracts were reduced to dryness using ultra pure nitrogen gas (N-EVEP Analytical Evaporator, Organomation, South Berlin, MA) and residues were resuspended in 1% Tween 80 in 5 mM TRIS buffer. Testosterone levels were then quantified by Testosterone ELISA and recoveries of ^{3}H-testosterone were determined by scintillation spectrometry (Packard Liquid Scintillation Analyzer Tri-Carb 2100TR). No testosterone was detected in blank samples processed through the extraction protocol, indicating that neither solvent residues nor buffer interfered with the ELISA.

2.5. Rat LH assay

Serum LH was determined by radioimmunoassay in duplicate using a commercial RIA kit (Biocode Hycel, France). The cross reactivity percent with other rat pituitary hormones by radioimmunoassay was quoted as: rat LH 100%, rat TSH 3.6%, rat PRL <0.1%, rat GH 0.1%, rat FSH 0.1%.

2.6. Rat FSH assay

Serum FSH was determined by radioimmunoassay in duplicate using a commercial ELISA kit, Alpco (Salem, NH, USA). The cross reactivity percent with other rat pituitary hormones by ELISA was quoted as: rat FSH 100%, rat GH <0.1%, rat LH <0.1%, rat TSH, <0.1%, rat PRL, <0.1%.

2.7. Testicular microsomal steroidogenic enzyme activities

Decapsulated testes were homogenized in 100 mM Tris–HCl, pH 7.4 (containing 250 mM sucrose, 25 mM KCl, 5 mM $MgCl_2$, 7 mM mercaptoethanol) and 500 μM NADPH and centrifuged at $10{,}000 \times g$ for 10 min at 4 °C. The supernatant was re-centrifuged under the same conditions and then the post-mitochondrial supernatant was centrifuged at $176{,}000 \times g_{max}$ for 1 h at 4 °C. Microsomal pellets were resuspended in 100 mM Tris–HCl buffer pH 7.4 containing 500 μM NADPH for immediate use in radiometric assays of 3β-HSD, 17α-hydroxylase-C17,20-lyase and 17β-HSD. Assays of the conversion of [4-^{14}C]-labelled steroid substrates were done according to the modified methods of Cooke et al. [22,23]. Thin layer chromatography plates were exposed to phosphorimaging tritium screens (GE Health Care, Montreal, Canada) overnight and then the screens were imaged using a Storm 280 (GE Health Care, Montreal, Canada) and quantified using Image Quant TLV2005 software. Carrier steroids were located by exposure of the thin layer chromatography plates to UV light (254 nm) and iodine vapour. Microsomal protein estimates were determined using the method of Bradford using the Bio Rad Protein Assay and measured using a POLARstar Optima, BMG Labtech plate reader.

2.8. Isolation of RNA and qRT-PCR for targeted gene expression

Total RNA was isolated from frozen testes and purified using RNeasy Lipid Mini Kits (Qiagen, Mississauga, ON, Canada) as per manufacturer's instructions. Integrity of RNA samples was determined using a Bioanalyzer (Agilent Technologies, Mississauga, ON, Canada), whereby samples with RNA integrity numbers (RIN) over 8.0 were used for further analysis. RNA concentrations were determined at A_{260} using the ND-1000 NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Transcriptionally analysis of five genes of interest and β-actin endogenous control gene (Actβ) was performed using pre-designed and optimized TaqMan® Gene Expression Assays (Applied Biosystems, Burlington, ON, Canada). These genes were chosen based on their known biological function (probe/primer sets are listed in Table 1). Six biological samples were chosen from each of the 6 groups (control and 5 treatments). Briefly for cDNA synthesis, 2.5 μg of total RNA was used in the High Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Burlington, ON, Canada). TaqMan® Gene Expression Assays were run according to manufacturer's protocol (with TaqMan® Fast Advanced Master Mix;