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Evaluation of serum and liver toxicokinetics for furan and liver DNA adduct formation in male Fischer 344 rats

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ABSTRACT

Furan is a food processing contaminant found in many common cooked foods that induces liver toxicity and liver cancer in animal models treated with sufficient doses. The metabolism of furan occurs primarily in the liver where CYP 2E1 produces a highly reactive *bis*-electrophile, *cis*-2-butene-1,4-dial (BDA). BDA reacts with nucleophilic groups in amino acids and DNA in vitro to form covalent adducts. Evidence for BDA-nucleoside adduct formation in vivo is limited but important for assessing the carcinogenic hazard of dietary furan. This study used controlled dosing with furan in Fischer 344 rats to measure serum and liver toxicokinetics and the possible formation of BDA-nucleoside adducts in vivo. After gavage exposure, furan concentrations in the liver were consistently higher than those in whole blood (~6-fold), which is consistent with portal vein delivery of a lipophilic compound into the liver. Formation of BDA-2′-deoxycytidine in furan-treated rat liver DNA was not observed using LC/MS/MS after single doses as high as 9.2 mg/kg bw or repeated dosing for up to 360 days above a consistent background level (1-2 adducts per 10^8 nucleotides). This absence of BDA-nucleoside adduct formation is consistent with the general lack of evidence for genotoxicity of furan in vivo.

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1. Introduction

Furan is an important thermal processing contaminant present in a number of common foods, including canned meats, vegetables, soups, and brewed coffee, at levels that can exceed 100 ppb (U.S. Food and Drug Administration, 2007). The lack of correlation between furan levels and food composition is consistent with the complexity associated with many formation pathways, which is corroborated by results from model pyrolytic systems showing that ascorbate, reducing sugars with and without amino acids, and polyunsaturated fatty acids can serve as precursors to furan (Perez-Locas and Yaylayan, 2004). Dietary intake assessment, derived from measured levels of furan in foods and consumption estimate distributions, shows that mean exposure to furan for the U.S. population above the age of 2 is 0.25 μg/kg body weight (bw)/day and 90th percentile exposure is 0.61 μg/kg bw/day, of which approximately one half is provided by coffee (U.S. Food and Drug Administration, 2007; Morehouse et al., 2008). Morehouse et al. (2008) also reported remarkably similar dietary intake assessments for potentially susceptible population groups, including boys and girls (age 2–5 years, 0.23 μg/kg bw/day) and women of child-bearing age (15–45 years, 0.24 μg/kg bw/day). Similar estimates of furan intake worldwide were reported by the World Health Organization/Food and Agriculture Organization Joint Expert Committee on Food Additives (JECFA), in which mean intake in European countries was estimated at 0.27–1.17 μg/kg bw/day and high consumers at 0.60–2.22 μg/kg bw/day (JECFA, 2011). The presence of furan in foods is a toxicological concern because sufficient doses of furan are hepatotoxic in rodents (Moser et al., 2009; Gill et al., 2010) and chronic lifetime exposure increases the incidences of tumors in male and female rats and mice, most notably in the liver (National Toxicology Program, 1993; Moser et al., 2009).

Furan is rapidly and extensively metabolized in the liver (Burka et al., 1991; Kedderis et al., 1993), primarily via cytochrome P450 (CYP) 2E1, following either oral or inhalational administration to Fischer 344 (F344) rats. Following a single gavage dose of ^{14}C-labeled furan to F344 rats, 14% of administered ^{14}C was exhaled as furan, another 26% was exhaled as CO_2, 25% was excreted in the urine, and 20% in the feces during the first 24 h (Burka et al., 1991). Most of the radiolabel remaining in the animal after 24 h was found in the liver (13% of dose) and the preponderance of

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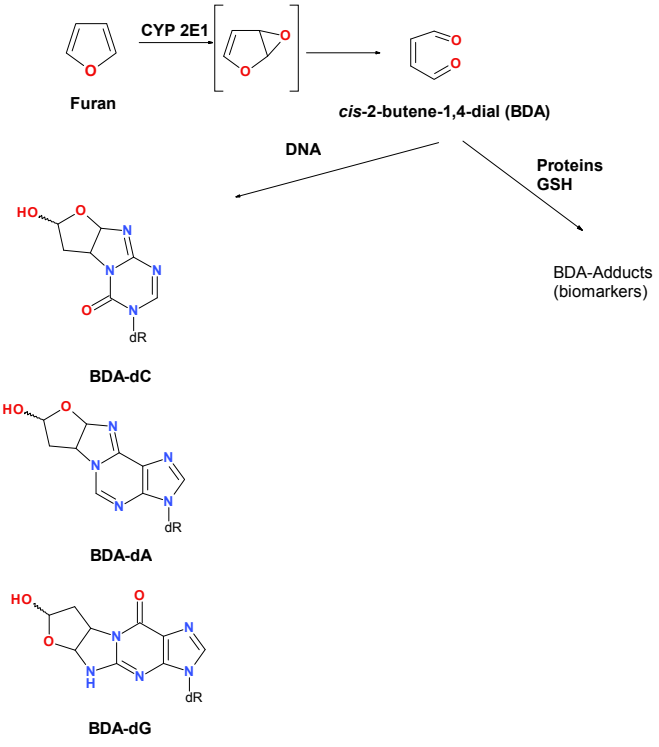
that radiolabel could not be extracted by organic solvents, which is consistent with extensive macromolecular binding. The absence of detectable radiolabel associated with the resulting nucleic acid fraction of liver homogenates was interpreted as resulting from the binding of reactive furan metabolites predominantly to proteins. Even though binding to DNA was undetectable, the low specific activity of the ^{14}C-furan (10 μCi/kg bw for 8 mg/kg bw or 0.085 mCi/mmol) make lower bound estimates of DNA adduct formation quite high by current standards of DNA adduct analysis using LC/MS/MS (i.e., assuming 1 fmol binding per μg DNA or 32 adducts per 10^8 nucleotides, only 0.2 dpm per mg DNA would have resulted). Scheme 1 shows that CPY 2E1 converts furan to the highly reactive *bis*-electrophilic species, *cis*-2-butene-1,4-dial (BDA, Byrns et al., 2002), which covalently modifies nucleophilic groups in amino acids (Chen et al., 1997) and nucleic acid bases (Byrns et al., 2006) under proper conditions in vitro. Furan administration does not appear to be directly mutagenic in rat liver in vivo (McDaniel et al., 2012; Ding et al., 2012), and it has long been proposed that furan exerts its hepatocarcinogenicity via a primary mechanism involving protein damage and cytotoxicity, with secondary effects including inflammation, oxidative stress, and cell proliferation (Kedderis et al., 1993; Moser et al., 2009; Dong et al., 2015); however, some evidence for DNA-associated binding from accelerator mass spectrometric measurements of rat tissue DNA (Neuwirth et al., 2012) and for formation of DNA-protein crosslinks in ovo (Jeffrey et al., 2012) suggests that directly genotoxic mechanisms cannot be ignored.

The current study sought to identify toxicokinetic factors that predispose the liver as the primary site of toxicity following furan administration, with a focus on the oral route, by evaluating the time courses for furan in male F344 rat serum and liver following a single gavage dose. Information about this route of exposure extends and complements that available for inhalation (Kedderis et al., 1993), which is directly relevant to furan exposures through either cigarette smoke (Grill et al., 2015) or possible industrial exposures (National Toxicology Program, 1993). Secondly, this study evaluated the possibility of liver DNA adduct formation in vivo following single or repeated oral dosing of F344 rats with furan by validating sensitive analytical methodology to quantify the major, and most stable, nucleoside adduct formed in vitro by reaction of 2′-deoxycytidine (dC) and BDA (Gingipalli and Dedon, 2001).



Scheme 1. Metabolic activation of furan.

2. Experimental

Water and methanol were Optima LC/MS grade purchased from Fisher Scientific (Pittsburgh, PA). Furan (redistilled and stored at −20 °C, d_4 –furan, adenosine deaminase, alkaline phosphodiesterase, ammonium acetate, Bis-Tris, ethylenediaminetetraacetic acid (EDTA), 2′-deoxycytidine, 2,5-dimethoxy-2,5-dihydrofuran (*cis* and *trans* mixture), DNase I, methoxylamine hydrochloride, sodium phosphate, snake venom phosphodiesterase, and sodium dodecyl sulfate (SDS) were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO). Ammonium hydroxide was purchased from JT Baker, Phillipsburg (NJ).

2.1. Preparation of standards

Stock standards were prepared in methanol at 50 nmol/μL and 500 pmol/μL and stored at −20 °C for up to four weeks. Working standards were prepared daily in water at 5 pmol/μL and kept on ice or at 4 °C. Due to the small but measurable content of unlabeled furan (d_0 content 0.01%), internal standard blanks were analyzed for each sample set and the calculated concentrations were subtracted from all samples in the set (range 0.4–1.5 pmol/mL).

2.2. Animal handling procedures

Procedures involving care and handling of rats were reviewed and approved by the National Center for Toxicological Research (NCTR) Institutional Animal Care and Use Committee. Male F344 rats were obtained from the NCTR colony at approximately 50 days of age. All furan dosing was performed by gavage in corn oil and the furan concentrations in all dosing solutions were quantified using headspace gas chromatography/mass spectrometry (GC/MS) prior to use. A dose of 0.92 mg/kg bw was used for the single dose toxicokinetic study. The requirement to collect a large volume of whole blood (1 mL) for furan time course analysis and the need for timed removals of livers made it necessary to conduct the toxicokinetic study by analyzing 6 rats at each time point, rather than taking serial blood samples from individual rats. A furan dose-adduct formation study was conducted using single doses of 0.92–9.2 mg/kg bw with a common 24 h post-dose exposure time for liver removal. A repeat furan dosing study was conducted using a common dose of 4.4 mg/kg bw/day (n = 6 rats per dose) for 45–360 days of daily gavage treatment (5 days per week) with liver removal 24 h after the last dose. A corn oil vehicle-treated group of rats was included in each sub-study.

2.3. Furan quantitation method development

The method of Nyman et al. (2006) was adapted for quantifying furan in whole blood and liver. Various GC columns were evaluated, with a PLOT-Q column giving the best chromatographic results. Also, both split and splitless injection modes were evaluated, and a 10:1 split ratio gave the best response. Autosampler agitation time and incubation temperatures were evaluated from 40 to 80 °C and 5–30 min, with 10 min at 40 °C giving the best response. The effect of adding salt to the headspace vial was evaluated by the addition of 500 mg ammonium sulfate per mL to spiked blood or liver samples; however, since no improvement in recovery of furan was noted and because the addition of salt tended to plug