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The role of epigenomic alterations in furan-induced hepatobiliary pathologies☆

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ABSTRACT

Furan, a volatile heterocyclic organic chemical found in a wide spectrum of common human foods, is a liver toxicant and carcinogen in mice and rats. The carcinogenic effects of furan have been attributed to genotoxic and non-genotoxic mechanisms. Among the broad range of non-genotoxic alterations induced by furan, epigenetic alterations are of special interest because of their connection to all other non-genotoxic events. This review summarizes current evidence of alterations for epigenetic mechanisms, including cytosine DNA methylation, histone modifications, and microRNA expression, caused by furan exposure and highlights the role of these aberrations in furan-associated hepatobiliary pathologies. It also illustrates the potential role of epigenetic alterations as indicators for carcinogen exposure and for identification of carcinogens, especially those with non-genotoxic mechanisms of action.

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1. Introduction

Furan is a volatile heterocyclic organic "high-production-volume" chemical widely used as a synthetic intermediate and in the production of pesticides, stabilizers, and pharmaceuticals (International Agency for Research on Cancer, 1995). Furan is also a constituent of tobacco smoke (International Agency for Research on Cancer, 1995; International Agency for Research on Cancer, 2004; Grill et al., 2015), and is produced during the cooking of many common foods, including coffee, baked or fried cereal products, canned and jarred foods, baby food, and infant formula (Hasnip et al., 2006; Nyman et al., 2006; Zoller et al., 2007; Morehouse et al., 2008). Tobacco products and food are the two major sources of furan exposure for the general public. Mainstream cigarette smoke contains up to 65 μg furan per cigarette (Smith et al., 2000; International Agency for Research on Cancer, 2004), and the average daily dietary consumption of furan by adults has been estimated to be 0.25 μg per kg body weight (bw) in the U.S (Morehouse et al., 2008). and 0.78 μg per kg bw in Europe (European Food Safety Authority, 2009). Coffee contributes approximately 50% of the total population-based furan exposure in the U.S. in individuals two years of age and older (Morehouse et al., 2008). Importantly, the average daily consumption of furan by children during their first year of life is 0.41 μg per kg bw and 0.9 μg per kg bw in infants consuming only formula (DiNovi and Mihalov, 2007). The presence of furan in food is a public health concern for the U.S. Food and Drug Administration and the European Food Safety Authority, prompting extensive research on furan by academic and government organizations, including the U.S. National Toxicology Program.

2. Toxicity and carcinogenicity of furan

The results of extensive studies have demonstrated clearly that the exposure of experimental animals to furan results in a broad range of toxicities, with liver toxicity being the most common and consistent finding in mice (National Toxicology Program, 1993; Moser et al., 2009; Cordelli et al., 2010; Gill et al., 2011; Terrell et al., 2014) and rats (National Toxicology Program, 1993; Gill et al., 2010; Hickling et al., 2010a; Ding et al., 2012; Von Tungeln et al., 2017). For example, a recently completed comprehensive bioassay (Von Tungeln et al., 2017) demonstrated that administration of 0.02, 0.044, 0.092, 0.2, 0.44, 0.92, and 2 mg furan per kg bw by gavage in corn oil 5 days per week to male Fischer 344 (F344) rats for 2 years (104 weeks) resulted in the development of cholangiofibrosis, a precancerous state of cholangicarcinoma (Thoolen et al., 2010). The incidence of cholangiofibrosis was 76% in rats treated with 0.2 mg furan per kg bw, and increased to 100% in rats

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treated with 0.44, 0.92, and 2 mg furan per kg bw (Von Tungeln et al., 2017).

In addition to the toxic effects of furan exposure, there is well-established evidence of furan carcinogenicity, in particular, hepatocarcinogenicity in mice and rats (National Toxicology Program, 1993; Moser et al., 2009). Male and female B6C3F1 mice administered 0, 8, or 15 mg furan per kg bw for two years showed a dose-dependent increase in hepatocellular adenoma and carcinoma (National Toxicology Program, 1993). In a subsequent study, significant increases in hepatocellular adenoma or carcinoma occurred in female B6C3F1 mice given 4 or 8 mg furan per kg bw (Moser et al., 2009). Significant increases in hepatocellular adenoma or carcinoma were also observed in male and female F344/N rats administered 4 or 8 mg furan per kg bw for two years. In addition, nearly a 100% incidence of cholangiocarcinoma was observed at doses as low as 2 mg furan per kg bw (National Toxicology Program, 1993), although a re-assessment of the livers from this study suggests that the cholangiocarcinoma incidence was lower than originally reported (Von Tungeln et al., 2017). In additional to liver neoplasms, furan induced dose-dependent increases in mononuclear cell leukemia in both sexes of F344/N rats (National Toxicology Program, 1993; Von Tungeln et al., 2017) and malignant mesothelioma of the epididymis or testes in male F344/N rats (Von Tungeln et al., 2017). Although there is substantial evidence for the carcinogenicity of furan in experimental animals, there are no data pertaining to the carcinogenicity of furan in humans, which resulted in furan being classified as a "possibly carcinogenic to humans" (Group 2B) by the International Agency for Research on Cancer (International Agency for Research on Cancer, 1995) and as "reasonably anticipated to be a human carcinogen" by the U.S. National Toxicology Program (National Toxicology Program, 2016).

3. Mechanisms of furan carcinogenicity

Despite of a wealth of existing data for furan liver toxicity and carcinogenicity, the mechanisms associated with these effects are still unclear and subject for discussion. Two main mechanisms, genotoxic and non-genotoxic, especially *epigenotoxic*, have been considered to explain the pathogenesis of the furan-induced hepatobiliary lesions.

3.1. Genotoxic effects of furan

The liver is the main organ for furan metabolism. After absorption, furan undergoes hepatic cytochrome P450 2E1-catalyzed oxidation to a highly reactive *cis*-2-butene-1,4-dial intermediate, that is capable of reacting with glutathione, proteins, and DNA, thereby generating protein and DNA adducts (Peterson, 2013).

Several studies have demonstrated the formation of DNA adducts from *cis*-2-butene-1,4-dial *in vitro* (Gingipalli and Dedon, 2001; Byrns et al., 2002, 2004, 2006; Bohnert et al., 2004); nonetheless, there is minimal evidence of the formation of *cis*-2-butene-1,4-dial-DNA adducts in hepatic DNA from animals treated with furan. For example, no *cis*-2-butene-1,4-dial DNA adducts were detected in the livers of rats treated with 4.4 mg furan per kg bw for 52 weeks (Churchwell et al., 2015), and although there was a dose-related increase in ^{14}C associated with liver DNA in rats administered [2,3−^{14}C]furan, only very low amounts of radioactivity corresponded to the previously characterized *cis*-2-butene-1,4-dial deoxynucleoside adducts (Neuwirth et al., 2012). In contrast to data regarding DNA adducts, there is evidence for the formation of *cis*-2-butene-1,4-dial-protein adducts in the livers of furan-treated animals (Lu et al., 2009). Furthermore, in a recent study, Nunes et al. (2016) demonstrated, in the livers of rats treated with furan, a dose-dependent reaction of 2-(*S*-glutathionyl)butanedial, a reaction product of *cis*-2-butene-1,4-dial a glutathione, with lysine residue 107 of histone H2B, an amino acid that is critical for nucleosome stability.

Similar to DNA adduct formation, there is no evidence for the role of genetic aberrations with respect to carcinogenesis in furan-treated animals. For example, Terrell et al. (2014) reported no changes in the frequency or spectrum of mutations at the *cII* transgene in liver tissue of female Big Blue B6C3F1 transgenic mice treated with 15 mg furan per kg bw 5 times per week for 6 weeks. Similarly, *Pig-a*, *Hprt*, and *cll* genetic tests were negative in Big Blue transgenic rats treated with 2 or 8 mg furan per kg bw 5 times per week for 8 weeks (McDaniel et al., 2012), and there were no furan-specific *gpt* mutations in F344 *gpt* delta transgenic rats treated with 2 or 8 mg furan per kg bw 5 times per week for 13 weeks (Hibi et al., 2017).

3.2. Epigenotoxic effects of furan

The lack of evidence for furan genotoxicity *in vivo* suggests that non-genotoxic mechanisms may be involved in the pathogenesis of furan-induced liver toxicity and carcinogenicity. Exposure to furan at tumorigenic doses induces a broad spectrum of molecular and cellular non-genotoxic aberrations, including changes in the expression of protein-coding and non-coding genes, epigenetic alterations, sustained cell proliferation, cell cycle alterations, oxidative stress, inflammation, and apoptosis. Among these alterations, epigenetic mechanisms are of special interest because of their intimate connection to the other non-genotoxic events.

Epigenetic mechanisms signify the fundamental molecular principles of how genetic information is organized and read, and consist of cytosine DNA methylation and hydroxymethylation, covalent modification of histone proteins, nucleosome positioning along DNA, and expression of non-coding RNAs (ncRNAs) (Chen et al., 2017). These epigenetic mechanisms are well-balanced and tightly and interdependently regulated in normal cells, but become compromised and disrupted in a wide range of pathological states and upon exposure to harmful insults. Several reports have demonstrated significant epigenomic alterations in liver tissue after exposure to furan. For example, Chen et al. (2012) reported extensive gene-specific DNA methylation changes and altered expression of microRNAs (miRNAs) in cholangioma samples from female Sprague-Dawley rats treated with 2 mg furan per kg bw for 500 days. They also demonstrated that treatment of male Sprague-Dawley rats with furan at a high dose (30 mg furan per kg bw) for 3 months resulted in substantial alterations in the expression of critical cancer-related genes mediated by abnormal DNA methylation. Additionally, altered expression of 30 miRNAs, with 18 being up-regulated and 12 down-regulated, was also found in the livers of furan-treated rats. Similar marked alterations of the hepatic transcriptome have been reported in the F344 rats exposed to 0.12, 0.5, and 2 mg furan per kg bw for 3 months, with the vast majority of gene expression changes being found in the highest dose group (Dong et al., 2016). Importantly, the results of this sub-chronic repeat-dose exposure study demonstrated marked sex differences in gene expression responses to furan treatment, even at low doses, as evidenced by a greater number of differentially-expressed genes in male rats than female rats. This corresponds to the greater sensitivity of male rats compared to female rats to liver tumor induction in 2-year carcinogenicity studies with furan (National Toxicology Program, 1993).

Substantial transcriptomic changes have been reported also in the livers of mice upon furan treatment. In particular, Jackson et al. (2014) demonstrated that exposure of female B6C3F1 mice to a tumorigenic dose of furan (8.0 mg per kg bw) for 3 weeks resulted

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