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Genotoxic, epigenetic, and transcriptomic effects of tamoxifen in mouse liver☆

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ABSTRACT

Tamoxifen is a non-steroidal anti-estrogenic drug widely used for the treatment and prevention of breast cancer in women; however, there is evidence that tamoxifen is hepatocarcinogenic in rats, but not in mice. Additionally, it has been reported that tamoxifen may cause non-alcoholic fatty liver disease (NAFLD) in humans and experimental animals. The goals of the present study were to (i) investigate the mechanisms of the resistance of mice to tamoxifen-induced hepatocarcinogenesis, and (ii) clarify effects of tamoxifen on NAFLD-associated liver injury. Feeding female WSB/EiJ mice a 420 p.p.m. tamoxifen-containing diet for 12 weeks resulted in an accumulation of tamoxifen-DNA adducts, (*E*)-α-(deoxyguanosin-N^2-yl)-tamoxifen (dG-TAM) and (*E*)-α-(deoxyguanosin-N^2-yl)-*N*-desmethyltamoxifen (dG-DesMeTAM), in the livers. The levels of hepatic dG-TAM and dG-DesMeTAM DNA adducts in tamoxifen-treated mice were 578 and 340 adducts/108 nucleotides, respectively, while the extent of global DNA and repetitive elements methylation and histone modifications did not differ from the values in control mice. Additionally, there was no biochemical or histopathological evidence of NAFLD-associated liver injury in mice treated with tamoxifen. A transcriptomic analysis of differentially expressed genes demonstrated that tamoxifen caused predominantly down-regulation of hepatic lipid metabolism genes accompanied by a distinct over-expression of the lipocalin 13 (Lcn13) and peroxisome proliferator receptor gamma (Pparγ), which may prevent the development of NAFLD. The results of the present study demonstrate that the resistance of mice to tamoxifen-induced liver carcinogenesis may be associated with its ability to induce genotoxic alterations only without affecting the cellular epigenome and an inability of tamoxifen to induce the development of NAFLD.

1. Introduction

Tamoxifen, a non-steroidal anti-estrogenic drug, is commonly used to prevent the re-occurrence of breast cancer or its occurrence in healthy women at high risk of developing breast cancer. Despite the indisputable benefits of tamoxifen, a number of clinical reports have demonstrated that the use of tamoxifen increases the incidence of endometrial cancer (Fisher et al., 1994; Jones et al., 2012) and induces NAFLD (Bruno et al., 2005; Murata et al., 2000; Oien et al., 1999; Saphner et al., 2009) in humans. Additionally, it has been demonstrated that tamoxifen is a potent hepatocarcinogen in rats with both initiating and promoting activities. This was evidenced by results of several independent studies showing that long-term tamoxifen exposure induces a formation of various types of neoplastic lesions in the livers of different rat strains (Davies et al., 1997; Greaves et al., 1993).

Abbreviations: NAFLD, Non-alcoholic fatty liver disease; dG-TAM, (*E*)-α-(deoxyguanosin-N^2-yl)-tamoxifen; dG-DesMeTAM, (*E*)-α-(deoxyguanosin-N^2-yl)-*N*-desmethyltamoxifen; ALT, alanine transaminase; AST, aspartate transaminase; LDH, lactate dehydrogenase; α-SMA, α-smooth muscle actin; Col1a1, collagen, type I, alpha 1; Lcn13, lipocalin 13; Pparγ, peroxisome proliferator receptor gamma; Dnmt1, DNA methyltransferase 1; Dnmt3a, DNA methyltransferase 3a; Dnmt3b, DNA methyltransferase 3b; SAM, S-adenosyl-L-methionine; SAH, S-adenosyl-L-homocysteine; LINE 1, long interspersed elements 1; SINE B1, short interspersed nuclear elements B1; SINE B2, short interspersed nuclear elements B2; H3K4, histone H3 lysine 4; H3K9, histone H3 lysine 9; H3K27, histone H3 lysine 27; H3K79, histone H3 lysine 79; H4K20, histone H4 lysine 20; Gapdh, glyceraldehyde-3-phosphate dehydrogenase; qRT-PCR, quantitative reverse transcription-PCR.

☆ The views expressed in this paper do not necessarily represent those of the U.S. Food and Drug Administration.

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It is widely believed that the mechanism of tamoxifen-induced rat hepatocarcinogenesis is associated with various genotoxic alterations. Tamoxifen and its *N*-demethylated derivative *N*-desmethyltamoxifen are metabolized in the liver through α-hydroxylation by cytochrome P450-dependent monooxygenases (Kiyotani et al., 2012). The α-hydroxylated metabolites are subsequently esterified (e.g., sulfation) to reactive derivatives capable of interacting with DNA to cause the formation of (*E*)-α-(deoxyguanosin-N^2-yl)-tamoxifen (dG-TAM) and (*E*)-α-(deoxyguanosin-N^2-yl)-*N*-desmethyltamoxifen (dG-DesMeTAM) DNA adducts. Previously, we demonstrated that hepatocarcinogenic activity of tamoxifen in female rats is associated with its ability to induce both genotoxic and epigenetic abnormalities in the liver (Tryndyak et al., 2006, 2007).

In contrast to a well-established hepatocarcinogenicity of tamoxifen in rats, two reports by Tucker et al. (1984) and Martin et al. (1997) have demonstrated that tamoxifen is not hepatocarcinogenic in mice. However, because of some limitations of these studies, such as shorter than required 2-year treatment (Tucker et al., 1984) and small size of experimental group (Martin et al., 1997), the tamoxifen liver carcinogenicity in mice remains unclear. Umemoto et al. (2001) has attributed this species-dependent difference in tamoxifen hepatocarcinogenicity to a substantially lower level of tamoxifen-DNA adducts in the livers of mice than in rats. However, there is insufficient knowledge to clarify these existing hepatocarcinogenicity and hepatotoxicity discrepancies in mice.

In light of this, the goals of the present study were to (i) investigate the mechanisms of the resistance of mice to tamoxifen-induced liver carcinogenesis, and (ii) clarify effects of tamoxifen on NAFLD-associated liver injury in female mice. The results of our study demonstrate that treatment of mice with tamoxifen for 12 weeks resulted in a substantial accumulation of tamoxifen-DNA adducts in the livers. In contrast, tamoxifen treatment did not affect the status of hepatic epigenome.

These findings suggest that distinct rat and mouse differences in tamoxifen liver toxicity and carcinogenicity may be attributed to the absence of tamoxifen-induced epigenetic alterations and to lack of tamoxifen ability to induce the development of NAFLD-associated liver injury in mice.

2. Material and methods

2.1. Animals and treatments

WSB/EiJ female mice were obtained from the Jackson Laboratory (Bar Harbor, ME), housed four per cage in a temperature-controlled (24 °C) room with a 12-h light/dark cycle, and given ad libitum access to water and NIH-31 laboratory diet. Mice were allocated randomly to receive either NIH-31 diet containing 420 p.p.m. tamoxifen (Dyets, Bethlehem, PA) or control NIH-31 diet. Diets were stored at 4 °C and given ad libitum, with biweekly replacement. Body weights of the mice were

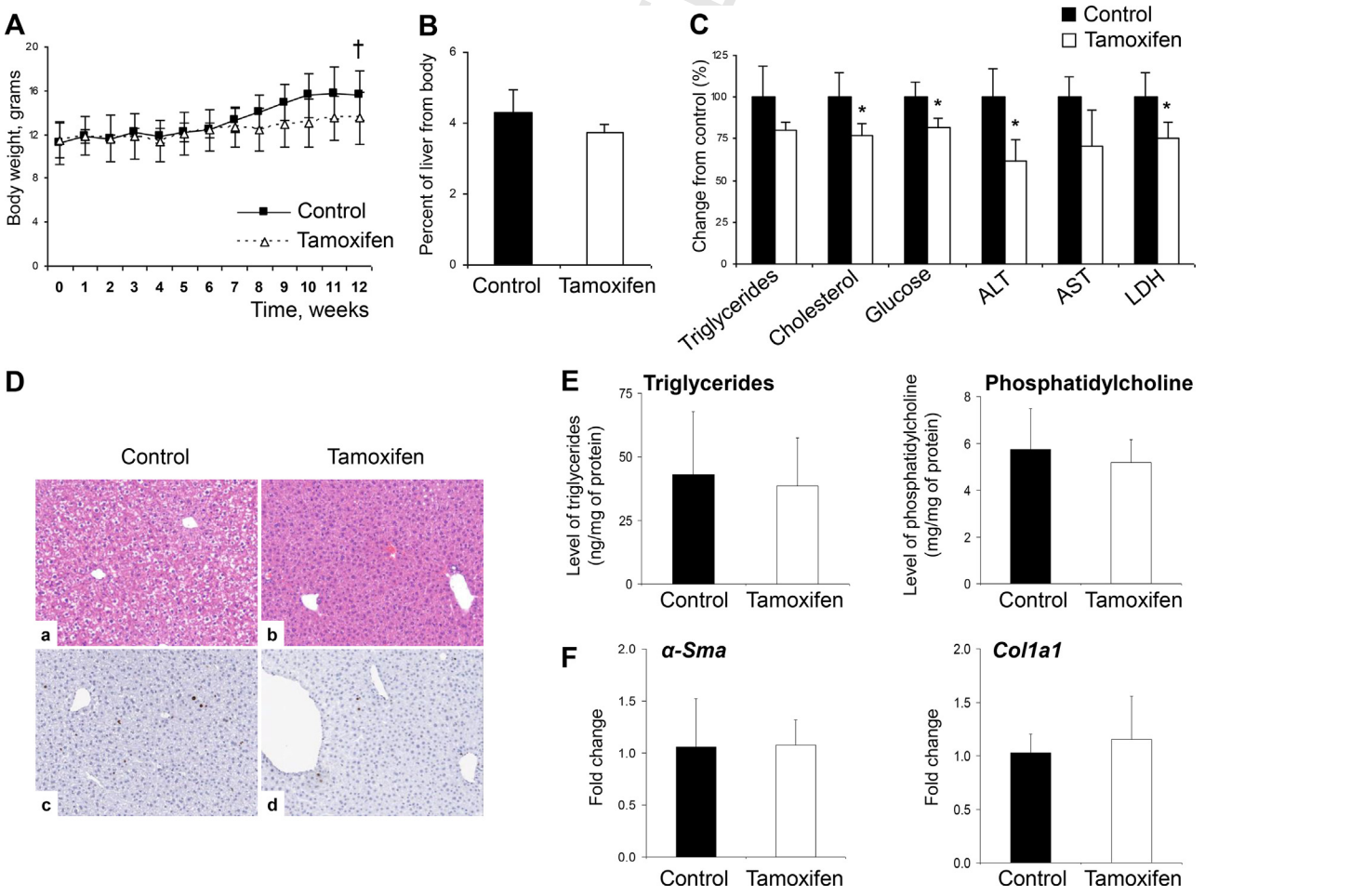

Fig. 1. Pathology endpoints and biochemical and morphological changes in control mice and mice fed tamoxifen. (A) Body and (B) relative liver weight. (C) Plasma levels of triglycerides, cholesterol, glucose, and activity of ALT, AST and LDH. (D) Representative images of hematoxylin and eosin (a,b) and Ki-67 staining (c,d) in liver sections. Original magnification, 20×. (E) Liver content of triglycerides and phosphatidylcholine (ng/mg protein). (F) Expression of *α-Sma* and *Col1a1* in the livers as measured by qRT-PCR. Values are means ± S.D. ($n = 5$). * – Denotes a significant ($p < 0.05$) difference from the control mice; † – denotes significant ($p < 0.001$; $r = 0.758$) trend.

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