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Epigenetic effects of the continuous exposure to peroxisome proliferator WY-14,643 in mouse liver are dependent upon peroxisome proliferator activated receptor α ☆

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Abstract

Peroxisome proliferators are potent rodent liver carcinogens that act via a non-genotoxic mechanism. The mode of action of these agents in rodent liver includes increased cell proliferation, decreased apoptosis, secondary oxidative stress and other events; however, it is not well understood how peroxisome proliferators are triggering the plethora of the molecular signals leading to cancer. Epigenetic changes have been implicated in the mechanism of liver carcinogenesis by a number of environmental agents. Short-term treatment with peroxisome proliferators and other non-genotoxic carcinogens leads to global and locus-specific DNA hypomethylation in mouse liver, events that were suggested to correlate with a burst of cell proliferation. In the current study, we investigated the effects of long-term exposure to a model peroxisome proliferator WY-14,643 on DNA and histone methylation. Male SV129 mice were fed a control or WY-14,643-containing (1000 ppm) diet for one week, five weeks or five months. Treatment with WY-14,643 led to progressive global hypomethylation of liver DNA as determined by an HpaII-based cytosine extension assay with the maximum effect reaching over 200% at five months. Likewise, trimethylation of histone H4 lysine 20 and H3 lysine 9 was significantly decreased at all time points. The majority of cytosine methylation in mammals resides in repetitive DNA sequences. In view of this, we measured the effect of WY-14,643 on the methylation status of major and minor satellites, as well as in IAP, LINE1 and LINE2 elements in liver DNA. Exposure to WY-14,643 resulted in a gradual loss of cytosine methylation in major and minor satellites, IAP, LINE1 and LINE2 elements. The epigenetic changes correlated with the temporal effects of WY-14,643 on cell proliferation rates in liver, but no sustained effect on c-Myc promoter methylation was observed. Finally, WY-14,643 had no effect on DNA and histone methylation status in *Pparα*-null mice at any of the time points considered in this study. These data indicate the importance of epigenetic alterations in the mechanism of action of peroxisome proliferators and the key role of Pparα.

Keywords: Liver; Peroxisome proliferators; Epigenetics; DNA methylation; Cancer

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

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1. Introduction

Recent evidence shows that epigenetic changes play an important role in carcinogenesis induced by a variety of factors that act via either genotoxic or non-genotoxic mechanisms [1,2]. Genotoxic carcinogens or products of their metabolic activation interact with DNA directly causing tumor formation [3], whereas non-

genotoxic carcinogens are a diverse group of chemicals that induce neoplastic cell transformation by mechanisms other than direct DNA damage [4]. One of the most extensively studied classes of non-genotoxic carcinogens are peroxisome proliferators, a structurally diverse group of chemicals and therapeutic agents [5]. Long-term exposure to these chemicals results in the development of liver tumors in male and female mice and rats [6,7]. The mode of action of peroxisome proliferators in rodent liver includes increased cell proliferation, decreased apoptosis, secondary oxidative stress, and other events [8]. It is widely recognized that the molecular target for their action in liver is the peroxisome proliferators-activated receptor (*Ppar*)α, and most of the effects, including the hepatocarcinogenic response, are the result of a *Ppar*α-mediated mechanism [9,10]. However, it is not well understood how peroxisome proliferators are triggering the plethora of molecular signals leading to cancer in rodent liver and why prolonged activation of *Ppar*α-induced pathways leads to hepatocarcinogenesis.

Short-term treatment with non-genotoxic rodent carcinogens, including peroxisome proliferators, is known to lead to hypomethylation of DNA and proto-oncogenes in mouse liver, events that were suggested to correlate with a burst of cell proliferation [11–13]. Our previous studies using a methyl-deficient model of non-genotoxic hepatocarcinogenesis in rats showed that alteration of cellular epigenetic processes, such as DNA hypomethylation and loss of histone H4 lysine 20 trimethylation, are key steps in the carcinogenic process induced by methyl deficiency [14,15]. Additionally, it has been demonstrated recently that global DNA hypomethylation was associated with the development of multiple liver tumors, while protecting from colon tumors, in Apc(Min/+)/Dnmt1(chip/c) mice providing additional evidence for the importance of epigenetic alterations in the origin of liver tumors [16]. These findings have led to a suggestion that sensitivity to tumorigenesis may be inversely related to the capacity to maintain normal patterns of the cellular epigenetic landscape [17].

In order to further characterize the long-term epigenetic effects of peroxisome proliferators in mouse liver and their dependence upon *Ppar*α, we examined the hypothesis that epigenetic dysregulation is an important contributing factor to the mechanisms of carcinogenicity of these agents. The effects of WY-14,643, a model peroxisome proliferator, were examined in wild type or *Ppar*α-null mice treated for up to five months. Global methylation of DNA, histone modification changes and other endpoints were evaluated.

2. Materials and methods

2.1. Animals and treatments

*Ppar*α-null male mice (SV129 background; [18]), and corresponding wild type counterparts (6–8 weeks of age at the beginning of treatment) were used. Animals were housed in sterilized cages in a facility with a 12 h night/day cycle. Temperature and relative humidity were held at 22 ± 2 °C and 50 ± 5%, respectively. The UNC Division of Laboratory Animal Medicine maintains these animal facilities, and veterinarians were always available to ensure animal health. All animals were given humane care in compliance with NIH and institutional guidelines and studies were performed according to protocols approved by the appropriate institutional review board. Prior to experiments, animals were maintained on standard lab chow diet and purified water *ad libitum*. 4-Chloro-6-(2,3-xylidino)-pyrimidynylthioacetic acid (WY-14,643) was obtained from Aldrich (Milwaukee, WI). NIH-07 was used as the base for the pelleted diet (prepared by Harlan Teklad, Indianapolis, IN) containing either 0 ppm (control), or 1000 ppm of WY-14,643. Dietary concentration of WY-14,643 was measured by high performance liquid chromatography after the pellets were made and determined to be ±18% of the target concentration. Diet was administered *ad libitum* for one week, five weeks or five months. Animals had free access to water throughout the study and the health status of the animals was monitored every second day throughout the study. At sacrifice, mice were anesthetized with pentobarbital (100 mg/kg, i.p.) and following exsanguination livers were removed, weighed, placed in microcentrifuge tubes and snap frozen in liquid nitrogen. The samples were stored at −80 °C until assayed.

2.2. Global DNA methylation analysis

The extent of the global DNA methylation was evaluated with a radiolabeled [^{3}H]dCTP extension assay as described previously [19]. Briefly, 1 μg of genomic DNA was digested with 20 U of methylation-sensitive HpaII restriction endonuclease (New England Biolabs, Beverly, MA) for 16–18 h at 37 °C. A second DNA aliquot (1 μg) was digested with methylation-insensitive iso-schizomer MspI, which cleaves CCGG sites in DNA regardless of CpG methylation status, to serve as a control for the digestion efficiency. Undigested DNA served as a background control. The single nucleotide extension reaction was performed in a 25 μl reaction mixture containing 1.0 μg DNA, 1X PCR buffer, 1.0 mM $MgCl_2$, 0.25 U Ampli-