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Nonylphenol induces liver toxicity and oxidative stress in rat

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ABSTRACT

Background: Nonylphenol (NP) is one of the most widely used synthetic xenoestrogens in detergents, plastic products, paints and the most important environmental degradation factor. In this study, the effects NP was investigated on hepatic oxidative stress-related gene expression in rats.
Method: Wistar male rats weighing 150–200 g were divided into control and NP receiving groups. NP was given in three doses (5, 25, and 125 μg/kg). All doses were given by gavage and the experiment continued for a consecutive 35 days. AST, ALT and ALP determined by the colorimetric method. The RNA was extracted from the rats liver tissue and RT- PCR was used to investigate the changes in gene expression. For this purpose, primers and specific probes of HO1 and Gadd45b genes as well as B-actin as control were prepared and the expression of each gene was separately assessed with ABI-7300. Hematoxylin and eosin staining was performed for evaluating of cell death.
Finding: The data from our study indicated nonylphenol increased alkaline phosphatase level but not changed aspartate aminotransferase and alanine aminotransferase in serum. That various doses of NP result in a dose-dependent increase in the expression of HO-1 gene. The intensified expression of HO-1 was statistically significant just at the doses of 25 and 125 μg/kg compared to control group ($p < 0.05$). In addition, it was shown that different doses of nonylphenol raised the expression of Gadd45b gene and this increase was significantly evident at 5 μg/kg ($p < 0.05$). Histological evaluation also indicated that NP increased hepatocytes cell death.
Conclusion: We conclude that NP increased serum alkaline phosphatase, lead to liver damage and can increase the expression of HO1 and Gadd45b genes and may modify the toxic effects on liver through induction of oxidative stress.

1. Introduction

Nonylphenol (NP) is a synthetic estrogen-like compound that is produced in large quantities [1]. It has a very wide use in detergents, plastic products, paints. NP has a very little stability in acidic and basic solutions as well as under exposure to UV light. It can be released from polymers to the environment and from plastic containers to the contents in very cold and hot conditions [2]. In addition, surfactants leaked from waste poured into rivers as leachate are also observed in aquatics in large quantities. The levels of NP in samples of fish show the high solubility of this compound in water [3].

This compound increases oxidative stress and may induce organ toxicities such as liver toxicity [4–6]. It is also associated with diabetes, obesity, cardiovascular disorders, reproductive disorders, and cancer [7–11]. Oxidative stress is related to produce reactive oxygen species (ROS) such as superoxide, hydroxyl radicals, and hydrogen peroxide [12] that lead to an imbalance between pro-oxidants and antioxidants [13] and this may induce hepatotoxicity [14].

Hepatotoxicity has been reported following exposure to high doses of NP in animals [15]. Although, liver as a major organ in biotransformation of environmental pollutants and can inactivate NP [16,17].

Particular estrogen receptor has been recognized in the liver cells and cellular responses with the estrogen interactions have

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been identified. NP is widely metabolized by microsomal UDP-glucuronosyltransferase [18] [19]. It has been reported the NP can decrease hepatic testosterone hydroxylation and CYP2C expression following oral administration, and also inhibit in vitro CYP1A1 activity in rat liver microsomes [20].

Many studies have examined the effect of NP through the induction of oxidative stress on some genes [5,7]. HO-1 and Gadd45b are two hepatic genes may involve in oxidative stress and changes in their expression have been investigated [12,21–23]. However, the effect of NP has not been fully determined on these genes so far. On the other hand, only a few studies have reported the effects of low doses of NP, and no study was carried out regarding the impacts of NP on the mentioned genes. For this reason, the present study was done to investigate the effect of NP on the expression of HO-1 and Gadd45b, liver damage.

2. Materials and methods

2.1. Chemicals and reagents

Nonylphenol (NP) with purity >99% (Kento, japan), tripure and DEPC (Roche, Sigma, Germany), C-DNA kit (Amplisens Co., Russia) were obtained. The primers for RT-PCR analysis were synthesized by Bioneer, Korea. All other chemicals were purchased from Merck (Germany).

2.2. Animals

Male Wistar rats (150–200 g) were prepared from animal house of Babol University of Medical Science (Babol, Iran). The rats were housed at a controlled temperature (24 ± 3 °C) under 12 h light-dark cycles, humidity (50 ± 5%), and free access to food and tap water *ad libitum*. This study was approved by the University Ethics Committee and all the experiments were done in according to the guidelines for the Care and Use of Lab Animals.

2.3. Treatment

Rats were randomly divided into four groups (n = 5). NP was given to rats in three doses (5, 25, and 125 μg/kg in olive oil as vehicle). It was orally given every 24 h for 35 days. The control group received only olive oil.

2.4. Preparing the blood samples

The blood samples (1 ml) were taken from cutting of axillary artery for each rat and were transferred into 2-ml tubes. Then the samples were separately centrifuged and the serums were kept at −20 °C for further analysis.

2.5. Tissue collection

Following oral administration of Wistar rats with the last doses of NP, they were fasted overnight. Thereafter, they were anesthetized using sodium thiopental and then sacrificed. Subsequently, the liver of each rat was taken off and quickly cleaned from adhering fat and connective tissues. The samples were fastly flash-freezed in liquid nitrogen and stored at −80 °C for further analysis.

2.6. Liver function

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to the method of Reitman and Frankel [24]. Serum alkaline phosphatase (ALP) was determined by the colorimetric method of Kind and King [25].

2.7. Histological staining

Hematoxylin and eosin staining was uses for cell death assessment. Animals were deeply anesthetized with ketamine and xylaxin and transcardially perfused with formalin 10% and then processed for routine embedding in paraffin. Serial sections (5 μm) were prepared using a rotary microtome (Leica RM2135, Germany). For hematoxylin and eosin staining, sections were stained with 0.1% hematoxylin (Ciba, Basle, Switzerland) for 10 min, rinsed in tap water for 15 min, immersed in 0.1% eosin (Ciba, Basle, Switzerland) for 5 min and washed again with distilled water. Dehydration was performed by ascending ethanol passages, cleared with xylene and cover slipped and screened for cell death assessment by Olympus microscope.

2.8. RNA extraction

Total RNA was extracted from frozen rat liver tissues with using Tripure reagent kit and then solved in diethyl pyrocarbonate-treated deionized water. The quantity and quality of extracted RNA were determined using Spectrophotometer (Thermo, USA) at wavelength of 260 and 280 nm.

2.9. cDNA synthesis and real-time PCR

First-strand cDNA was synthesized by reverse transcription using Reverta-L RT Reagent kit, according to the manufacturer's instruction. The thermocycler for cDNA synthesis was set up at a temperature of 37 °C for 30 min.

Quantitative PCR (q-PCR) was performed byan Applied Biosystems 7300 Real Time PCR System (Applied Biosystems, Branchburg, NJ, USA) at three conditions, 95 °C for 5 min, 45 cycles at 95 °C for 30 s and 60 °C for 1 min. Then the levels of DNA product were normalized to *GAPDH* gene as the internal control. Subsequently, the relative differences between treatment and control groups were calculated and expressed as percentage of controls. Primers and probes for the q-PCR were designed using Allele ID 6. All primers were listed in Table 1.

2.10. Statistical analysis

Statistical analyses were performed using SPSS software version 19, one-way ANOVA followed by Tukey post-hoc. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Weight changes

Body weight of animals was recorded at the beginning and the end of experiments. The body weight of animals decreased in NP receiving groups compared to control group (Table 2).

3.2. Liver function

The results presented in Table 3 indicated that NP caused a significant increase ($p < 0.01$) in serums ALP level in rats. Serum levels of ALT and AST did not show any significant changes compared to control group.

3.3. Histology results

Hematoxylin and eosin staining was used to evaluate the effect of NP on liver damage. Results indicated that NP produced disruption in liver parenchyma, nucleus aggregation, infiltration of