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Supplemental dietary phytosterin protects against 4-nitrophenol-induced oxidative stress and apoptosis in rat testes

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ABSTRACT

4-Nitrophenol (PNP), is generally regarded as an environmental endocrine disruptor (EED). Phytosterin (PS), a new feed additive, possesses highly efficient antioxidant activities. The transcription factor, nuclear factor-erythroid 2-related factor 2 (Nrf2), is an important regulator of cellular oxidative stress. Using rats, this study examined PNP-induced testicular oxidative damage and PS-mediated protection against that damage. The generation of MDA and H_2O_2 upon PNP and PS treatment was milder than that upon treatment with PNP alone. This mitigation was accompanied by partially reversed changes in SOD, CAT, GSH and GSH-Px. Moreover, PNP significantly reduced the caudal epididymal sperm counts and serum testosterone levels. Typical morphological changes were also observed in the testes of PNP-treated animals. PNP reduced the transcriptional level of Nrf2, as evaluated by RT-PCR, but it promoted the dissociation from the Nrf2 complex, stabilization and translocation into the nucleus, as evaluated by immunohistochemistry and Western blotting. In addition, PNP enhanced the Nrf2-dependent gene expression of heme oxygenase-1 (HO-1) and glutamate–cysteine ligase catalytic subunit (GCLC). These results suggest that the Nrf2 pathway plays an important role in PNP-induced oxidative damage and that PS possesses modulatory effects on PNP-induced oxidative damage in rat testes.

1. Introduction

It is well established that the exposure of immature animals to chemicals during crucial developmental periods may result in growth alteration, structural abnormalities or functional deficits. 4-Nitrophenol (PNP), is in popular use worldwide for agriculture and industrial manufacturing [1]. PNP is a nitrophenol derivative that has been isolated from diesel exhaust particles (DEP) – 1 kg of DEP contains an average of 169 mg of PNP [2]. PNP is also a degradation product of the insecticide parathion [3]. Due to the stability of PNP, its non-biodegradable nature and its consequent persistence in the environment [4], increasing

Abbreviations: EED, environmental endocrine disruptor; PNP, 4-nitrophenol; DEP, diesel exhaust particles; ROS, reactive oxygen species; GSH, glutathione; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; ARE, antioxidant response element; Nrf2, nuclear factor erythroid 2-related factor 2; Keap1, kelch-like ECH-associated protein 1; PS, phytosterins; HO-1, heme oxygenase 1; GCLC, γ-glutamylcysteine synthetase; NQO1, NAD(P)H: quinone oxidoreductase 1; NF-κB, nuclear factor-κB.

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attention has been focused on understanding the toxicology of this class of compounds. EPA regulations require the concentration in water to be less than 10 ng/L [5]. The general population can be exposed to PNP via inhalation of ambient air or via the ingestion of contaminated water. Occupational exposure to PNP can occur by both inhalation and dermal contact at workplaces where this compound is produced or used. Previous studies indicated that PNP had potential adverse effects, such as disturbing the endocrine and reproductive system [6,7]. Exposure to DEP is associated with oxidative damage [8], including protein oxidation and lipid peroxidation [9]. Reactive oxygen species (ROS) were thought to be the cause of oxidative stress following DEP exposure [10]. Therefore, the toxic influence of PNP is likely due to the formation of excessive free radicals, causing oxidative stress and leading to cell damage.

ROS have been known to cause a loss of membrane polyunsaturated fatty acids and an increase in the lipid-peroxides in spermatozoa [11]. Oxidative stress may induce free-radical-mediated decomposition of vital molecules, such as proteins and lipids, and ultimately cell death [12]. Oxidative stress is frequently cited to explain cell damage in various disease. Cells are endowed with an array of protective antioxidants, such as the glutathione (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), which scavenge ROS to prevent possible cellular damage.

Expression of most antioxidant enzymes is controlled by the antioxidant response element (ARE) and activated by nuclear factor erythroid 2-related factor 2 (Nrf2) [13]. Nrf2 remains inactive in the cytoplasm through its interaction with Kelch-like ECH-associated protein 1 (Keap1) [14]. The release of Nrf2 from Keap1 can be induced via direct attack by multiple environmental factors and a variety of chemicals or via indirect actions, such as phosphorylation, allowing Nrf2 to translocate to the nucleus and activate the expression of ARE-containing genes [15]. Thus, a disruption in this pathway might affect organ toxicity caused by environmental chemicals.

Phytosterins (PS) are commonly found as minor constituents of edible vegetable oils and are natural constituents of the human diet. PS have attracted much attention in recent decades because of their health benefits for humans. There is a wide variety of PS structures but the most frequent PS found in nature are β-sitosterol, campesterol and stigmasterol [16]. Dietary intake of PS has been estimated to be 200–300 mg/day in northern European countries [17] and approximately 300–450 mg/day in Japan [18]. It is well established that certain PS reduce plasma cholesterol levels, ostensibly by inhibiting enterocytic cholesterol uptake through competition with dietary and biliary cholesterol for absorption [19]. In addition, PS are recognized to exert antioxidative actions [20,21].

To the best of our knowledge, PNP toxicity in gonads has been reported mainly based on endocrine mechanisms with low dosages of PNP, and the reproductive toxicity of high dose PNP has yet to be investigated. Because the consumption of PNP in industrial and agricultural activity is dramatically increasing and PNP is accumulating in water and soil, it is necessary to assess the toxicity of high doses of PNP. In the present study, we investigated the PNP-induced testicular oxidative damage in rats using a variety of assays. The attenuating effects of PS on PNP-induced testicular toxicity were also studied.

2. Materials and methods

2.1. Chemicals and antibodies

PNP monomer dry crystals ($C_6H_5NO_3$, >99.9% purity, CAS 100-02-7) were purchased from Chengdu Kelong Chemical Reagent Factory, China (Fig. 1A).

Commercial grade PS (mixtures of β-sitosterol, campesterol and stigmasterol, >90% purity) were provided by Jiangsu Chunzhigu Biological products company (Fig. 1B).

The primary antibodies used for tissue immunohistochemistry and Western blotting were anti-Nrf2 (Abcam, ab53019; rabbit anti-human), anti-caspase-3 (Cell Signaling, Asp175; rabbit anti-human), anti-β-actin (Beyotime Institute of Biotechnology, AA128; mouse antibody), anti-Histone3 (Beyotime Institute of Biotechnology, AH433; rabbit antibody). The secondary antibodies used in this study were goat anti-rabbit IgG (H + L) (Beyotime Institute of Biotechnology, A0208) and goat anti-mouse IgG (H + L) (Beyotime Institute of Biotechnology, A0216).

2.2. Animals

Twenty-one-day-old male Sprague-Dawley rats supplied by QingLongShan Laboratory Animal Company (NanJing, China) were maintained under controlled environmental conditions at room temperature (22 ± 2 °C) with 50 ± 10% humidity and an automatically controlled cycle of 12 h light and 12 h dark. Feed (purchased from a commercial supplier) and sterile distilled water were provided ad libitum. During the experiment, animals were allocated into polypropylene cages with laboratory-grade pine shavings as bedding. Animals were acclimatized to the experimental conditions for a period of one week prior to the beginning of the experiment. The experimental protocol was approved in accordance with the Guide for

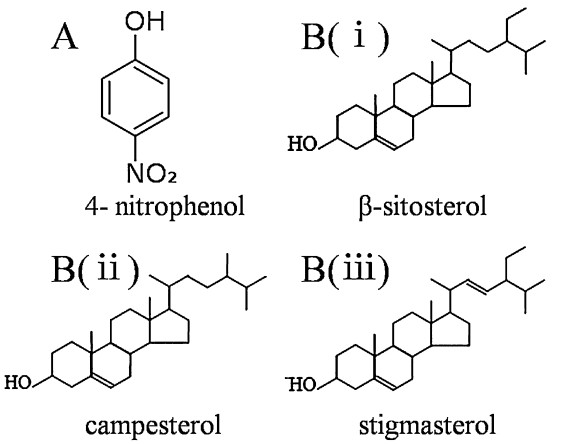

Fig. 1. Chemical structures of 4-nitrophenol, β-sitosterol, campesterol and stigmasterol.