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Lycopene protects against atrazine-induced hepatic ionic homeostasis disturbance by modulating ion-transporting ATPases☆

Jia Lin[a], Hua-Shan Zhao[a], Li-Run Xiang[a], Jun Xia[a], Li-Li Wang[a], Xue-Nan Li[a], Jin-Long Li[a,*], Ying Zhang[b,*]

[a]College of Veterinary Medicine, Northeast Agricultural University, Harbin 150030, People's Republic of China
[b]School of Resources and Environment, Northeast Agricultural University, Harbin 150030, People's Republic of China

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Abstract

The aim of this study was to evaluate the possible chemoprotective role of lycopene (LYC) against atrazine (ATR)-induced ionic disorder and hepatotoxicity in mice. Male kunming mice were treated with LYC (5 mg/kg) and/or ATR (50 mg/kg or 200 mg/kg) by lavage administration for 21 days. Ionic disorder was assessed by determining the Na^+, K^+ and Ca^{2+} content and the alteration in ATP enzymes (ATPases) including Na^+-K^+-ATPase, Ca^{2+}-ATPase, Mg^{2+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase and the mRNA levels of ATPase's subunits in liver. ATR caused the increases of alanine aminotransferase and aspartate aminotransferase activities and histological changes. LYC pretreatment significantly protected liver against ATR-caused alternation. The significant effect of ATR and LYC on the K^+ and Mg^{2+} content in liver was not observed, but ATR increased hepatic Na^+-K^+-ATPase activity and decreased Mg^{2+}-ATPase and Ca^{2+}-Mg^{2+}-ATPase activity. The mRNA expressions of Na^+-K^+-ATPase subunits were regulated significantly by ATR. A significant increase of Ca^{2+} content and seven down-regulated mRNA expressions of Ca^{2+}-ATPase subunits and a decrease of Ca^{2+}-ATPase activity were observed in the ATR-treated mice. Notably, LYC modulated these ATR-induced alterations of ATPase activity and mRNA expression of their subunits. These results suggest that ATR presents hepatotoxicity via regulating hepatic ATPase's activities and their subunit transcriptions and inducing ionic disorder. LYC protects liver against ATR-induced hepatotoxicity, significantly. LYC modulated hepatic ionic homeostasis disturbance via regulation of ATPase activities and their subunits' (1a1, 1b3, 1b4 and 2b4) transcriptions. In summary, these effects play a critical role of LYC-mediated chemoprevention against ATR-induced hepatotoxicity.

Keywords: Atrazine; Lycopene; Ionic homeostasis; ATPases activities; ATPase's subunits transcriptions; Mouse liver

1. Introduction

Atrazine (ATR; 2-chloro-4-ethylamino-6-isopropylamine-1,3,5-triazine) has become an increasingly serious source of environmental chemical pollution due to its widespread presence and continued use in most countries in recent decades. As a result of indiscriminate use, residues of ATR are found not only in plants and agricultural products [1] but also in wild animals [2]. There is an increasing concern about ATR's potential adverse health effects in human and animals [3–6]. They are bioavailable to aquatic organisms and can bioaccumulate through every step of the food chain up to humans. Investigations on uptake, accumulation, biotransformation and excretion of xenobiotics including pesticides are fundamental for understanding the effects that these chemicals have on organ/tissue systems in mice, especially on liver. Growing evidences have demonstrated that the herbicide of ATR has the potential to induce oxidative stress, by increasing the concentration of reactive oxygen species and products of oxidative damage such as lipid peroxides and therefore influencing the activity of antioxidant enzymes [7]. Liver is the first target of ingested oxidants and also very important tissue in defense against oxidative stress [7], DNA damage [8] and endocrine disruption [9,10] to different organs. Therefore, ATR is hazardous to the health of fish and amphibians wild life. Effects reported in mammals (human and experimental animals) include increased serum lipids and liver enzymes and liver histopathology. However, ATR impacts on the liver of mammals, particularly ionic homeostasis disturbance, is less clear.

The liver is an essential organ involved in the metabolism of endogenous or exogenous substances. Most of xenobiotics are metabolized in the liver by detoxification processes involving several activation, conjugation and elimination mechanisms [11]. The ionic pumps are essential for the regulation of cell volume, uptake of nutrients, cell growth and differentiation and are critical for the normal functioning of liver cells [4,12]. It is reported that ATR could alter plasma ion levels and Na^+-K^+-ATPase activity in gill of Atlantic salmon (*Salmo salar*) or in liver of mice; therefore, it causes

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* Corresponding authors. J. L. Li is to be contacted at: tel.: +86-451-5519-0407; Y. Zhang, tel.: +86-451-5519-0993.
E-mail addresses: Jinlongli@neau.edu.cn (J.-L. Li), zhangyinghr@hotmail.com (Y. Zhang).

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disturbance of osmoregulation or disturbance of liver function [4]. Thus, despite growing concerns over the toxicological effects of ATR, which is considered an endocrine disruptor of different organisms, its molecular interactions with biological targets in mammalian endocrine systems are not fully understood.

Lycopene (LYC), which is an actually occurring carotenoid that is present in tomatoes and tomato products, has attracted considerable attention as a potential chemopreventive agent. It has many biochemical functions as an antioxidant scavenger, antihyperlipidemic agent and inhibitor of proinflammatory and prothrombotic factors [13]. Experimental rodent models suggest that LYC plays an important role in the modulation of organ injury evoked by inflammatory process [14,15]. There are a number of potential mechanisms through which tomato products may reduce the risk for chronic diseases, including common forms of cancer and heart disease [16]. Thus, LYC may prevent oxidative damage, toxicity and disease. Nevertheless, little is known about the role of LYC in the regulations of ionic homeostasis balance and the underlying protective mechanisms during ATR exposure. Therefore, the aims of the study were to ascertain the protective effects of LYC on ATR-induced hepatic ionic homeostasis disturbance and to assess whether ion-transporting ATPases were involved in these effects.

2. Materials and methods

2.1. Animals and experimental design

Three-week-old male kunming mice (18–23 g) were acclimatized for 10 days before the start of the study (Institutional Animal Care and Use Committee No. 2012-0010) at constant temperature of 22±2°C, relative humidity of 50±15% and light/dark cycle of 12 h. Temperature and relative humidity were monitored daily. Beginning at Day 0, body weight was measured every day, and the consumption of drinking water and diets was measured every day. These mice were housed individually in polycarbonate cages, given access to both food and water *ad libitum*. The entire experiment was repeated three independent times; each replicate for each group contained 10 mice, for a total of 60 mice. The mice were divided into six groups as follows:

Group 1 (Control) was treated with autoclaved water and corn oil for 21 days.
Group 2 (LYC) was treated with autoclaved water. This group received 5 mg/kg LYC (North China Pharmaceutical Group, China) for 21 days.
Group 3 (ATR1) was exposed to 50 mg/kg of ATR (Shandong Binnong Technology Co., Ltd., China). This group received corn oil for 21 days.
Group 4 (ATR2) was exposed to 200 mg/kg of ATR. This group received corn oil for 21 days.
The mice in Group 5 (ATR1+LYC) were exposed to 50 mg/kg of ATR, with the simultaneous administration of 5 mg/kg LYC for 21 days.
The mice in Group 6 (ATR2+LYC) were exposed to 200 mg/kg of ATR, with the simultaneous administration of 5 mg/kg LYC for 21 days.

The rationale for this dose regimen selection and its relevance to occupational exposure have been addressed in detail in previous studies with similar or the same exposure paradigms [9,17–19]; these doses are also in line with other short-term exposure studies of ATR's effects on the endocrine systems, allowing parallel comparisons of study findings.

2.2. Hematological and biochemical analysis

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities are frequently associated with hepatotoxic effects. At the end of ATR exposure, mice were sacrificed by enucleation for blood. Moreover, whole blood was centrifuged at 2400*g* for 10 min to obtain serum for biochemical analysis, and serum was stored at −80°C. Biochemical parameters including AST and ALT were measured using an autoanalyzer.

2.3. Histopathological assessment

The livers of control and treated group were fixed in 10% neutral buffered formalin and processed using routine histological techniques. After paraffin embedding, 5-μm sections were cut and stained with hematoxylin and eosin to assess tissue structure. The slides were visualized using light microscopy (Nikon, Japan). The image acquisition parameters and microscope settings were kept the same throughout the process.

2.4. Hepatic ionic concentration

The liver was excised immediately on an ice-cold plate washed in physiological saline solution. K^+, Ca^{2+} and Mg^{2+} concentration in liver tissue was measured with detection kits (Nanjing Jiancheng Bioengineering Institute, China).

2.5. Hepatic ATPase activities assay

The activities of the Na^+-K^+-ATPase, the Ca^{2+}-ATPase, the Mg^{2+}-ATPase and the Ca^{2+}-Mg^{2+}-ATPase were determined using the appropriate assay kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions. The activities of the Na^+-K^+-ATPase, the Ca^{2+}-ATPase and the Mg^{2+}-ATPase were measured by quantifying the inorganic phosphorus (Pi) production from the conversion of ATP into ADP at 636, 636, 660 and 660 nm, respectively, using the molybdenum blue spectrophotometric method and were expressed as units per milligram (U/mg) of protein.

2.6. Quantitative real-time polymerase chain reaction

Total RNA was extracted from liver tissue (50 mg) with RNA out reagent (Beijing Tiandi, Inc., China) according to the manufacturer's instructions. RNA amount and quality was determined using the spectrophotometer (GE, USA). Samples with ratios of absorbance at 280/260 nm between 1.9 and 2.0 were judged to be of acceptable quality and integrity. Total RNA (2.0 μg) was then reverse-transcribed to cDNA using the TransScript Reverse Transcriptase (Beijing TransGen Biotech Co., Ltd., China) for real-time polymerase chain reaction (PCR) according to the manufacturer's instructions, using random hexamers as primers. Real-time quantitative PCR was performed with GoTaq qPCR Master Mix (Promega, USA) on BIOER LineGene 9620 (Hangzhou bioer Technology Co., Ltd., China). The primer sequences used for the gene expressions analyses are found in Table S1. The housekeeping gene β-actin was used as a reference gene for normalization. For each gene, relative expression levels in steady-state mRNA in samples was determined using the $2^{-\Delta\Delta Ct}$ method, corrected for the housekeeper.

2.7. Statistical analysis

The results are expressed as the mean±standard deviation and analyzed with GraphPad Prism 5.0 (GraphPad Software, USA) and SPSS 19.0 software (SPSS Inc., USA). Statistical analyses were performed using Student's *t* test (GraphPad Software, USA) and one-way ANOVA test followed by Tukey's *post hoc* pairwise comparison. Asterisks (*) indicated statistically significant differences to the control group, $^*P<.05$, $^{**}P<.01$ and $^{***}P<.001$. In addition, principal component analysis (PCA) was used as an effective tool for simplifying the information from intercorrelated variables through linear transformation of the original variables into a few principal components. PCA was done in this work to define the most important parameters, which could be used as key factors for individual variations using the same software. The observed relationships among the parameters were confirmed and quantified according to Spearman's test.

3. Results

3.1. Body and liver weights

The body weight and liver weight in ATR-treated groups were slightly decreased, compared to the control group. When mice were treated with LYC+ATR, the body and liver weight recovered was observed (Table 1). However, there were no significant alteration of

Table 1
Summary weights of liver in mice.

Group	Body weight (g)	Liver weight (mg)	Relative weight (mg/g)
Control group	33.07±2.782	1449±178.0	43.6±3.52
5 mg/kg LYC	34.88±1.107	1560±122.9	44.7±3.8
50 mg/kg ATR	33.70±2.761	1433±179.4	42.5±2.7
200 mg/kg ATR	31.48±1.288	1362±167.8	43.3±4.5
50 mg/kg ATR+LYC	34.40±1.916	1495±182.9	43.3±3.8
200 mg/kg ATR+LYC	34.49±2.713	1468±157.5	42.5±2.3

Each value represents mean±S.D. (N=10, males).