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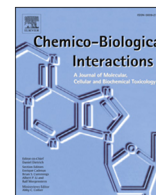

Protective effect of lycopene on fluoride-induced ameloblasts apoptosis and dental fluorosis through oxidative stress-mediated Caspase pathways

Weishan Li [a,*], Binghua Jiang [b], Xianglin Cao [b], Yongjiang Xie [c], Ting Huang [a]

[a] *The Affiliated Stomatological Hospital of Jiamusi University, Jiamusi 154007, China*
[b] *Hainan Stomatological Hospital, Haikou 570105, China*
[c] *Daqing Oilfield General Hospital, Daqing 163000, China*

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ABSTRACT

Fluoride is an environmental toxicant and induces dental fluorosis and oxidative stress. Lycopene (LYC) is an effective antioxidant that is reported to attenuate fluoride toxicity. To determine the effects of LYC on sodium fluoride (NaF) -induced teeth and ameloblasts toxicity, rats were treated with NaF (10 mg/kg) and/or LYC (10 mg/kg) by orally administration for 5 weeks; ameloblasts were treated with NaF (5 mM) and/or LYC (2 μM) for 6 h. We found that the concentrations of fluoride, malondialdehyde (MDA) and reactive oxygen species (ROS), gene expressions and activities of Caspase-9 and Caspase-3, and the gene expressions of Bax were significantly decreased, while the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPX), the gene expression of Bcl-2 were significantly increased in the LYC + NaF-treated rats group; concentrations of MDA and ROS, gene expressions and activities of Caspase-9 and Caspase-3, and the gene expression of Bax, and ameloblasts apoptosis rate were significantly decreased, while the activities of SOD and GPX, the gene expression of Bcl-2 were significantly increased in the LYC + NaF-treated ameloblasts group. These results suggest that LYC significantly combated NaF-induced ameloblasts apoptosis and dental fluorosis by attenuation oxidative stress and down-regulation Caspase pathway.

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1. Introduction

Fluoride is well-known water contaminant that raises serious health issues all over the world [1,2]. Millions of people worldwide are reported to drink water containing high concentration of fluoride [3]. Exposure to fluoride can result in enamel and skeletal fluorosis, renal toxicity, neurotoxicity, cardiovascular system toxicity, reproductive toxicity and epithelial lung cell toxicity [1,3–6]. Dental fluorosis is a developmental disorder caused by fluoride exposure during enamel formation, which manifests as mottled, discolored, and porous enamel [7]. Recent reports indicate that in the United States the prevalence of mild to severe dental fluorosis among children aged 6–11 was 33.4% and in adolescents aged 12–15 was 40.6% [8], in India the prevalence of dental fluorosis was 73% among 12 years and 70.1% among 15 years children [9].

Ameloblasts are enamel organ cells that are responsible for enamel formation [10]. Ameloblasts form enamel by secreting enamel matrix proteins and directing matrix mineralization [11]. Studies have shown that high-dose fluoride causes dental fluorosis in the ameloblasts and enamel organ [12–15]. During the last four decades, many experimental animal and organ culture studies have investigated mechanisms by which fluoride affects ameloblasts and enamel formation [16,17]. Generation of reactive oxygen species (ROS) is considered to play an important role in the toxic effects of fluoride [18,19]. Oxidative stress occurs when the generation of ROS in a system exceeds the system's capacity to neutralize and eliminate them. Shivarajashankara et al. reported there was an increased oxidative stress based on increased malondialdehyde (MDA), ascorbic acid, glutathione peroxidase (GPX), and decreased glutathione levels in children aged 3–10 years with endemic fluorosis [20]. Li et al. revealed that people living in areas of endemic fluorosis had a decrease in superoxide dismutase (SOD) [21]. Furthermore, studies have suggested that oxidative stress activates cell

* Corresponding author.
E-mail address: weishanli666@126.com (W. Li).

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apoptosis signaling pathways *in vivo* and *vitro* [22]. The Caspase, a major signaling pathway in cell death, is sensitive to ROS [23]. The Caspase pathway plays a pivotal role in mediating apoptosis by modulating the transcription of apoptosis-related genes. Previous results demonstrated that Caspase was strongly activated by ROS, leading to apoptosis [23]. Moreover, studies have suggested that sodium fluoride (NaF) induces cell apoptosis through ROS-dependent and Caspase- pathways *in vitro* [24].

Lycopene (LYC), a naturally occurring hydrocarbon carotenoid that is found in red foods such as tomatoes, pink guavas, watermelons, and papayas, has attracted considerable attention as a potential chemopreventive agent against oxidative stress [25]. Tomatoes are the largest contributor to dietary intake of LYC in humans [26]. LYC is an effective antioxidant with a singletoxygen-quenching capacity 47 and 100 times stronger than that of vitamin E and beta-carotene, respectively [27]. Indeed, LYC has been shown to be a very efficient singlet oxygen quencher, as well as a scavenger of other ROS such as superoxide radicals, peroxyl radicals, and hydroxyl radicals. Previous studies have proposed that LYC may be important in preventing fluoride toxicity through a mechanism related to antioxidative effects [1]. There is also increasing evidence to suggest that LYC attenuates oxidative stress [25,28,29]. Moreover, recently, the LYC has been shown to modulate redox-sensitive molecular pathways involved in apoptosis [30]. Nevertheless, little is known about the role of LYC in the oxidative and apoptotic damages caused by fluoride on the dental and ameloblasts.

Therefore, the aim of this study was to investigate the association between fluoride-induced oxidative and apoptotic damages and the chemopreventive potential of LYC on the dental and ameloblasts. Finally, we offer a conclusion on the chemopreventive potential of LYC in dental health and the amelioration of fluoride-induced dental fluorosis.

2. Materials and methods

2.1. Animals and treatments

This experimental protocol was approved by the Ethics Committee on the Use and Care of Animals, Northeast Agricultural University, China. The housing conditions were maintained at a constant temperature (24 ± 1 °C), relative humidity at 55 ± 5%, and in a 12/12-h light/dark cycle. Food (standard diet) and water were available *ad libitum*. Forty 4-week-old healthy male Sprague-Dawley rats weighed 72–95 g were acclimatized for 1 week then the rats were randomly divided into four groups, each ten rats.

Group 1 (Control), control rats were treated with corn oil and distilled water; Group 2 (LYC), rats were given LYC (North China Pharmaceutical Group, China) orally (10 mg/kg body weight/day), which was dissolved in 0.5 ml of corn oil as it was reported to be effective in achieving antioxidant defense and lowering the toxicity of various xenobiotics according to previous studies [31–33]; Group 3 (NaF), rats were given NaF orally (10 mg/kg body weight/day), which was chosen according to previous studies [1,34,35]; Group 4 (LYC + NaF), rats were first treated with LYC (10 mg/kg body weight/day) and then with NaF after an hour (10 mg/kg body weight/day); All rats received their respective doses daily by tubes for 5 weeks. At the end of the experimental durations, animals were sacrificed by decapitation. Incisor teeth were collected and stored at −70 °C.

2.2. Cell culture and treatments

The mouse ameloblast-derived cell line (LS8) was maintained in α minimal essential medium (Invitrogen) supplemented with fetal bovine serum (10%), penicillin (50 units/ml), and streptomycin (50 μg/ml). LYC was delivered to the cells using THF[4] as a solvent. The cells were divided into four groups. Group 1 (C), cells were cultured in α minimal essential medium and THF[4], the final concentration of THF[4] in the culture medium was not greater than 0.5% (v/v) [25]; Group 2 (LYC), cells were cultured in α minimal essential medium plus LYC (2 μM) for 6 h; Group 3 (NaF), cells were cultured in α minimal essential medium plus NaF (5 mM) for 6 h, which was chosen according to previous study [13]; Group 4 (LYC + NaF), cells were cultured in control medium plus LYC (2 μM) and NaF (5 mM) for 6 h.

Until now, no studies have examined the chemoprotective role of LYC against fluoride-induced toxicity in ameloblasts. A dose of 2 μM LYC was chosen to alleviate various types of poison-induced toxicity in a large number of studies [25,28]. Thus, supplementation with LYC (2 μM) served as the dosage to ameliorate fluoride-induced toxicity in ameloblasts. We also conducted a preliminary experiment before the formal experiments to demonstrate that the dosage of LYC was appropriate.

2.3. Measurement of fluoride concentration in incisor teeth

The fluoride concentration in incisor teeth was detected according to the research of Błaszczyk et al. [35]. Five different rat incisors in each treatment group were assayed in duplicate. The determination was performed by the potentiometric method using an Orion 96–09 fluoride ion-selective electrode (USA).

2.4. Measurement of ameloblasts apoptosis

Ameloblasts were harvested and washed twice with ice-cold phosphate-buffered saline (PBS). Ameloblasts apoptosis was measured using an annexin V-FITC/propidium iodide apoptosis detection kit (BD PharMingen, San Jose, CA, USA) as previously described [36]. The apoptosis rate was detected by flow cytometry (Becton Dickinson, San Jose, CA, USA).

2.5. Measurement of MDA, SOD, and GPX in ameloblasts and incisor teeth

Ameloblasts were harvested and washed twice with ice-cold PBS and then lysed using the SL-1000D ultrasonic cell disruption apparatus (Shunliu Instrument Company, Nanjing, China). Rats incisor enamel organs were crushed using a mortar and pestle and then homogenized in ice-cold sodium, potassium phosphate buffer (0.01 M, pH 7.4) containing 1.15% KCl using an Ultra-Turrax T25 Homogenizer (IKA-Labortechnik, Staufen, Germany). The lysate was centrifuged for 5 min at 12,000×g at 4 °C, and the supernatant was used to determine the MDA concentration and activities of the antioxidant enzymes (i.e., SOD, and GPX) according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) [37].

2.6. Reactive oxygen species measurements in ameloblasts and incisor teeth

The ROS concentration in the ameloblasts and incisor teeth were determined using Reactive oxygen species Assay Kit (chemistry fluorescence method) from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to the protocol.

2.7. Real-time quantitative PCR (qPCR) analysis

Total RNA were extracted from rats incisor enamel organs and cells using Direct-zol RNA MiniPrep (Zymo Research Corp., Irvine,

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