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Rescuing fluoride-induced damages in liver with gamma aminobutyric acid

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ABSTRACT

Fluorine poisoning affects human health all over the world and an urgent task is to develop alleviative medicine to recover or ameliorate the damages to the body. Here we studied the effects of gamma-aminobutyric acid (GABA), a liver protector reported previously, on fluoride-induced damage in the mouse liver. Through microscope imaging of the liver tissue, TUNEL immunostaining, real-time RT-PCR, enzyme immunoassay and colorimetric method, we found that GABA supplementation prevented the metabolic toxicity caused by fluoride treatment in mice. This detoxification was reflected by the reduced oxidative stress and apoptosis, enhanced neuron protection and liver function. Collectively, this study provided evidence of the beneficial effects of GABA supplement on liver damage, implicating its therapeutic potential in fluorosis.

1. Introduction

Fluorine is a widely distributed natural chemical. Normally, human body needs only a small amount of fluoride; fluorosis will be developed if high levels of fluorine cannot be metabolised effectively [1]. Endemic chronic fluorosis has been reported in more than 50 countries where source of drinking water and the well salt may be the causal factors [2,3]. Fluoride-induced damage to the body is systemic and involves multiple organs including liver, bone and teeth [4–6]. Liver is the most important detoxification organ in the body. A rising number of research studies demonstrated excessive intake of fluoride causes serious liver damage [7–9]. An urgent need is to find the chemical substances that can ameliorate this injury.

Recently, gamma-aminobutyric acid (GABA), as a functional food factor, has been drawn more and more attention. It is an important inhibitory neurotransmitter in the nervous systems of mammals, crustaceans, insects and some parasitic worms. GABA has anti-anxiety, anti-hypertensive, growth-promoting [10] and antioxidant properties [11,12]. A large number of clinical and basic studies have shown that GABA has a protective effect on liver damage. GABA reacts with α-ketoglutaric acid to form glutamic acid, which effectively reduces blood ammonia concentration and promotes the production of urea; the combination of glutamic acid and ammonia relieves ammonia toxicity and improves liver function [13–15]. Other experiment results showed that GABA activated renal function and reduces alkaline phosphatase activity, an important function index of liver [16]. Furthermore, this chemical can inhibit oxidative stress and hepatic steatosis in high-fat diet-fed obese mice [17].

However, the effects of GABA on fluoride-induced liver damage have not been reported. The present study used sodium fluoride as a chemical inducer to establish a fluoride-induced liver damage model. In this study, we demonstrated the ameliorating effects of GABA on the recovery of liver injury induced by fluoride through a systemic analysis and the markers detailed as follows. Oxidative stress was represented with genes of malondialdehyde (MDA), glutathione reductase (GR), and ascorbate peroxidase (APX); neuron protection was marked with nerve growth factor (NGF); liver function on thyroid hormone synthesis was marked with deiodinase (*Dio1*), monocarboxylate transporter 8 (*MCT8*); cell apoptosis signalling pathway was marked with the mitogen-activated protein kinase kinase1 (*MEK1*), the extracellular regulated kinase *ERK1*, *ERK2* and mitochondrial apoptosis pathway-

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related gene, 18 kDa translocator protein (*TSPO*). Also, we examined liver pathology with microscopic observations.

2. Materials and methods

2.1. GABA preparation and other reagents used

Scallops were purchased from Nanshan aquatic product market, Qingdao, China. A new *Enterococcus avium* strain 9184 with a high ability to produce GABA was isolated from a naturally fermented scallop solution. The scallop solution was mixed with 1% sodium glutamate and was used as a medium for culturing *E. avium* to produce GABA. Fermentation was performed for 3 days to obtain a solution rich in GABA (3.71 g/L of the solution). A 732-type cation-exchange resin was used to purify GABA from the fermented solution by using a conventional amino acid separation method. The purity of the end-product was 63%. This purified GABA was used for treatment in mice.

Liver-protecting tablets were purchased from Swisse Wellness pty Ltd (Australia). NaF, GABA and other chemicals were purchased from Sigma Reagent Company. MDA, GR, APX, NGF and HDL-C test kits were purchased from Nanjing Jiancheng Bioengineering Institute. TUNEL Staining Kit was purchased from Roche Company.

2.2. GABA treatment and animal group design

Adult male Kunming mice (weight, 18–22 g) were obtained from Institute of Drug Inspection of Qingdao. The animals (N = 180) were divided into two groups; one group (N = 20) were given pure water as the controls. The other group (N = 160) were given an oral dose of 50 mg/kg body weight (bw)/day of NaF for 30 days and served as models of fluoride injury. Determination of NaF concentration was determined according to Dai (1996) [18]. For sub-chronic toxicity test, NaF dosage should be 1/20 - 1/5 of LD_{50} [19]. Determination of GABA concentrations was also referred to the previous studies [15,20,21], and doses of 5, 25 and 50 mg/kg bw/day were chosen. The mice in the fluoride-exposed group consisted of 8 sub-groups (N = 20): negative control group (NCG, mice receiving only pure water for 14 days), positive control group (PCG, mice receiving liver-protecting tablet orally at a dose of 50 mg/kg bw/day for 14 days), low concentration of pure GABA (G1, mice receiving pure GABA orally at a dose of 5 mg/kg/day for 14 days), medium concentration (G2, 25 mg/kg GABA), high concentration of pure GABA (G3, 50 mg/kg GABA). Similarly, doses of the laboratory-separated GABA were classified as low concentration of laboratory-separated GABA (LSG1, mice receiving laboratory-separated GABA orally at a dose of 5 mg/kg bw/day for 14 days), medium concentration (LSG2, 25 mg/kg GABA) and high concentration (LSG3, 50 mg/kg GABA).

2.3. Analysis of liver tissues

The liver tissues from the GABA treated mice were collected. MDA, GR, APX and HDL-C protein levels in the liver tissues were assayed with the kits from Nanjing Jiancheng Bioengineering Institute. We measured mRNA expression with the reverse transcription and real-time PCR according to the kit instruction provided by Shanghai bioengineering Limited by Share Ltd. The sequences of the primers are listed in Supplemental Table 1.

For cytohistology of microstructure, liver tissues was fixed

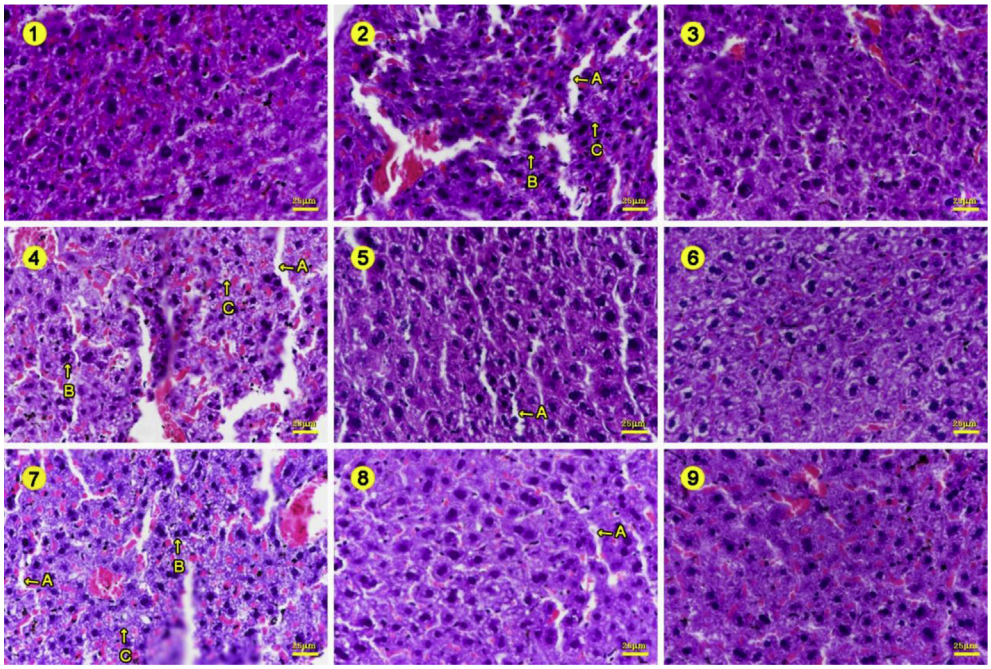

Fig. 1. Improved recovery in the rat liver from fluoride-induced damage by GABA supplementation.
Histological micrograph was performed through HE staining on the slides with rat liver section. Fluoride exposure in the rat caused liver damage manifested with the loose cell arrangement, vacuolization, and chromatin condensation (karyopyknosis) in the tissue, compared to the tightly connected liver cells in the control group. Feeding the rats with either self-made (④, ⑤ and ⑥) or commercially purchased (⑦, ⑧ and ⑨) GABA supplements helped liver to heal. Liver-protecting tablets avoided animals from the fluoride-induced injury (panel ③).
Rats in panel ① Control group were given with water only, without the oral NaF treatment, while all the animals in panels ②-⑨ were treated with NaF, plus GABA supplements from the commercial GABA: 5 mg/kg bw/day (Group G1, panel ④), 25 mg/kg bw/day (Group G2, panel ⑤), and 50 mg/kg bw/day (Group G3, panel ⑥), as well as GABA self-purified in the laboratory: 5 mg/kg bw/day (Group LSG1, panel ⑦); 25 mg/kg bw/day (Group LSG2, panel ⑧), and 50 mg/kg bw/day (Group LSG3, panel ⑨). Meanwhile, NaF-treated mice were received either pure water as the negative control group (NCG, panel ②), or the liver protecting-tablet as the positive control group (PCG, panel ③). A: loose cell arrangement; B: karyopyknosis; C: cell vacuolization.