Pharmacological Reports 68 (2016) 782–786

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original research article

Allopurinol decreases serum uric acid level and intestinal glucose transporter-5 expression in rats with fructose-induced hyperuricemia

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ARTICLE INFO

Article history:
Received 22 March 2016
Received in revised form 20 April 2016
Accepted 23 April 2016
Available online 6 May 2016

Keywords:
Hyperuricemia
Glucose transporters
Fructose
Allopurinol
Benzbromarone

ABSTRACT

Background: High fructose consumption is considered to be related to the increasing prevalence of hyperuricemia (HUA). Glucose transporters (GLUT) 2 and 5 are crucial for fructose absorption and transporter. Effects of anti-HUA drugs, allopurinol (API) and benzbromarone (BBR), on expressions of GLUT5 and GLUT2 are not evaluated.
Method: Wistar rats were given 10% fructose in drinking water for 60 days to induce HUA, and 5 mg/kg API and 10 mg/kg BBR were intragastricly treated for 30 days. Serum level of uric acid and xanthine oxidase (XOD) activity in liver were determined. Expressions of GLUT2 and GLUT5 in intestine were analyzed by immunohistochemistry staining assay and Western blot assay.
Results: Treatment with API or BBR significantly decreased the serum level of uric acid in HUA rats induced by fructose. Meanwhile, API treatment significantly reduced the XOD activity in liver and GLUT5 expression in intestine. However, BBR treatment did not show inhibitory effects on hepatic XOD activity and intestinal GLUT5 expression. In addition, treatment with API or BBR did not show any effect on GLUT2 expression in intestine.
Conclusion: API decreases serum level of uric acid in fructose-induced HUA rats. The mechanisms are associated with suppressing XOD activity in liver to reduce uric acid production, and inhibiting GLUT5 expression in intestine to reduce fructose absorption.

Introduction

Uric acid, as the end-product of purine metabolism in human body, is dominantly produced in liver by xanthine oxidase (XOD) and excreted through kidney. Hyperuricemia (HUA) is charactered by an elevated level of uric acid in blood. HUA is the precursor of gout and is demonstrated to be associated with diabetes [1], kidney diseases [2] and coronary heart diseases [3]. HUA is a result of either overproduction of uric acid or underexcretion of uric acid in the urine. According to the pathogenesis, allopurinol (API), which reduces uric acid production, and benzbromarone (BBR), which enhances uric acid excretion, are frequently-used drugs for HUA treatment in current clinic [4].

During the past few decades, the prevalence and incidence of HUA have rapidly increased throughout the world [5]. Although the underlying causes behind this fact are complicated, it is indisputable that the considerable increase in sugar-sweetened soft drink and associated fructose consumption has coincided with the increasing trend of HUA [6]. The fructose consumption has increased substantially since the high fructose corn syrup (HFCS) came out in 1967. HFCS is a product manufactured from corn, and usually has a composition of 42%, 55% or 90% fructose [7]. Fructose has been demonstrated to increase uric acid production through inducing adenosine triphosphate (ATP) degradation to adenosine monophosphate (AMP), a uric acid precursor and ultimately, serum level of uric acid rises within minutes after fructose infusion [8].

Dietary fructose is absorbed in intestine and transported to blood by the way of two glucose transporters (GLUT), named GLUT5 and GLUT2. GLUT5 is dominantly expressed in enterocytes and it possesses a high specificity for fructose. The primary

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http://dx.doi.org/10.1016/j.pharep.2016.04.014
1734-1140/

function of GLUT5 is to facilitate the uptake of dietary fructose across the apical membrane of the small intestine [9]. GLUT2, a sodium-independent glucose and fructose transporter, is responsible for the transport of fructose and glucose out of the enterocytes across the basolateral membrane [10].

Researchers have revealed some modulating factors on GLUT5 or GLUT2 expression in intestine. GLUT5 expression appears to be tightly regulated by developmental patterns, glucocorticoids, thyroid hormones, nutrition, diurnal rhythm and its own substrate, fructose [11]. Glucose and fructose likely stimulate the transcription of intestinal GLUT2 just as fructose alone stimulates transcription of intestinal GLUT5 [12]. To this day, the effects of anti-HUA drugs on expressions of GLUT5 and GLUT2 are not totally elucidated. Therefore, we investigated the effects of API and BBR, two classic anti-HUA drugs, on expressions of GLUT5 and GLUT2 in intestine of fructose-induced HUA rats.

Materials and methods

Chemicals and reagents

All chemicals and reagents were purchased from Sigma-Aldrich (USA) unless otherwise specified. BBR tablets were purchased from Excella GmbH (Germany). API tablets were purchased from Chongqing Qingyang Pharmaceutical Co., Ltd (China). Antibodies against GLUT2, GLUT5 and β-actin were purchased from Santa Cruz (USA). Polyvinylidene fluoride (PVDF) membrane and enhanced chemiluminescence (ECL) reagents were purchased from Milipore (USA). Ethylene diamine tetraacetic acid (EDTA)-free protease inhibitor cocktail was purchased from Roche (Switzerland). Enhanced bicinchoninic acid (BCA) protein assay kit was purchased from Beyotime Biotech (China). Uric acid detection kit was purchased from Nanjing Jiancheng Biotech (China). Two-step polymer (non-biotin) detection kit was purchased from GBI (USA).

Experimental animals

Male Wistar rats (180 ± 20 g) were purchased from Chongqing Medical University (China). The rats were housed in a temperature-controlled room (23 ± 2 °C) under a 12-h light/dark cycle with available food and water ad libitum. The rats were allowed one week to adapt to their environment before initiation of the experiments. All animal procedures were approved by the institutional animal care and use committee of Chongqing Technology and Business University (Ethics No. CTBU2015-019).

Induction of HUA and drugs treatment

In this study, rats were given 10% fructose in drinking water to induce HUA as previously described [13]. Rats were divided into 4 groups, which were (1) Control group, (2) HUA group, (3) 5 mg/kg API group, (4) 10 mg/kg BBR group. Each group had 10 rats. The doses of API and BBR used in this study were determined according to published literatures [13,14]. Rats of (2), (3) and (4) groups were given 10% fructose in drinking water, and rats of (1) group were given normal drinking water, for 60 consecutive days. From the 31st day to 60th day during 10% fructose treatment, rats of (3) and (4) groups were intragastricly treated with API or GTP, and rats of (1) and (2) groups were intragastricly treated with saline. On the day 60 all the rats were sacrificed under anesthesia 1 h after the last drugs treatment.

Blood, liver and intestine samples collection

Blood samples were collected from hearts under anesthesia. Blood coagulated at 4 °C overnight and serum was separated by centrifuge at 3000 g for 20 min. Serum samples were stored at −20 °C and used for the test of uric acid level.

Liver tissues of rats were rapidly separated on ice plate, snap-frozen in liquid nitrogen, and stored at −70 °C. For XOD activity assay, liver tissues were sufficiently homogenized in 9 volumes of 80 mmol/L sodium phosphate buffer (pH 7.4) in ice bath. Subsequently, the homogenate was centrifuged at 4,000 g for 20 min at 4 °C. After lipid layer was carefully removed, supernatant was further centrifuged at 10,000 g for 10 min at 4 °C and the final supernatant was used for XOD activity assay. Protein concentration of the final supernatants was determined by the BCA method and performed according to manufacturer's instructions.

The small intestine was quickly removed and rinsed in saline. Five small intestine samples from five rats were fixed in 4% paraformaldehyde for 24 h. Then the tissues were embedded in paraffin and serial paraffin sections (5 μm) were prepared for the subsequent histological analysis. Five small intestine samples from remaining five rats were opened and scraped with a glass slide. The mucosal scrapings were snap-frozen in liquid nitrogen and stored at −70 °C for the subsequent total protein extract. Briefly, the mucosal scrapings were sufficiently homogenized in lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, pH 7.6) and EDTA-free protease inhibitor cocktail in ice bath. Subsequently, the homogenate was centrifuged at 10,000 g for 10 min at 4 °C and the final supernatant was used for Western blot assay. Protein concentration of the supernatant was determined by the BCA method and performed according to manufacturer's instructions.

Uric acid assay

Serum level of uric acid was analyzed by the uric acid detection kit. The experimental process was performed according to manufacturer's instructions.

XOD activity assay

XOD activity in liver extracts was analyzed by the XOD activity detection kit. The experimental process was performed according to manufacturer's instructions.

Western blot assay

Equal amounts of protein samples were separated by SDS-PAGE electrophoresis and transferred onto PVDF membranes. After blocked with 5% nonfat milk in Tris-buffered saline (TBS) and Tween 20 buffer (10 mM Tris-HCl, 150 mM NaCl, 0.1% Tween 20, pH 7.6) for 1 h at room temperature, PVDF membranes were incubated with anti-GLUT2, anti-GLUT5 or anti-β-actin antibody at 4 °C overnight. Then the membranes were incubated for 1 h at room temperature with appropriate horseradish peroxidase-conjugated (HRP)-conjugated secondary antibody. The protein bands were detected with ECL reagents. Chemiluminescent signals were detected and analyzed using the ChemiDoc XRS imaging system (Bio-Rad, USA).

Immunohistochemistry (IHC) staining

Prepared serial paraffin sections were stained using a two-step polymer (non-biotin) detection kit according to manufacturer's instructions. Briefly, the sections were deparaffinized and blocked for 20 min with 3% hydrogen peroxide at room temperature. Heat-induced epitope retrieval was performed in Tris/EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, 0.05% Tween 20, pH 9.0) by a pressure cooker. Subsequently, the sections were incubated with anti-GLUT2 antibody or anti-GLUT5 antibody overnight at 4 °C.