Biomedicine & Pharmacotherapy 104 (2018) 315–324

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Hordenine protects against hyperglycemia-associated renal complications in streptozotocin-induced diabetic mice

Shuhao Su[a,1], Meng Cao[a,1], Guangyuan Wu[a], Zi Long[a], Xiaodong Cheng[a], Junshu Fan[a], Zhongrui Xu[a], Hongfei Su[a], Yiming Hao[b], Ge Li[a], Jie Peng[a], Shuang Li[c], Xin Wang[a,*]

[a] Department of Toxicology, Shaanxi Key Lab of Free Radical Biology and Medicine, School of Public Health, Fourth Military Medical University, Xi'an, 710032, China
[b] Department of Gastrointestinal Surgery, Xijing Hospital of Digestive Diseases, Fourth Military Medical University, Xi'an, 710032, Shaanxi, China
[c] Department of Cardiology, Chengdu Military General Hospital, Chengdu, 610083, China

ARTICLE INFO

Keywords:
Diabetes
Hordenine
Diabetic nephropathy
Inflammation
Fibrosis
Oxidative stress

ABSTRACT

The worldwide prevalence of diabetes and associated metabolic diseases has dramatically increased. Pharmacological treatment of diabetes is still limited. Hordenine (HOR), a phenethylamine alkaloid, is a natural constituent in many plants. The present study was designed to explore the possible anti-diabetic effect of HOR in streptozotocin (STZ)-induced diabetic mice. Combined treatment of HOR and insulin significantly reduced fasting and postprandial blood glucose level in diabetic mice. HOR and insulin did not show evident protective effect against structural and functional injuries of pancreas. Renal histological and functional injuries were significantly improved by HOR or insulin treatment. Moreover, combined treatment of HOR and insulin resulted in a more significant amelioration of renal histological and functional injuries in diabetic mice. HOR induced a decrease of renal IL-1α/β and IL-6 expression, and a reduction of Col1α1 and MMP9 expression and PAS-stained mesangial expansion in glomeruli of diabetic mice. In diabetic mice, HOR significantly decreased Nrf2 expression and increased hnRNPF and hnRNPK expression in kidney. Moreover, HOR showed a synergistic effect with insulin on the expression of these regulators. Renal ROS level and TBARS content in diabetic mice were decreased by HOR. The reduction of renal expression of antioxidant enzymes in diabetic mice was inhibited by HOR and insulin. Furthermore, HOR and insulin function synergistically to play an antioxidant role against oxidative injury in diabetic nephropathy. In conclusion, to the best of our knowledge, we, for the first time, found the anti-diabetic, anti-inflammatory, and anti-fibrotic role of HOR in combination with insulin. HOR functions synergistically with insulin and prevents diabetic nephropathy. However, the molecular mechanism of the synergistic effect of HOR and insulin needs to be elucidated.

1. Introduction

The worldwide prevalence of diabetes and associated metabolic diseases has dramatically increased in the last decades [1]. The prevalence of diabetes for all age-groups worldwide and the total number of diabetes patients are estimated to rise to 4.4% and 366 million, respectively, in 2030 [1]. China has the world's largest diabetes epidemic, which continues to increase. Among adults in China, the latest estimated overall prevalence of diabetes is 10.9%, and that for prediabetes is 35.7% [2]. As a disorder of glucose metabolism, diabetes is also associated with death from failure of multiple organ systems [3,4]. Diabetes has become the fifth leading cause of death, contributing to 5.2% of all deaths in 2000 [5]. However, to date, the pharmacological treatment of diabetes is still limited. Especially, in the management of type 1 diabetes mellitus (T1DM), the clinician has to balance between adequate glycemic control and adverse effects related to insulin up-

Abbreviations: ACR, albumincreatinine ratio; ALT, alanine aminotransferase; AST, aspartic transaminase; BUN, blood urea nitrogen; BW, body weight; CAT, catalase; CK, creatine kinase; Col1α1, collagen1α1; CREA, creatinine; GCLC, glutamate-cysteine ligase catalytic subunit; GCLM, glutamate-cysteine ligase modifier subunit; Gpx1, glutathione peroxidase 1; GSP, glycated serum protein; HDL, high density lipoprotein; HNE, 4-Hydroxynone; hnRNPF, heterogeneous nuclear ribonucleoprotein F; hnRNPK, heterogeneous nuclear ribonucleoprotein K; HOR, hordenine; LDH, lactate dehydrogenase; LDL, low density lipoprotein; MMP9, matrix metalloproteinase 9; NEFAs, non-esterified fatty acids; Nrf2, nuclear factor erythroid 2-related factor 2; PAMY, pancreatic amylase; PAS, periodic acid schiff; ROS, reactive oxygen species; RT-qPCR, real time-quantitative polymerase chain reaction; SOD1, superoxide dismutase 1; SOD2, superoxide dismutase 2; STZ, streptozotocin; T1DM, type 1 diabetes mellitus; TBARS, thiobarbituric acid reactive substances; TCHO, total cholesterol; TG, triglyceride; TGF-β1, transforming growth factor-beta 1; UUN, urine urea nitrogen

* Corresponding author at: Department of Toxicology, School of Public Health, Fourth Military Medical University, Changle West Road 169, Xi'an, 710032, Shaanxi Province, China.
E-mail address: xinwang@fmmu.edu.cn (X. Wang).
[1] Co-authors.

https://doi.org/10.1016/j.biopha.2018.05.036
Received 2 April 2018; Received in revised form 8 May 2018; Accepted 8 May 2018

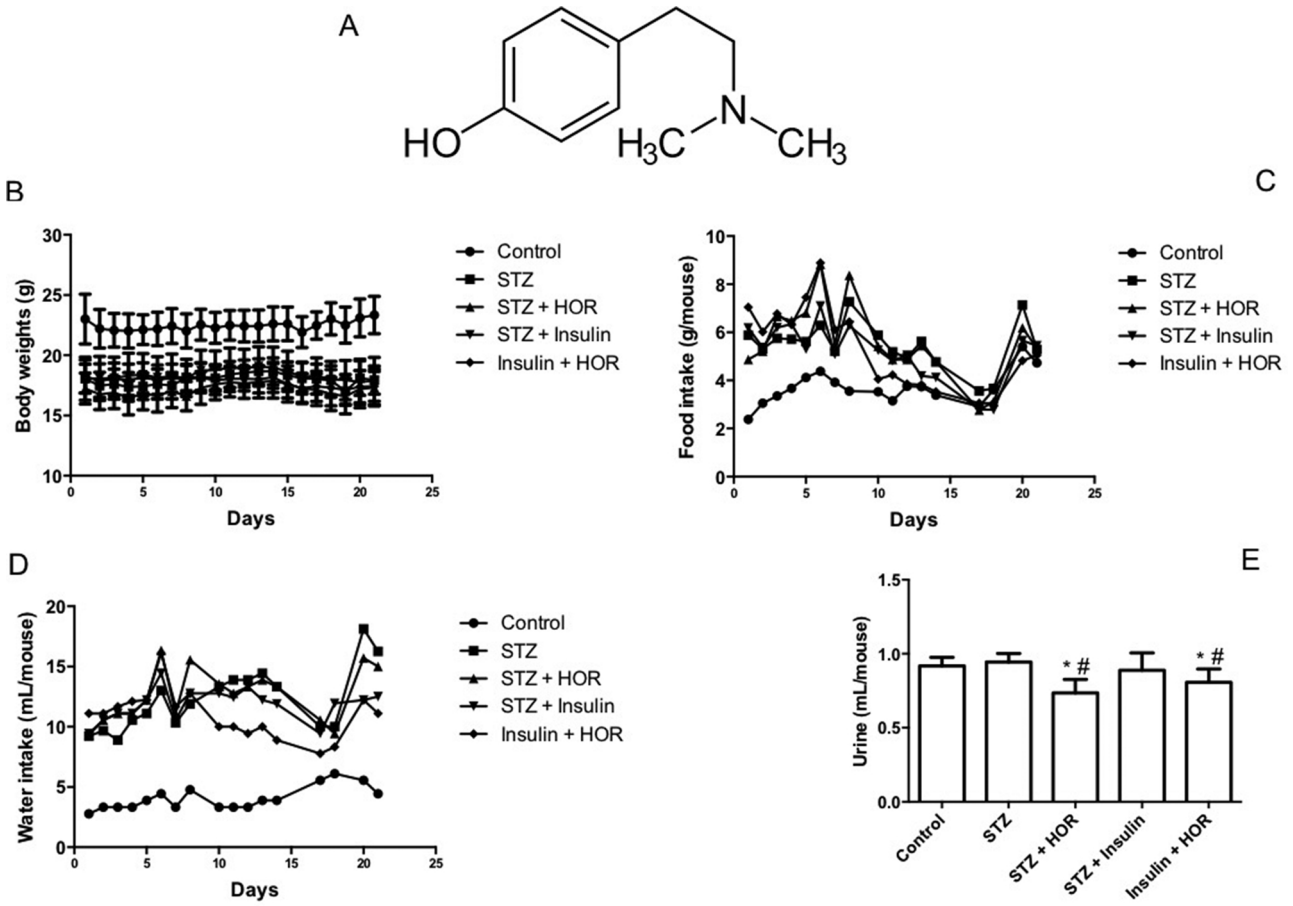

Fig. 1. The protective effect of HOR on general characteristics in diabetic mice.
STZ-induced diabetic mice were administrated with 50 mg/kg HOR or 0.75U/kg insulin or combined treatment of 25 mg/kg HOR and 0.375 U/kg insulin for 3 weeks. (A) Structure of HOR. (B) Daily body weights. (C) Daily food intake. (D) Daily water intake. (E) Content of urine at the end of experiment. *$P < 0.05$, compared with control. #$P < 0.05$, compared with STZ treatment.

titration [6]. Therefore, novel agents that will enhance or complement insulin actions are urgently needed [6].

Hordenine (HOR), (4-(2-Dimethylaminoethyl)) (Fig. 1A), a phenethylamine alkaloid, is a natural constituent in many plants, particularly in cactus [7], barley (Hordeum vulgare) [8], bitter orange [9], *Lophophora williamsii* [10] and certain grasses, and is probably present at low levels in most equine diets. In fact, HOR is occasionally found in post race urine samples of horses, and horses with rapid intravenous injection (but not oral administration) of HOR showed a transient flehmen response, respiratory distress, increased respiratory and heart rates [11]. During germination, HOR concentration in barley roots reaches a maximum within 3–9 days and slowly decreases until only traces remains after 1 month [8]. HOR is derived from tyramine through step-wise *N*-methylation, in which process tyramine is firstly converted to *N*-methyltyramine, and further methylated to HOR [12]. Several pharmacological properties of HOR have been reported, including inhibition of monoamine oxidase B (MAO-B) [13], stimulation of gastrin release in rats [14], and antibacterial and antibiotic properties [15]. However, no data is available on the possible effects of HOR on diabetes and its associated complications.

In the present study, we aimed to explore whether HOR exhibited an anti-diabetic effect and to evaluate the effect of combined use of HOR and insulin. Using streptozotocin (STZ)-induced diabetic mice, we investigated the effect of HOR alone or in combination with insulin injection on blood glucose level, serum insulin level and injury of liver and kidney. The influence of HOR on oxidative stress, inflammation, glycogen metabolism, and fibrosis has been evaluated.

2. Materials and methods

2.1. Materials and reagents

The Nrf2 antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Gpx1 and HNE antibodies were purchased from Abcam (MA, USA). HNF1b antibody was obtained from ProSci Technology (Poway, CA, USA). Hoechst and DHE were purchased from Beyotime Institute of Biotechnology (Jiangsu, China). HOR, STZ, and most of the chemicals and reagents used in this study were procured from Sigma Aldrich (St. Louis, MO, USA).

2.2. Animals and model of T1DM

Animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication no. 85–23, revised 1996) and treatment of animals were approved by the Animal Care and Use Committee of the Fourth Military Medical University. Male C57BL/6J mice (7–8 weeks of age, 18–20 g) received intraperitoneal injections of citrate buffer (0.05 mol/L sodium citrate, pH 4.5, Control) or streptozotocin (STZ, 90 mg/kg) dissolved in citrate buffer for three consecutive days at a 24 h interval during the first week of the study. In the second week of the experiment, mice received intraperitoneal injections of citrate buffer (0.05 mol/L sodium citrate, pH 4.5, Control) or STZ (60 mg/kg) dissolved in citrate buffer for another three consecutive days at a 24 h interval. At the end of the second week, blood glucose levels of a tail prick were measured twice at a 24 h interval using a human OTC