Original Article

MOLECULAR METABOLISM

Intestinal bitter taste receptor activation alters hormone secretion and imparts metabolic benefits

Bernard P. Kok [1], Andrea Galmozzi [1], Nicole K. Littlejohn [1], Verena Albert [1], Cristina Godio [1], Woojoo Kim [1], Sean M. Kim [1], Jeffrey S. Bland [3], Neile Grayson [3], Mingliang Fang [2], Wolfgang Meyerhof [4], Gary Siuzdak [2], Supriya Srinivasan [1], Maik Behrens [4,5], Enrique Saez [1,*]

ABSTRACT

Objectives: Extracts of the hops plant have been shown to reduce weight and insulin resistance in rodents and humans, but elucidation of the mechanisms responsible for these benefits has been hindered by the use of heterogeneous hops-derived mixtures. Because hop extracts are used as flavoring agents for their bitter properties, we hypothesized that bitter taste receptors (Tas2rs) could be mediating their beneficial effects in metabolic disease. Studies have shown that exposure of cultured enteroendocrine cells to bitter tastants can stimulate release of hormones, including glucagon-like peptide 1 (GLP-1). These findings have led to the suggestion that activation of Tas2rs may be of benefit in diabetes, but this tenet has not been tested. Here, we have assessed the ability of a pure derivative of a hops isohumulone with anti-diabetic properties, KDT501, to signal through Tas2rs. We have further used this compound as a tool to systematically assess the impact of bitter taste receptor activation in obesity-diabetes.

Methods: KDT501 was tested in a panel of bitter taste receptor signaling assays. Diet-induced obese mice (DIO) were dosed orally with KDT501 and acute effects on glucose homeostasis determined. A wide range of metabolic parameters were evaluated in DIO mice chronically treated with KDT501 to establish the full impact of activating gut bitter taste signaling.

Results: We show that KDT501 signals through Tas2r108, one of 35 mouse Tas2rs. In DIO mice, acute treatment stimulated GLP-1 secretion and enhanced glucose tolerance. Chronic treatment caused weight and fat mass loss, increased energy expenditure, enhanced glucose tolerance and insulin sensitivity, normalized plasma lipids, and induced broad suppression of inflammatory markers. Chronic KDT501 treatment altered enteroendocrine hormone levels and bile acid homeostasis and stimulated sustained GLP-1 release. Combined treatment with a dipeptidyl peptidase IV inhibitor amplified the incretin-based benefits of this pure isohumulone.

Conclusions: Activation of Tas2r108 in the gut results in a remodeling of enteroendocrine hormone release and bile acid metabolism that ameliorates multiple features of metabolic syndrome. Targeting extraoral bitter taste receptors may be useful in metabolic disease.

Keywords Bitter taste receptor; Diabetes; Enteroendocrine hormones; GLP-1; Intestinal signaling; Isohumulone

1. INTRODUCTION

Extracts of the hops plant (*Humulus lupulus* L.) used as flavoring agents in beer production have been shown to reduce weight and improve glucose homeostasis in rodents [1–8] and humans [1,9,10]. Thus, it is of considerable interest to identify the molecular mechanisms that mediate the benefits of bioactive ingredients of hops in metabolic disease. The humulones or α-acids are among the most abundant phytochemicals synthesized by female hop cones [11]. Hop α-acids are isomerized during brewing to iso-α-acids, or isohumulones, that impart beer its characteristic bitter flavor. There are three main isohumulones (*n*-humulone, cohumulone, and adhumulone) that account for more than 80% of the hops-derived matter in beer [11]. Each isohumulone occurs as a mixture of *cis* and *trans* isomers that can be reduced into rho, tetrahydro, or hexahydro analogs. This level of chemical complexity has hampered efforts to uncover the mechanism of action of isohumulones in metabolic syndrome.

An initial study [1] with mixtures of isohumulones showed that obese-diabetic KK-A^y mice treated with these compounds had reduced glycemia and decreased plasma triglycerides and free fatty acid levels. Similarly, mice with established Diet Induced Obesity (DIO) treated with

[1]Department of Molecular Medicine, USA [2]Center for Metabolomics, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA, 92037, USA [3]Kindex Pharmaceuticals, 800 Fifth Avenue, Seattle, WA, 98104, USA [4]Department of Molecular Genetics, German Institute of Human Nutrition Potsdam-Rehbruecke, Arthur-Scheunert-Allee 114-116, 14558, Nuthetal, Germany [5]Leibniz-Institute for Food Systems Biology at the Technical University of Munich, Lise-Meitner-Str. 34, 85354, Freising, Germany

*Corresponding author. Department of Molecular Medicine, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA, 92037, USA. E-mail: esaez@scripps.edu (E. Saez).

Received June 26, 2018 • Revision received July 23, 2018 • Accepted July 31, 2018 • Available online xxx

https://doi.org/10.1016/j.molmet.2018.07.013

isohumulones showed enhanced glucose tolerance and insulin sensitivity. These benefits were ascribed to direct activation of peroxisome proliferator-activated receptors (PPARs) α and γ. However, no changes in PPARγ target gene expression could be detected in isohumulone-treated mice, suggesting that isohumulones exert their benefits through alternative, unknown mechanisms. Interestingly, this study also included a small double-blind, placebo-controlled trial in people with diabetes that showed that treatment for 8 weeks with isohumulones significantly reduced blood glucose and hemoglobin A1c levels. Subsequent studies [2–5,8] have reported positive effects of isohumulones in reducing weight gain and fat mass, improving glucose homeostasis and insulin sensitivity, decreasing liver lipid accumulation, and reducing plasma triglycerides in multiple rodent models (e.g., DIO, KK-A^y, *db/db* mice, Wistar rats). Notably, a larger clinical trial in Japanese subjects with prediabetes showed that 12-week treatment with isohumulones significantly reduced body weight, body mass index, fat mass, fasting blood glucose, and hemoglobin A1c levels [9].

An important, enabling development towards the identification of the molecular players responsible for the effects of isohumulones was the synthesis of KDT501, a stereochemically pure substituted 1,3-cyclopentadione [12]. This derivative of hops tetrahydro iso-α-acids retained the desirable properties of natural isohumulone mixtures [13]. DIO mice and Zucker Diabetic Fatty rats treated orally with KDT501 showed decreased weight and fat mass, increased insulin sensitivity, and reduced plasma lipids. This study, however, did not decisively determine the molecular target(s) of KDT501 relevant for its action.

Given that hops-derived compounds are used as flavoring agents for their bitter properties, it is conceivable that the benefits of KDT501 and natural isohumulones on metabolic disease may be due to signaling through bitter taste receptors present in extraoral tissues, particularly the gastrointestinal (GI) tract. Beyond its role in nutrient absorption, the GI tract is emerging as a powerful regulator of systemic energy balance that secretes hormones that affect varied aspects of physiology and behavior [14,15]. Taste receptors were discovered in the tongue [16–18], but it is now well established that they are expressed in multiple tissues besides the oral cavity, including the GI tract [19,20]. Bitter taste receptors (mouse Tas2rs; human TAS2Rs) are G_α-gustducin/G_q-coupled GPCRs that were initially thought to have evolved as a defense mechanism to avoid ingestion of harmful substances [21]. However, it is now clear that in addition to this protective function in the mouth, extraoral bitter taste receptors play important roles in physiology [22,23]. For instance, exposure of cultured enteroendocrine cells to promiscuous bitter tastants (e.g., quinine) is known to increase intracellular calcium signaling [24] and stimulate release of hormones, including cholecystokinin (CCK) [25–27], Peptide YY (PYY) [28], and glucagon-like peptide 1 (GLP-1) [26,29]. The ability of bitter compounds to acutely stimulate intestinal hormone secretion, particularly incretins, has led to the suggestion that activation of Tas2rs may be of benefit in diabetes [30]. However, the extent to which these effects may be seen *in vivo* and in sufficient magnitude to be therapeutic is not known. Unfortunately, evaluation of the therapeutic potential of bitter taste receptor ligands has been hampered by a lack of selective Tas2r agonists. Quinine, for instance, signals through Tas2rs but is also known to activate the calcium-activated cation channel Trpm5 [31], several potassium channels [32,33], and to directly enhance ERK/S6 kinase [34] and G-protein signaling [35].

In this report, we provide evidence that KDT501 and, by inference natural isohumulones, exert their anti-diabetic effects via modulation of bitter taste receptor signaling in the gut. A principal mediator of these effects is GLP-1, which has broad effects on physiology and in reversing metabolic dysfunction. We show that KDT501 activates a single mouse (Tas2r108) or human (TAS2R1) bitter taste receptor (of 35 and 25, respectively). Enteroendocrine cells exposed to KDT501 secreted GLP-1, and this effect was blunted in cells depleted of Tas2r108. The excellent selectivity of KDT501 enabled us to use this compound as a tool to examine the impact of activation of bitter taste receptor signaling in obesity-diabetes. DIO mice treated with a single dose of KDT501 had enhanced glucose tolerance. Chronic dosing resulted in weight loss, reduced fat mass, increased energy expenditure, greater glucose tolerance and insulin sensitivity, and a normalization of plasma lipid profiles accompanied by broad suppression of circulating inflammatory markers. The basis of these benefits is a remodeling of gut hormone secretion and bile acid homeostasis that includes stimulation of GLP-1 release. These findings indicate that targeting gut bitter taste receptors may be of utility in metabolic disease. Further, co-treatment with a dipeptidyl peptidase IV (DPP-IV, the enzyme that degrades GLP-1) inhibitor considerably augmented the incretin-based benefits of this pure isohumulone, stressing the potential advantage of combined administration of an incretin secretagogue (e.g., KDT501) with DPP-IV inhibitors in clinical use. Our results illustrate an interesting paradigm by which naturally occurring compounds can harness gut signaling to alter whole body metabolism.

2. MATERIALS AND METHODS

2.1. Animal studies

C57BL/6N DIO and control mice were purchased from Taconic Biosciences at 17 weeks of age and maintained on a 60% and 10% kcal high fat diet (Research Diets D12492, D12450J), respectively. All experiments were conducted with male mice housed (2–4 mice per cage) in ventilated rack systems under regular housing temperatures (22–24 °C) with a 12 h light/12 h dark cycle (06:00 light on to 18:00 light off). Compounds were homogenized (Tissue-Tearor, Biospec) and bath-sonicated in 0.5% methylcellulose containing 0.2% Tween-80 (vehicle). Mice were dosed daily by oral gavage with vehicle, 150 mg/kg KDT501, or 10 mg/kg rosiglitazone. The dose of KDT501 was chosen based on prior work [13]. Weights were monitored weekly and mice were fasted for 16 h prior to blood collection. Animal experiments were approved by and conducted in accordance with the guidelines of The Scripps Research IACUC.

2.2. Cell culture

STC-1 cells were purchased from the American Type Culture Collection (RRID CVCL_J405). Cells were cultured in 10% FBS-DMEM and maintained in a 37 °C, 5% CO_2 incubator. Lentiviral shRNA constructs targeting mouse Tas2r108 (TRCN0000418872 and TRCN0000072142) and the corresponding controls (SHC202 and SHC002) were purchased from Sigma–Aldrich. Experimental results were similar with both shRNAs, and results from first- (TRCN0000072142) and second-generation (TRCN0000418872) TRC vectors are shown in the supplementary and main figures, respectively.

2.3. Plasma lipid and hormone measurements

Blood was collected from the tail of non-sedated mice, retro-orbital plexus of isoflurane-sedated mice, or by cardiac puncture upon euthanasia. The DPP-IV inhibitor KR-62436 (Sigma–Aldrich) was added at a final concentration of 5 μM for blood collection related to GLP-1 assays. Plasma triglycerides (Sigma–Aldrich), non-esterified free fatty acids (Sigma–Aldrich), ketone bodies (Bioassay Systems), and cholesterol (Wako Chemicals) were analyzed with colorimetric assay kits. Plasma insulin, GLP-1, PYY, and ghrelin levels were

