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A novel and selective melanin-concentrating hormone receptor 1 antagonist ameliorates obesity and hepatic steatosis in diet-induced obese rodent models

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ABSTRACT

Melanin-concentrating hormone (MCH), a cyclic neuropeptide expressed predominantly in the lateral hypothalamus, plays an important role in the control of feeding behavior and energy homeostasis. Mice lacking MCH or MCH_1 receptor are resistant to diet-induced obesity (DIO) and MCH_1 receptor antagonists show potent anti-obesity effects in preclinical studies, indicating that MCH_1 receptor is a promising target for anti-obesity drugs. Moreover, recent studies have suggested the potential of MCH_1 receptor antagonists for treatment of non-alcoholic fatty liver disease (NAFLD).

In the present study, we show the anti-obesity and anti-hepatosteatosis effect of our novel MCH_1 receptor antagonist, Compound A. Repeated oral administration of Compound A resulted in dose-dependent body weight reduction and had an anorectic effect in DIO mice. The body weight lowering effect of Compound A was more potent than that of pair-feeding. Compound A also reduced lipid content and the expression level of lipogenesis-, inflammation-, and fibrosis-related genes in the liver of DIO mice. Conversely, intracerebroventricular infusion of MCH caused induction of hepatic steatosis as well as increase in body weight in high-fat diet-fed wild type mice, but not MCH_1 receptor knockout mice. The pair-feeding study revealed the MCH-MCH_1 receptor system affects hepatic steatosis through a mechanism that is independent of body weight change. Metabolome analysis demonstrated that Compound A upregulated lipid metabolism-related molecules, such as acylcarnitines and cardiolipins, in the liver.

These findings suggest that our novel MCH_1 receptor antagonist, Compound A, exerts its beneficial therapeutic effect on NAFLD and obesity through a central MCH-MCH_1 receptor pathway.

1. Introduction

Melanin-concentrating hormone (MCH) is a cyclic 19-amino-acid peptide expressed predominantly in the lateral hypothalamic area and zona incerta, and MCH-producing neurons project throughout the brain (Bittencourt et al., 1992). Two distinct MCH receptors have been identified: MCH_1 receptor (Bachner et al., 1999; Chambers et al., 1999; Saito et al., 1999; Shimomura et al., 1999) and MCH_2 receptor (An et al., 2001; Mori et al., 2001; Sailer et al., 2001). MCH_1 receptor is widely distributed throughout the vertebrate brain including the hypothalamus, thalamus, olfactory cortex, amygdala, striatum, and hippocampus (Hervieu et al., 2000; Schlumberger et al., 2002). In contrast, MCH_2 receptor is expressed in similar areas of the brain in higher mammals, but not in rodents (Tan et al., 2002) and the physiological function of MCH_2 receptor is currently little understood.

A large number of studies demonstrate that the MCH-MCH_1 receptor pathway plays a key role in the regulation of feeding behavior and energy expenditure. Chronic intracerebroventricular (icv) infusion of MCH induces hyperphagia, body weight gain, and hyperinsulinemia, especially under high-fat diet conditions (Della-Zuana et al., 2002; Gomori et al., 2003; Ito et al., 2003). Transgenic mice overexpressing MCH in the lateral hypothalamus are found to be more susceptible to obesity and insulin resistance when fed a high-fat diet (Ludwig et al., 2001). In contrast, mice lacking MCH or MCH_1 receptor are lean with increased metabolic rate and resistant to diet-induced obesity (DIO) (Shimada et al., 1998; Marsh et al., 2002; Kokkotou et al., 2005). In

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human studies, the two MCH_1 receptor mutants (R210H and P377S) were identified in markedly underweight subjects, indicating the possibility that the lean phenotype may be linked to deficiency of MCH_1 receptor signaling (Goldstein et al., 2010).

Non-alcoholic fatty liver disease (NAFLD), a progressive liver disease that includes steatosis, steatohepatitis (non-alcoholic steatohepatitis [NASH]), fibrosis, cirrhosis, and hepatocellular carcinoma, is also strongly associated with obesity, and the prevalence of NAFLD and NASH in obese patients has been estimated to be about 90% and 30%, respectively (Williams et al., 2011; Bettermann et al., 2014). Despite the increasing prevalence, few drugs have fully met doctors' and patients' needs in the management of obesity and NAFLD because of insufficient efficacy, cost, and safety concerns (Moscatiello et al., 2011; Apovian et al., 2015).

In addition to its involvement in obesity, the central MCH-MCH_1 receptor pathway directly controls lipid metabolism in the liver. Rats centrally treated with MCH exhibited lipid accumulation in the liver due to parasympathetic stimulation independent of increased calorie consumption (Imbernon et al., 2013), and chronic icv infusion of MCH_1 receptor antagonist ameliorated hepatic steatosis without affecting body weight in NASH model mice (Ito et al., 2008), suggesting that MCH_1 receptor antagonist is an attractive target to meet the need for not only a new obesity treatment but also NAFLD/NASH treatment.

Recently we synthesized a novel MCH_1 receptor antagonist, Compound A, (1-(2-cyclopropyl-3-methyl-2*H*-indazol-5-yl)-4-{[5-(trifluoromethyl)thiophen-3-yl]methoxy}pyridin-2(1*H*)-one) (Igawa et al., 2016). In the present study, we assessed the promising anti-obesity and anti-hepatosteatosis effects of Compound A in diet-induced rodent models.

2. Materials and methods

2.1. Materials

Compound A (Fig. 1) was synthesized at Takeda Pharmaceutical Co., Ltd. Sibutramine and MCH were purchased from Alexis Biochemicals (Lausen, Switzerland) and Peptide Institute, Inc. (Osaka, Japan), respectively. All other chemicals were of analytical grade and purchased from Wako Pure Chemicals (Osaka, Japan). Compound A and sibutramine were suspended in 0.5% methylcellulose solution (Shin-Etsu Chemical Co., Ltd, Tokyo, Japan) for oral dosing and MCH was dissolved in distilled water for icv injection.

2.2. Animals

Male F344 rats and C57BL/6 J mice were purchased from CLEA Japan, Inc. (Tokyo, Japan). For DIO-rodent studies, animals were fed a high-fat diet (45 kcal% energy as fat, 20 kcal% as protein, and 35 kcal% as carbohydrate; 4.73 kcal/g; D12451, Research Diets, Inc., New Brunswick, NJ, USA) from 5 weeks of age to induce obesity. MCH_1 receptor knockout (KO) and wild-type mice were originally generated by targeted disruption of exon2 of the *Mchr1* gene and backcrossed 4 times to a C57BL/6 J background by speed congenic system and fed the high-fat diet. Animals had *ad libitum* access to diet and tap water unless otherwise stated. Animals were housed individually under controlled temperature (20–26 °C), humidity (40–70%) and a 12 h light-dark cycle (lights on 7 a.m.). All animal experiments were conducted in accordance with the protocol reviewed by the Institutional Animal Care and Use Committee of Takeda Pharmaceutical Co., Ltd.

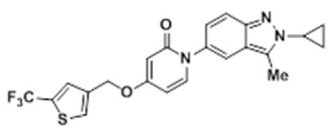

Fig. 1. Chemical structure of Compound A.

2.3. In vivo target selectivity of Compound A in MCH_1 receptor knockout mice

MCH_1 receptor KO and wild-type mice were habituated to oral dosing before the start of the experiment and divided into 3 groups at 36 weeks of age. The mean initial body weights in wild-type and KO mice were 49.8 ± 3.4 g and 45.3 ± 4.9 g, respectively. Each group was orally given either vehicle or Compound A (10, 30 mg/kg) once-daily for 3 days (n=6). The drug was administered after measurement of body weight and food intake, 1–3 h before the onset of the dark period.

2.4. Ex vivo receptor occupancy study in DIO-F344 rats

Prior to the start of treatment, DIO-F344 rats were given powdery high-fat diet (45% fat; D12451M, Research Diets, Inc.) and were habituated to oral dosing for 2 weeks. The rats (48 weeks of age) were divided into 4 groups and there was no between-group difference in body weight. The mean initial body weight of all groups was 499.6 ± 9.3 g. Each group was orally administered either vehicle, or Compound A (5, 10 mg/kg) once-daily for 2 weeks (n=6). The drug was administered after measurement of body weight, 1–3 h before the onset of the dark period. Food intake was measured every 2 or 3 days. Blood samples were collected from the tail vein and the rats were killed under isoflurane anesthesia after 2.7 h (T_{max}) or 24 h of final dosing (n=3). The brain was harvested and separated into hemispheres, followed by immediate freezing in isopentane at −30 to −40 °C. The frozen coronal sections (20 μm), including the striatal region where MCH_1 receptor was densely located (Able et al., 2009; Gehlert et al., 2009), were mounted on microscope slides. Brain sections were incubated for 2 h with 20 pM [^{125}I]Tyr-S36057 (Audinot et al., 2001; Able et al., 2009) in the assay buffer (25 mM HEPES [pH 7.4] containing 0.1% bovine serum albumin, 10 mM $MgCl_2$, 5 mM $MnCl_2$ and 10 mM NaCl) and dried. Non-specific binding was determined in the presence of 10 nM MCH. The radioligand bound to the tissue was visualized using BAS 2500 (Fujifilm, Kanagawa, Japan) and autoradiographic images in the striatal region were quantified with Multi-Gauge software (Fujifilm). Receptor occupancy was calculated by following formula: Receptor occupancy (%) =[1−(radioactivity of Compound A-treated group)/(radioactivity of vehicle-treated group)]. The compound concentrations in the plasma and brain were measured by high-performance liquid chromatography/tandem mass spectrometry (LC/MS/MS).

2.5. Four-week administration of Compound A in DIO-C57BL/6 J mice

DIO-C57BL/6 J mice (46 weeks of age) were habituated to oral dosing for 2 weeks before the start of the experiment. The mice were divided into 6 groups and there was no between-group difference in body weight. The mean body weight at day 0 of all animals was 50.9 ± 2.0 g. Each group received orally administered vehicle, Compound A (10, 30, 100 mg/kg), or sibutramine (10 mg/kg) once-daily for 4 weeks (n=5–8). A pair-fed group was also prepared to clarify whether the body weight reduction elicited by administration of 30 mg/kg of Compound A was due only to decreased food intake or not. In the pair-fed group, mice were given the same amount of high-fat diet as was consumed by the 30 mg/kg Compound A treatment group. Vehicle was administered to these animals. The drug was administered after measurement of body weight and food intake, 1–3 h before the onset of the dark period. At the end of the experiment, blood samples were collected from the facial vein for measurement of plasma parameters. Subsequently, the mice were killed and the liver was excised for measurement of hepatic lipid contents and gene expressions.