1. Introduction

The ATP-sensitive potassium (K<sub>ATP</sub>) channel in pancreatic β-cells is a validated anti-diabetic drug target to stimulate insulin secretion. We recently identified a novel thiosemicarbazide compound by in silico screening in combination with phenotypic screening followed by chemical modifications. The compound possesses a high stimulatory effect on insulin secretion both in vitro and in vivo through inhibition of the K<sub>ATP</sub> channels. The compound significantly suppressed a rise in blood glucose levels after oral glucose load in wild-type mice in a dose-dependent manner. In this report, we analyzed the pharmacokinetic profiles including dynamics of plasma concentrations of compound and after its oral administration using a conventional LC/MS/MS.

2. Methods and Materials

In silico-similarity search. Similarly searching using two-dimensional structural fingerprints (TSGFOP) was applied. 2D structural modifications of active drugs were used as a query, and 38 compounds were retrieved by similarity search. This list was further screened by commercially available databases.

In vitro experiments. C268-Na was washed twice and preincubated for 30 min in KRBH containing 0.1% BSA with 2.8 mmol/L glucose. After preincubation, the cells were incubated for 30 min with KRBH containing each stimulus. Incubation or perfusion buffer was measured by insulin assay (CIS Bio International).

In vivo experiments. Male C57BL/6J mice of 16–22 weeks of age were fasted for 16 hours and given C268-Na at 20 mg/kg prior to glucose loading at 1.5 g/kg body weight. Blood glucose levels were measured by AmiScan Bioglyc analyzer (Bayer Yotsuba); ELISA system was used for measurement of serum insulin (Merck). A glucose tolerance test (OGTT) was performed after 60 min. The orally administered compound was measured using external standard spiked into rat plasma (Fig. 5). Maximal concentrations were measured. **P < 0.01 (Student unpaired t-test).

3. Results

3-1. novel insulin secretagogues

We recently identified a novel thiosemicarbazide compound by in silico screening in combination with phenotypic screening followed by chemical modifications. Based on the information of the structure-activity relationship (SAR), we synthesized and obtained a novel compound referred to as C268. We also synthesized the sodium salt form of C268 (referred to as C268-Na).

3-2. Inhibition of the i-β<sub>2</sub>/K<sub>ATP</sub> channels by C268

As shown in the displacement curve, 300 μM and 1 mM of C268 inhibited interaction between 3H-labeled glibenclamide and human SUR1, indicating that C268 and glibenclamide share the same binding site in SUR1. Electrophysiological experiments showed that C268 inhibited the insulin-dependent outward current of the human i-β<sub>2</sub>/K<sub>ATP</sub> channel subunits, XH6.2 and SUR1 in COS-1 cells (IC50: 2.3 μM). These results suggest that C268 inhibits the K<sub>ATP</sub> channels in pancreatic β-cells by binding SUR1.


The glucose-lowering effect of C268-Na was then evaluated by oral glucose tolerance test in wild-type mice. C268 was administered orally 20 mg/kg to mice (C57ByJg mice; CMC, carboxymethyl cellulose; C268-Na dose-dependently suppressed the rises in glucose levels, compared to that in vehicle-treated mice.

4. Conclusions

Pharmacokinetic analysis using LC/MS/MS system revealed that C268 has well-defined pharmacokinetic profiles of the novel anti-diabetic compound.

The LC/MS/MS system enables us to evaluate in vivo pharmacokinetic characteristics of the novel anti-diabetic compound.