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Evaluation of the pharmacokinetics of a novel anti-diabetic agent using conventional LC/MS/MS

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1. Introduction

The ATP-sensitive potassium (K_{ATP}) 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_{ATP} 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 pharmacokinetic profiles including dynamics of plasma concentration of compound after its oral administration using a conventional LC/MS/MS.

2. Methods and Materials

***In silico* similarity search**

Similarity search using two-dimensional structural fingerprint (TGTFOP) was applied. 2D structures of sulfonylurea drugs were used as a query, and 38 compounds were retrieved by similarity searches (Daylight) from commercially available database.

Insulin secretion.

MIN6-K8 cells were washed twice and preincubated for 30 min in KRBH containing 0.1% BSA with 2.8 mM glucose. After preincubation, the cells were incubated for 30 min with KRBH containing each stimuli. Insulin released in the incubation or perfused buffer were measured by insulin assay kits (CIS Bio international).

Oral glucose tolerance test

Mice at 16-22 weeks of age were fasted for 16 hours and given C268-Na at 20 min prior to glucose loading (1.5 g/kg body weight). Blood glucose levels were measured by Antsense III glucose analyzer (Bayer Yakuhin); ELISA system was used for measurement of serum insulin (Morinaga).

[^{3}H]-glibenclamide displacement experiments

MIN6-K8 cells transfected with human SUR1 were incubated with 10 nM [^{3}H]glibenclamide and with different concentrations of C268-Na for 30 min. The percent inhibition of the interaction between ^{3}[H]-glibenclamide and SUR1 was obtained as described in a previous report with minor modifications (Sunaga et al., 2001).

Electrophysiology

The β-cell K_{ATP} channels were reconstituted in COS-1 cells transfected with human SUR1 and human Kir6.2 as previously described (Inagaki et al., 1995). Effects of C268 on the outward current induced by diazoxide (300 μM) (Sigma Chemical, St. Loius, MO) were examined with the whole-cell clamp and compared with those of glibenclamide and gliclazide (Sigma Chemical).

Quantitative analysis of plasma concentration of compounds

Hydrophilic fractions were extracted from mouse plasma by homogenization with extraction solution (Methanol/H_2O/CH_3Cl) containing internal standards (20 μM methionine sulfone) and centrifugation, filtered through 5-kD filter, and lyophilized. The dried metabolites were reconstituted with MilliQ water and then subjected to mass spectrometry. The compound and gliclazide, a sulfonylurea drug, are analyzed using a triple quadrupole mass spectrometer (LCMS-8050; Shimadzu Corporation) coupled with conventional flow liquid chromatography (Nexera UHPLC; Shimadzu Corporation). The LC separation was performed using Discovery HS F5 column (3 μm, 2.1 mm × 150 mm, Sigma-Aldrich) with binary gradient of 0.1% formic acid in water and 0.1% formic acid in acetonitrile.

UHPLC conditions (Nexera X2)
Column: Discovery HS F5 (3 μm, 2.1 mm × 150 mm, Sigma-Aldrich)
Column temperature: 40 ℃
Mobile phase A: 0.1 % formic acid / water
Mobile phase B: 0.1 % formic acid / acetonitrile
Solvent for sample loading: 0.1% formic acid / water
Flow rate: 0.25mL/min
Gradient [time (%A/%B)] : 0 min (100/0)–1.0 min (100/0)–9.0 min (5/95)–9.5 min (100/0)–10.0 min (100/0)
Injection vol.: 5 μL
MS conditions (LCMS-8050)
Ionization: ESI, Positive

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, we designed and synthesized a novel compound referred to as C268. We also synthesized the sodium-salt form of C268 (referred to as C268-Na), which has higher aqueous solubility than C268.

3-2. Inhibition of the β-cell K_{ATP} channels by C268

As shown in the displacement curve, 300 μM and 1 mM of C268-Na 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 diazoxide-induced K+ currents reconstituted from the human β-cell K_{ATP} channel subunits, Kir6.2 and SUR1, in COS-1 cells (IC50, 2.3 μM). These results suggest that C268 inhibits the K_{ATP} channels in pancreatic β-cells by binding to SUR1.

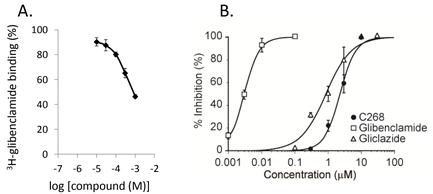

Figure 1. Effects of C268 and C268-Na on β-cell K_{ATP} channels. A: Inhibition of [^{3}H]glibenclamide binding to human SUR1 by C268-Na. [^{3}H]glibenclamide binding to human SUR1 was displaced by unlabeled C268-Na. Values are presented as mean ± SEM (n = 4). B: Concentration-response curves for the inhibitory effects of C268, gliclazide, and glibenclamide on diazoxide (300 μM)-induced potassium current at 0 mV. The IC_{50} values for the inhibitory effects of C268, gliclazide, and glibenclamide on the diazoxide-induced current were 2.3 μM, 0.86 μM and 0.003 μM, respectively. Each point represents mean ± SEM of 1-6 cells.

3-3. Glucose-lowering effect of C268 in vivo.

The glucose-lowering effect of C268-Na was then evaluated by oral glucose tolerance test in wild-type mice. Glucose was administered orally 20 min after treatment with C268-Na or vehicle (CMC, carboxymethyl cellulose). C268-Na dose-dependently suppressed the rises in glucose levels, compared to that in vehicle-treated mice.

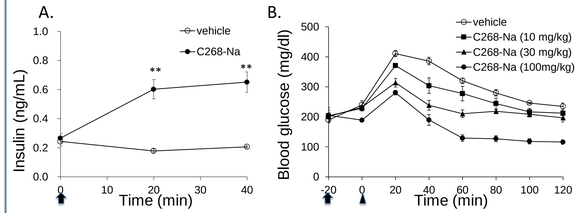

Figure 2 Effects of C268-Na in vivo. A: Insulinotropic effect of C268-Na in vivo. 30 mg/kg of C268-Na was orally administered to wild type mice and plasma insulin concentrations were measured. **P < 0.01 (Student unpaired *t* test). B Glucose-lowering effect of C268-Na in OGTT. Changes in blood glucose levels after oral glucose load following administration of C268-Na. Data are expressed as mean ± SEM (n = 4 for each group). Arrow and arrowhead indicate the administration of compound and glucose, respectively.

3-4. Quantitative analysis of plasma concentrations of compound and gliclazide.

To analyze pharmacokinetic property of C268-Na, plasma concentrations of the compound after oral administration were measured using a conventional LC/MS/MS. Mice at 16-28 weeks of age were administered orally 30 mg/kg of the compound, then whole blood samples were collected from the tail vein. The absolute concentration was calculated from the calibration curve using external standard spiked into rat plasma (Fig. 5). Maximal concentration of the compound was 196.1 μM at 30 min after administration, which in turn was rapidly decreased at 60 min. On the other hand, plasma concentration of gliclazide, a commonly used anti-diabetic sulfonylurea drug, reached a maximal concentration at 30 min and remained almost unchanged at 60 min. The compound may have a suitable pharmacokinetic profile for the treatment of post-prandial hyperglycemia in patients with type 2 diabetes.

Compound	Type	Transition	CE
C268-Na	Quantifier	370.3 > 168.1	-21
	Reference	370.3 > 185.1	-15
Gliclazide	Quantifier	324.2 > 110.2	-22
	Reference	324.2 > 127.2	-21
	Reference	324.2 > 153.3	-23

Table 1. MRM transitions for anti-diabetic agents

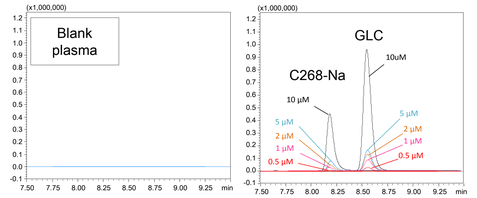

Figure 3. MRM chromatogram of antidiabetic agents spiked in plasma

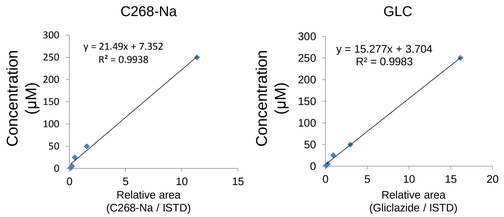

Figure 4. Representative calibration curve of C268-Na and gliclazide.

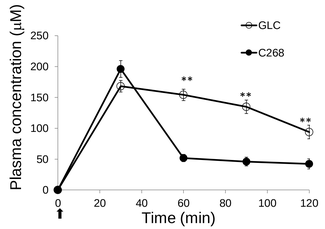

Figure 5. Plasma concentrations of C268-Na and gliclazide (GLC). 30 mg/kg of C268-Na was orally administered to wild type mice and plasma concentration of compounds were measured.Data are expressed as mean ± SEM (n = 4 for each group). Arrow indicates the oral administration of compound. **P < 0.01 (Student unpaired *t* test).

4. Conclusions

Pharmacokinetic analysis using LC/MS/MS system revealed that C268 had shorter half-life than gliclazide.

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