Special Review Article

Inhibitory Effect of Oxovanadium(IV), Copper(II), and Zinc(II) Ions on the Activity of an Alpha-glucosidase from a *Saccharomyces sp.*

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Abstract

We investigated the *in vitro* and *in vivo* effects of metal ions on the activity of an alpha-glucosidase (*Saccharomyces sp.*). $CuSO_4$, $ZnSO_4$, and $VOSO_4$ significantly inhibited the alpha-glucosidase activity *in vitro*. Additionally, we examined their effects on the blood glucose level by performing oral carbohydrate tolerance tests in both normal ddY mice and streptozotocin-induced diabetic mice. After oral administration of these three metal compounds, the elevation in the blood glucose levels in mice administered disaccharide (sucrose) was significantly suppressed in comparison with untreated mice. On the other hand, the elevation in the blood glucose levels in mice administered monosaccharide (glucose) was not suppressed when such compounds or a vehicle was administered. The results suggested that some metal ions suppress disacharide digestion probably due to the inhibition of the alpha-glucosidase activity in the epithelium of the small intestine.

Keywords : Alpha-glucosidase inhibition, Anti-diabetic effect, STZ-mice, Oral carbohydrate tolerance test, Metal ions

Introduction

Type 2 insulin-resistant diabetes mellitus (DM) accounts for 95% of all DM cases. The worldwide frequency is estimated to continue increasing by 6% each year and has the potential to reach a total of 200-300 million cases by 2010 [1, 2]. The main reason for the increasing incidence of DM is the staggering increase in the rate of obesity－the most important contributor to DM pathogenesis [3]. It is now clear that intensive control of hyperglycemia in patients with type 2 DM can attenuate the development of chronic complications such as retinopathy and nephropathy [4, 5]. At present, therapy for type 2 DM mainly relies on several approaches intended to reduce hyperglycemia. For example, the therapy may include the use of medicines such as sulfonylureas, which increase insulin release from pancreatic islets ; metformin, which reduces hepatic glucose production ; thiazolidinediones, which enhance insulin action ; and alpha-glucosidase inhibitors, which interfere with

Table 1 Anti-diabetic medicines and their mechanisms of action

Classification	Mechanism of action
Sulfonylureas	Promote insulin secretion in the pancreas
Phenylalanine derivatives	
Biguanides	Depress gluconeogenesis in the liver and improve insulin sensitivity in the peripheral tissues
Thiazolidine derivatives	Improve insulin resistance
Alpha-glucosidase inhibitors	Suppress the degradation of carbohydrates and delay the absorption of monosaccharides from the gastrointestinal tract

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Received : 31 August 2006
Accepted 1 November 2006

glucose absorption in the small intestine (Table 1) [6−9]. These therapies have limited efficacy and tolerability and have significant mechanism-based side effects. Thus, novel approaches to treat type 2 DM are urgently required. Since 1962, the antidiabetic effects of metal ions have been reported by many researchers [10−19]. Although the inhibition of enzymes by metal ions has been studied extensively [20, 21], there are few studies on the relationship between metals and alpha-glucosidase [22−25]. Therefore, we examined the effect of some metal ions on an alpha-glucosidase from a *Saccharomyces sp.* ; this is a key enzyme in the small intestine.

Methods

Materials

$ZnSO_4 \cdot 7H_2O$ (Zn(II)), $CuSO_4 \cdot 5H_2O$ (Cu(II)), $VOSO_4 \cdot nH_2O$ (VO(II)), $MnSO_4 \cdot 5H_2O$, $FeSO_4 \cdot 7H_2O$, $CoSO_4 \cdot 7H_2O$, $NiSO_4 \cdot 6H_2O$, sucrose, acarbose, and alpha-glucosidase (from a *Saccharomyces sp.*) were purchased from Wako Pure Chemical Industries (Osaka, Japan). The glucose concentration was determined by a Glucose CII test Wako kit. The purity of $VOSO_4 \cdot 1.6H_2O$ was determined by chelatometry using the Cu-1-(2-pyridyl-azo-2-naphthol) complex (Dojindo, Kumamoto, Japan).

Inhibitory effects of metal ions on alpha-glucosidase activity

Solutions of metal ions at various concentrations were prepared, and their alpha-glucosidase inhibitory effects were evaluated by a modified Dahlqvist method [26]. In brief, 0.1 ml of the test compounds in 0.15 M HEPES buffer (pH 6.8) and 0.1 ml of 5 units/ml alpha-glucosidase in 0.015 M HEPES buffer were added to 0.1 ml of the substrate (0.1 M sucrose in 0.15 M HEPES buffer), and then incubated at 37ºC for 60 min. After incubation, the reactions were stopped in a dry bath incubator, and the glucose concentration was determined by a Glucose CII-test Wako kit. Acarbose, which is one of the alpha-glucosidase inhibitor, was used as the positive control. The inhibition of alpha-glucosidase activity was calculated by using the following equation :

$$\text{Inhibition of alpha-glucosidase activity (\%)} = [(Ac-As)/Ac] \times 100$$

where Ac is the glucose concentration in the control solution (alpha-glucosidase and substrate) and As is the glucose concentration in the test solutions (alpha-glucosidase, substrate, and test compounds). The IC_{50} value, the concentration of the test compounds at which 50% of the enzyme activity is inhibited, was determined graphically from a plot of percent inhibition *vs.* concentration of the test compounds.

Inhibitory effects of metal ions on the increase in blood glucose levels in oral carbohydrate-loaded normal mice

Six-week-old male ddY mice (Shimizu Experimental Material Co., Kyoto, Japan) were kept under a light schedule of 8 : 00 a.m.-8 : 00 p.m. at 23±1℃. The mice were allowed access to drinking water and a laboratory diet (MF, Oriental Yeast Co., Tokyo, Japan). After overnight fasting at seven weeks of age, the test compounds (5 and 15 mg metal/kg body weight) were dissolved in saline and then orally administered to the mice. After 30 min, a saline solution of glucose (2.0 g/kg body weight) or sucrose (2.0 g/kg body weight) was orally administered. Blood samples were obtained from the lateral tail vein at various time points for 0-120 min. The blood glucose levels were measured by a glucose oxidase method (Glucocard, Arkray, Kyoto, Japan).

Inhibitory effects of metal ions on the increase in blood glucose levels in oral carbohydrate-loaded streptozotocin (STZ)-induced diabetic mice

STZ dissolved in cold 0.1 M sodium citrate buffer at pH 5 was used within 3 min of preparation. Six-week-old male ddY mice, which were starved for 6 h, received intraperitoneal injections of STZ (100 mg/kg body weight) twice a week. Seven days after the second STZ administration, the non-fasting blood samples were obtained, and the blood glucose levels of these were measured. Individual mice with a blood glucose level greater than 300 mg/dL were used for the oral carbohydrate tolerance test. STZ-induced diabetic mice were again starved for 12 h, and the test compounds were then orally administered at 15 mg metal/kg body weight. After 30 min, a saline solution of glucose (1.0 g/kg body weight) or sucrose (1.0 g/kg body weight) was orally administered. The blood glucose levels were measured as described from the blood samples obtained from the lateral tail vein at various time points from 0-180 min.

The animal study was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University (KPU) and was performed according to the Guidelines for Animal Experimentation of KPU.

Statistical analysis

The results of the *in vitro* experiments are expressed

as the mean ± standard deviations (s.d.) for three repeated runs. The oral carbohydrate tolerance level of normal mice and STZ-induced diabetic mice are expressed as the mean±s.d. for five animals. Statistical analysis was performed using a Student's *t*-test with the significance level of the difference at 5% ($p < 0.05$) or 1% ($p < 0.01$).

Results and Discussion

Inhibitory effects of metal ions on alpha-glucosidase activity

The inhibitory effects of acarbose (AC : positive control) and metal ions on alpha-glucosidase activity were concentration dependent. From the results, the apparent IC_{50} values of the test compounds with respect to the alpha-glucosidase activity were estimated (Fig. 1). In

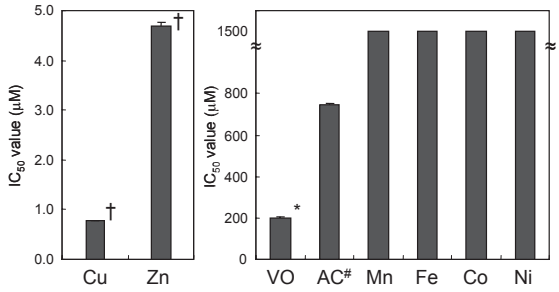

Fig. 1 The estimated IC_{50} values of acarbose and various metal ions. #AC : acarbose. Significance : †$p < 0.01$, *$p < 0.05$ *vs.* acarbose

comparison with AC, Cu(II), Zn(II), and VO(II) showed strong inhibition of alpha-glucosidase activity. It has been reported that cysteine is located in the active site of alpha-glucosidase [27]. The stability constant (log K_1) of the Cu-His complex (28.0) is higher than that of the Cu-Cys complex (16.0) [28]. Therefore, Cu(II) does not appear to bind to Cys in alpha-glucosidase. On the other hand, the log K_1 values of the Zn-His and Zn-Cys complexes are 12.0 and 18.2, respectively [28]. The Zn(II) ion may have a greater effect on Cys of the active site than on His in this enzyme. Further investigations are required to understand the mechanism of the interaction between metals and the enzyme.

Inhibitory effects of metal ions on the blood glucose levels in carbohydrate-loaded mice

We examined the effects of Cu(II), Zn(II), and VO(II) on the blood glucose levels in mice. The elevation in the blood glucose levels in normal ddY mice that were administered disaccharide (sucrose) was significantly suppressed after a single oral administration of Cu(II), Zn(II), and VO(II) in a concentration dependent manner as compared with the control mice that were administered vehicle (Fig. 2). On the other hand, no changes were observed in the blood glucose level in mice administered monosaccharide (glucose) after Cu(II), Zn(II), VO(II), or vehicle was administered (Table 2).

The effects of Cu(II), Zn(II), and VO(II) on the postprandial blood glucose level was also examined in the STZ-induced diabetic mice. The elevation in the blood glucose levels in diabetic mice that were administered sucrose was significantly suppressed after a single oral administration of Cu(II), Zn(II), and VO(II) at 15 mg metal/kg body weight as compared with the control mice that were administered vehicle (Fig. 3 a). In the case of glucose administration, the three ions did not show any significant effect (Fig. 3 b).

These results suggested that Cu(II), Zn(II), and VO(II) inhibited the alpha-glucosidase action in the epithelium of the small intestine and reduced disaccharide digestion.

Conclusion

We observed the *in vitro* and *in vivo* inhibitory effects of insulinomimetic metal ions on alpha-glucosidase ac-

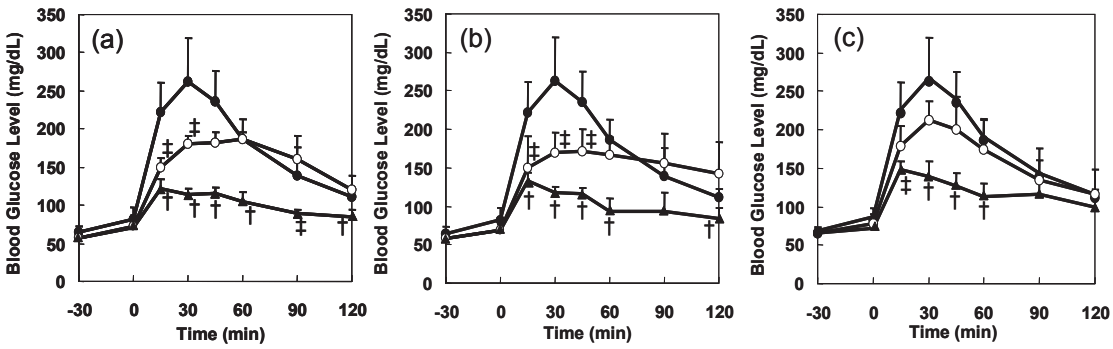

Fig. 2 Effects of $CuSO_4$ (a), $ZnSO_4$ (b), $VOSO_4$ (c), and vehicle (control) on the postprandial blood glucose level in disaccharide (sucrose)-loaded normal ddY mice. 0 mg (control : ●), 5 mg (○), and 15 mg (▲) metal /kg body weight. Significance : †$p < 0.01$, ‡$p < 0.05$ *vs.* control

Table 2 Effects of $CuSO_4$, $ZnSO_4$, $VOSO_4$, and vehicle on the postprandial blood glucose level (mg/dL) in monosaccharide (glucose)-loaded normal ddY mice
Data are expressed as the mean±s.d. for five animals.

Compound	Time (min)						
	-30	0	15	30	60	90	120
Vehicle	83 ± 7	102 ± 5	261 ± 24	272 ± 36	192 ± 35	137 ± 38	97 ± 20
$CuSO_4$	91 ± 27	106 ± 29	282 ± 28	257 ± 54	180 ± 40	139 ± 28	96 ± 23
$ZnSO_4$	81 ± 10	107 ± 20	266 ± 52	243 ± 49	172 ± 27	119 ± 20	98 ± 19
$VOSO_4$	77 ± 17	95 ± 20	274 ± 47	254 ± 48	189 ± 48	109 ± 16	90 ± 10

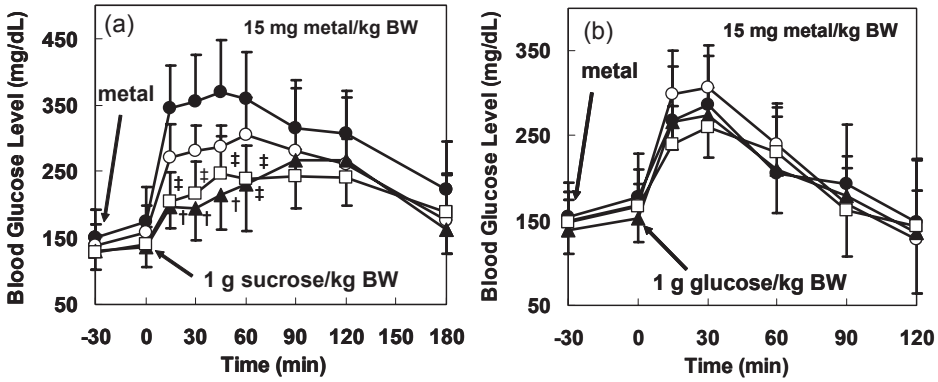

Fig. 3 Effects of $CuSO_4$ (□), $ZnSO_4$ (▲), $VOSO_4$ (○), and the vehicle (control : ●) on the postprandial blood glucose level in disaccharide (sucrose)-loaded (a) and monosaccharide (glucose)-loaded (b) STZ-induced diabetic mice. Significance : $^{\dagger}p < 0.01$, $^{\ddagger}p < 0.05$ *vs.* control

tivity. In *in vitro* enzyme experiments, Cu(II), Zn(II), and VO(II) strongly inhibited alpha-glucosidase activity, and their effects were approximately 1000, 200, and 4 times, respectively, higher than that of acarbose. Further, we evaluated the change in the blood glucose levels in normal and STZ-induced diabetic mice. Cu(II), Zn(II), and VO(II) suppressed the elevation in the blood glucose levels in normal and diabetic mice when they were administered disaccharide (sucrose). These results will be useful when they are clinically applied to DM therapy in the future.

Acknowledgements

This study was partly supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of the Japanese Government (Grant-in-Aid for Scientific Research, Scientific Research on Priority Areas, and Specially Promoted Research to H.S.) Furthermore, it was partially supported by a Grant-in-Aid for Young Scientists (B) from the Ministry of Education, Culture, Sports, Science and Technology to Y. Y.

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