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Novel α-Glucosidase Inhibitors, CKD-711 and CKD-711a Produced by *Streptomyces* sp. CK-4416

II. Biological Properties

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CKD-711 and CKD-711a are aminooligosaccharide α-glucosidase inhibitors discovered during the bioactive material screening for antibacterial agent. Their inhibitory activities were studied and compared with those of acarbose *in vitro* and *in vivo* with animals. In *in vitro* study, CKD-711 showed similar effects to acarbose on porcine intestinal maltase and sucrase, IC_{50}s of 2.5 and 0.5 μg/ml, respectively, whereas it had about 2 fold lower α-amylase inhibitory activity (IC_{50}, 78.0 μg/ml) than acarbose (IC_{50}, 36 μg/ml). CKD-711a showed less inhibitory activity than CKD-711 against all the enzymes tested. In rat fed on starch and sucrose meals, the dose of CKD-711 which reduced the postprandial blood glucose increment by 50 percent in comparison to control rats (ED_{50}) were 3.07 and 1.15 mg/kg, respectively, and acarbose had ED_{50}s of 1.94 and 1.15 mg/kg, respectively. CKD-711 and CKD-711a also showed antibacterial activity against *Comamonas terrigena*.

α-Glucosidases are the enzymes responsible for hydrolysis of carbohydrates into glucose to be absorbed in the intestine[1]. However, the absorbed glucose causes hyperglycemia in diabetic patients[2]. Acarbose (BAYg 5421)[3,4], an aminooligosaccharide which was isolated from the fermentation broth of *Actinoplanes* sp. inhibits *in vivo* brush-border enzymes of human[5]. Thus, the inhibition of carbohydrate-digestive enzymes by acarbose resulted in a significant decrease in the postprandial rise of blood glucose level after a mixed carbohydrate diet[6]. Over the past 20 years, inhibitors of intestinal brush-border α-glucosidases showing a broad spectrum of activity have been described and are partially in clinical use. These inhibitors include miglitol[7], voglibose[8], isoacarbose[9], diketopiperazine[10] and genistein[11]. Among these α-glucosidase inhibitors, acarbose remains the most thoroughly investigated. Indeed, acarbose intended mode of action is to delay carbohydrate digestion and absorption without prompting the overspill of nutrients into the colon[12]. However, the major adverse effects of acarbose are abdominal distention, flatulence, meteorism and possibly diarrhea[13]. It has been suggested that such adverse effects might be caused by the excessive inhibition of pancreatic α-amylase and the delay of carbohydrate digestion. Undigested carbohydrates and solid meals will enter the colon and, as a consequence of bacterial fermentation, give rise to side-effects[14,15].

In our continuing search for bioactive microbial metabolites, we found new α-glucosidase inhibitors, CKD-711 and CKD-711a produced by *Streptomyces* sp. CK-4416[16]. In this paper, we describe the biological properties of CKD-711 compared to those of acarbose.

Materials and Methods

Inhibitory Effects of the Enzymes

Porcine pancreatic α-amylase was purchased from Sigma

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Chemical Co. and α-glucosidases (maltase and sucrase) were partially purified from porcine small intestinal mucosa. The enzymes were prepared as follows[17]. Porcine small intestine was washed with cold phosphate-buffered saline. The brush-border was dissected and weighted (2.2 g). Tissue was homogenized in 0.2 M phosphate buffer (10 ml) at 4°C, and then centrifuged at 12,000 rpm for 20 minutes. The protein content was determined by Lowry's method[18]. The supernatant (50 μl) was incubated with 0.1 M sucrose (500 μl) or 0.05 M maltose (500 μl) at 37°C for 30 minutes. Sucrase and maltase activities were calculated by glucose concentration converted from sucrose or maltose, and were indicated as μmol/mg protein/hour[19]. The inhibitory effects of CKD-711 and CKD-711a were determined by incubating a solution of an enzyme (50 μl), phosphate buffer (pH 7.0, 500 μl) containing 0.4 mg/ml sucrose or maltose, or 1% soluble starch, and a solution (50 μl) with various concentrations of CKD-711 and CKD-711a at 37°C for 30 minutes. The reaction mixture was heated in a boiling water bath to stop the reaction for 2 minutes, and then the amount of liberated glucose was measured by the glucose oxidase method[17]. The inhibitory activity was calculated from the formula as follows. Inhibition (%)=(C−T)/C×100, where C is the enzyme activity without inhibitor and T is the enzyme activity with inhibitor.

Effect on Hyperglycemia

Effect on hyperglycemia induced by carbohydrate loads in Wistar rat was determined by the inhibitory action of CKD-711, CKD-711a and acarbose on postprandial hyperglycemia. After 11 groups of 7 male Wistar rats (180~200 g) were fasted for 20 hours, 1.5 g/kg of starch and 2.0 g/kg of sucrose were orally administered concurrently with 0~40 mg/kg of inhibitors (CKD-711, CKD-711a and acarbose). The blood samples were then taken from the tail vein after 30 minutes from administration. The glucose level in blood was determined by the glucose oxidase method[17] and compared with that of the control group, which has not taken the inhibitors.

Antimicrobial Activity Assay

Antimicrobial activities of CKD-711 and CKD-711a were determined against fungi, yeast and Gram-positive and negative bacteria by agar diffusion method using paper discs (8 mm diameter). Fifty micrograms of CKD-711 and CKD-711a were applied onto each paper disc. Inhibition zones were measured after incubation at 28°C and 48 hours for fungi, 37°C and 24 hours for bacteria, and 28°C and 24 hours for yeast.

Results

Characterization of Enzyme Inhibitory Activity

CKD-711 and CKD-711a were tested *in vitro* against intestinal α-glucosidase and pancreatic α-amylase of porcine origin (Table 1). As a result, the inhibition effect of CKD-711 was comparable to that of acarbose on porcine intestinal maltase and sucrase with IC_{50}s of 2.5 and 0.5 μg/ml, respectively, but it had about 2-fold less α-amylase inhibitory activity (IC_{50}, 78.0 μg/ml) than acarbose (IC_{50}, 36 μg/ml). CKD-711a, which has two more glucose units than CKD-711 showed less inhibitory activity than CKD-711 against all tested enzymes[20].

Effect on Hyperglycemia

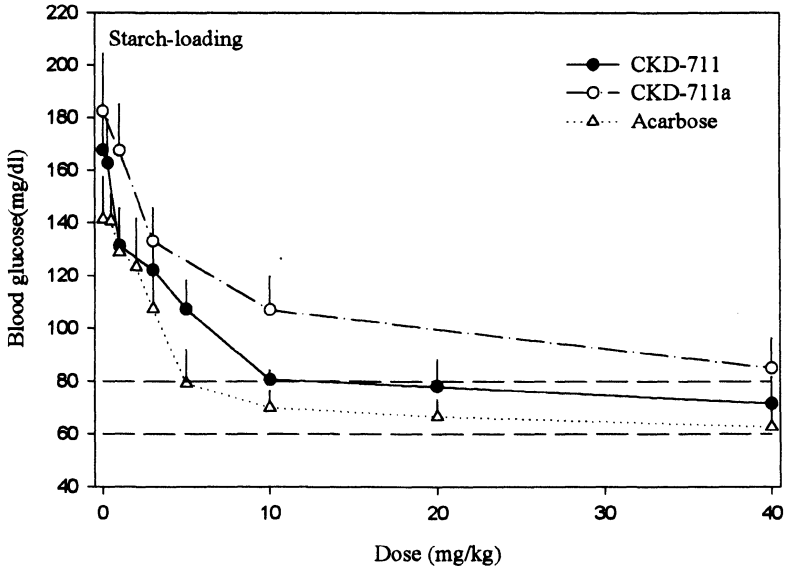
The results were illustrated in Figs. 1, 2 and Table 2. When cooked starch or sucrose was given with CKD-711 or CKD-711a, the hyperglycemia was significantly diminished with the respective ED_{50} of 3.1 and 1.1 mg/kg for CKD-711 and 4.3 and 1.6 mg/kg for CKD-711a. CKD-711 showed less potent inhibition in the starch-loading test than acarbose (ED_{50}, 1.9 mg/kg), whereas it had same effect as acarbose on the sucrose-loading test (ED_{50}, 1.1 mg/kg).

Indeed, acarbose intended mode of action is to delay carbohydrate digestion and absorption without prompting

Table 1. Inhibitory activity of CKD-711, CKD-711a and acarbose against porcine intestinal enzymes *in vitro* (IC_{50}: μg/ml).

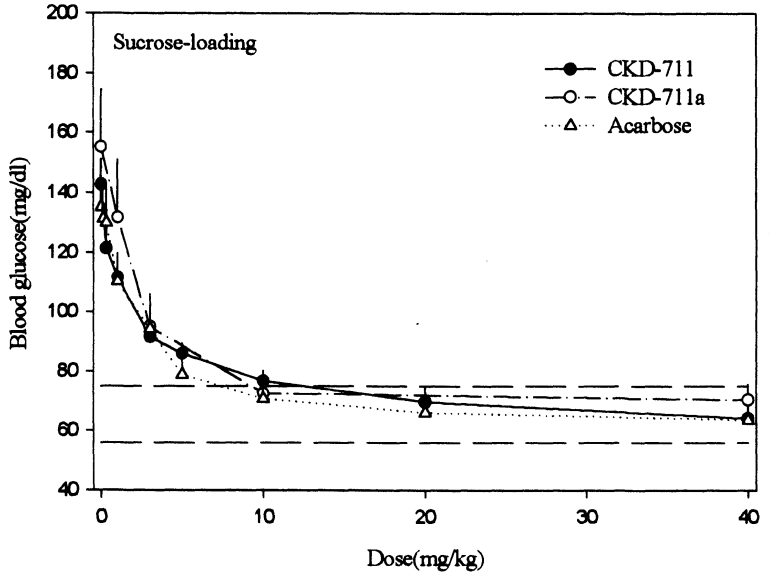
Enzymes	CKD-711	CKD-711a	Acarbose
α-amylase	78.0	104.0	36.0
maltase	2.5	6.5	2.5
sucrase	0.5	1.5	0.6

Fig. 1. Effect of CKD-711, CKD-711a and acarbose on starch tolerance test.



After fasted for 20 hours, 6-week-old, male Wister rats were orally administered with starch solution (1.5 g/5 ml/kg) with or without inhibitors (0~40 mg/kg). Each point represents Mean±S.D. (n=5). Significantly different from control (* $p<0.05$, ** $p<0.001$).

Fig. 2. Effect of CKD-711, CKD-711a and acarbose on sucrose tolerance test.



After fasted for 20 hours, 6-week-old, male Wister rats were orally administered with sucrose solution (2.0 g/5 ml/kg) with or without inhibitors (0~40 mg/kg). Each point represents Mean±S.D. (n=5). Significantly different from control (* $p<0.05$, ** $p<0.001$).

Table 2. Inhibition of blood glucose increment in Wistar rats ingestion with each carbohydrate (ED_{50}: mg/kg).

Loaded carbohydrates	CKD-711	CKD-711a	Acarbose
Starch	3.1	4.0	1.9
Sucrose	1.1	1.6	1.1

Table 3. Antimicrobial spectrum of CKD-711, CKD-711a and acarbose.

Organisms	Inhibitory diameter (paper disc, mm)*		
	CKD-711	CKD-711a	Acarbose
E. coli NIHJ No.34	0	0	0
Comamonas terrigena ATCC 8461	23.4	25.4	0
Bacillus subtilis ATCC 6633	0	0	0
Sarcina lutea ATCC 9341	0	0	0
Alcaligenes faecalis ATCC 8750	0	0	0
Aspergillus niger ATCC 9642	0	0	0
Candida albicans ATCC 7491	0	0	0
Candida utilis ATCC 9950	0	0	0

* 50 μg/ml of Samples

the overspill of nutrients into the colon. However, the major adverse effects of acarbose are abdominal distention, flatulence, meteorism and possibly diarrhea. It has been suggested that such adverse effects might be caused by the excessive inhibition of pancreatic α-amylase and the delay of carbohydrate digestion. Undigested carbohydrates and solid meals will enter the colon and, as a consequence of bacterial fermentation, give rise to side-effects.

Consequently, CKD-711, which showed lower pancreatic α-amylase inhibition than acarbose in *in vitro* and *in vivo* studies, is expected to improve the glycemic response in diabetes mellitus with less major adverse effects than acarbose.

Antimicrobial Activity

As shown in Table 3, CKD-711 and CKD-711a showed selective antibacterial activity against *Comamonas terrigena*, but no antimicrobial activity against fungi, yeast and Gram-positive bacteria.

Discussion

From the results of *in vitro* and *in vivo* tests, CKD-711 may have an advantage over acarbose, if abdominal side effects are caused by the excessive inhibition of pancreatic α-amylase, which results in the abnormal bacterial fermentation of undigested carbohydrates in the colon.

Furthermore, we discovered that CKD-711 and CKD-711a, in contrast to acarbose, showed the selective antibacterial activity against *Comamonas terrigena* among tested microorganisms. More detail investigation to characterize the precise antibacterial activity against *C. terrigena* is planned.

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