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Carboxymethylcellulose sodium improves the pharmacodynamics of 1-deoxynojirimycin by changing its absorption characteristics and pharmacokinetics in rats

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1-Deoxynojirimycin (DNJ) has excellent inhibitory activity against α-glucosidase and can therefore decrease the postprandial blood glucose level in humans. However, a major limitation of DNJ is its fast absorption rate compared with other α-glucosidase inhibitors. In this study, we investigated the effect of adjuvants on the pharmacokinetics of DNJ, and found that carboxymethylcellulose sodium (CMCNa) can remarkably improve the activity of DNJ on glucose levels. When DNJ was used in combination with CMCNa, its absorption was suppressed and delayed by CMCNa. CMCNa can also change the pharmacokinetics of DNJ in rats. Pharmacodynamics were further studied using the oral glucose tolerance test, and the results confirmed that CMCNa can enhance DNJ's modulation of glucose level. All the results indicate that carboxymethylcellulose sodium can improve the pharmacodynamics of 1-deoxynojirimycin by changing the absorption characteristics and pharmacokinetics of DNJ in rats.

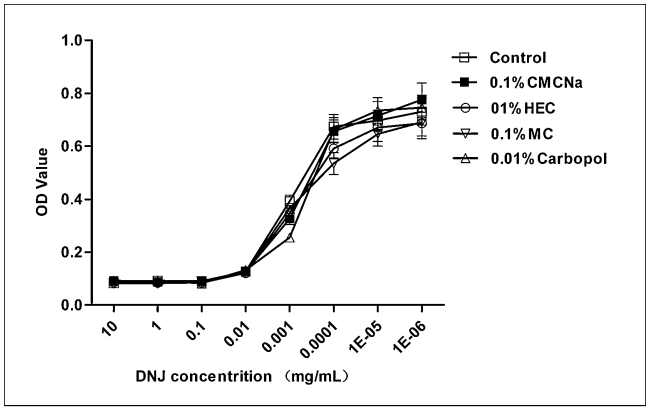
1. Introduction

1-Deoxynojirimycin (DNJ) is a D-glucose analogue with an NH group substituting the oxygen atom from the pyranose ring (Newbrun et al. 1983). DNJ was initially isolated from nojirimycin via catalytic hydrogenation by Inoue et al. (1968). DNJ was also isolated from the root bark of a *Morus* species by Yagi et al. (1976), and it exists in abundance in the leaves of *Morus* species and larvae of *Bombyx mori* (silkworm). DNJ is a characteristic constituent of mulberry (Moraceae) leaves, which is one of its best characterized bioactive components. DNJ has multiple biological activities including strong inhibitory activity to glucosidase and glucoamylase (Bembi and Deegan 2008; Kuriyama et al. 2008; Newbrun et al. 1983; Yatsunami et al. 2008), antiviral activity (Chang et al. 2009; Tanaka et al. 2006) and it can improve insulin sensitivity (Kong et al. 2008; Monte et al. 2010).

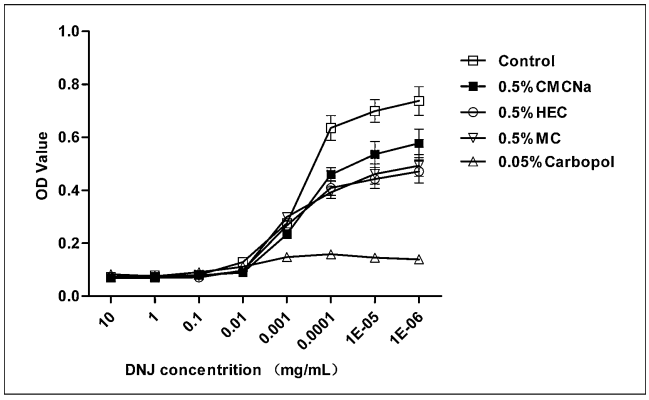
Controlling the blood glucose level is an effective way to prevent hyperglycemia and exacerbation of diabetes (Ramachandran and Snehalatha 2011; Takiya and Chawla 2002). DNJ can bind to the active center of α-glycosidase and subsequently reduces glucose metabolism and inhibit postprandial hyperglycemia, therefore, the anti-diabetic effect of DNJ was widely studied (Johnston et al. 1998; Konno et al. 2006). Kimura et al. (2007) demonstrated that food grade mulberry powder suppressed the elevation of postprandial blood glucose in humans. Miyahara et al. (2004) reported the inhibitory effects of an aqueous ethanol extract of mulberry leaves on postprandial hyperglycemia in normal Wistar rats. All these studies suggested an anti-diabetic potential of DNJ isolated from the mulberry leaves.

The absorption rate of DNJ is critical for its anti-diabetic effect. Available information on the absorption and metabolism of DNJ is still preliminary. Nakagawa et al. (2007) studied the plasma levels of DNJ after oral administration. They found that mulberry derived DNJ was rapidly absorbed (T_{max} = 30 min) in its intact form from the intestinal tract and then rapidly excreted from the body. Kim et al. (2010) compared the absorption of DNJ from mulberry extract with purified DNJ in rats. They suggested that a peak serum level is achieved 30 min after single oral administration followed by a rapid decline. These studies indicated that DNJ is rapidly absorbed. However, the absorption rates of two commercial α-glucosidase inhibitors acarbose (T_{max} = 1.27 h) (Ahr et al. 1989) and miglitol (T_{max} = 2.5 h) (Nirogi et al. 2006), were both lower than that of DNJ. This suggests that the biological effect of DNJ may not persist as long as that of acarbose and miglitol, and can be improved by changing its absorption kinetics. Currently, there has been no report on improving the inhibitory activity and pharmacodynamics of DNJ with adjuvants, and the relationship between the pharmacodynamics and the pharmacokinetics of DNJ.

In this study, we evaluated the effects of various concentrations of different adjuvants on the DNJ inhibition of α-glucosidase enzymatic activities, and the everted intestinal sac method was used to determine the effect on the hydrolysis of starch and the transport of glucose when DNJ was used in combination with adjuvants. In addition, the absorption characteristics of DNJ in small intestine and its pharmacokinetics in rats with or without adjuvant were investigated to characterize the interaction between DNJ and adjuvant. Lastly, the pharmaco-

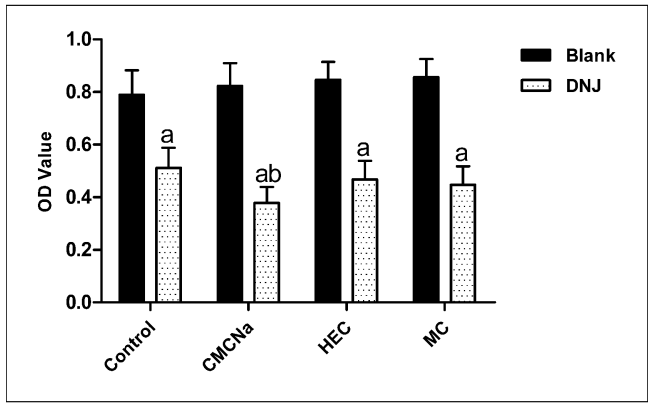


(A)

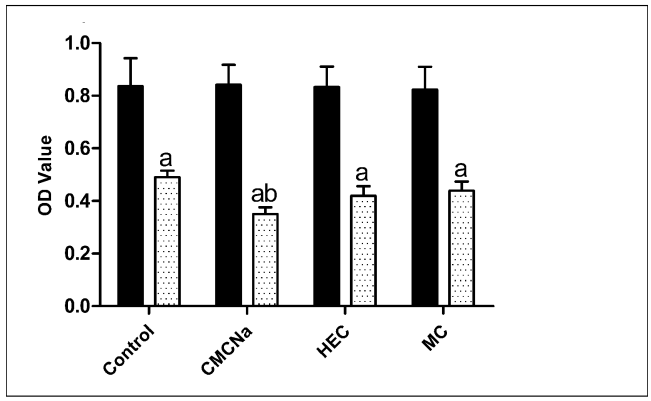


(B)

Fig. 1: Effect of different adjuvants on inhibitory activities of DNJ at low concentrations (A) and high concentrations (B). Data represent means ± SD for triplicate samples



(A)



(B)

Fig. 2: Effects of DNJ added with different adjuvants on the hydrolysis of starch (A) and transport of glucose (B). Values are means ± SD, $n = 5$ a represents significant difference ($P < 0.05$) compared with blank group, b indicates significant difference ($P < 0.05$) compared with control group

dynamics hypoglycemic activity of DNJ adjuvant combination was validated by the oral glucose tolerance test *in vivo* and the relationship between the pharmacodynamics and the pharmacokinetics of DNJ was discussed.

2. Investigations and results

2.1. Inhibition of α-glucosidase by DNJ/adjuvant combination

The effects of adjuvants on DNJ inhibition of α-glucosidase were evaluated by varying adjuvants concentrations (0.1% and 0.5% for CMCNa, HEC, MC, 0.01% and 0.1% for Carbopol). The inhibition profiles of adjuvant were shown in Fig. 1. The OD values indicated the production of glucose in the enzymatic reaction. More glucose production reflected a lower inhibitory activity. As shown, the inhibition profiles of all of the four adjuvants at low concentrations suggested that none of them affects DNJ inhibitory activity towards α-glucosidase at low concentrations. However, the adjuvants obviously decreased the production of glucose at higher concentrations, suggesting that they enhanced the inhibitory activity of DNJ. As Carbopol completely inhibited the activity of α-glucosidase, it was therefore not chosen for the subsequent tests, while CMCNa, HEC, MC at the high concentration (0.5%) remained included in the subsequent studies.

2.2. The inhibition of glucose production and transport by DNJ and adjuvants

To confirm that the adjuvants (CMCNa, HEC, MC) can enhance the DNJ inhibitory activity at organ level, an everted intestinal sac model was used. The solution outside the everted sac reflected the reaction inside the intestine while the solution inside the sac indicated the transport of glucose to blood. This model can indicate not only the inhibitory capacity of α-glucosidase hydrolysis, but also the inhibitory potency of the transport characteristics of glucose by DNJ combined with adjuvants. The inhibitory activities of DNJ combined with adjuvants are shown in Fig. 2. The OD values indicate the amount of glucose in the everted sac. More glucose reflects lower inhibitory activity and less transport. As shown by the OD values of the solution outside the sac, CMCNa, HEC, or MC alone had no influence on the glucose amount as compared with the blank. Adding CMCNa to DNJ significantly decreased the glucose amount and showed significant inhibitory potency on the hydrolysis of starch. The OD values of the solution inside the sac suggested that, CMCNa, HEC, or MC alone also had no influence on glucose amount while adding CMCNa to DNJ decreased glucose amount and showed significant inhibitory potency on glucose transport. These results indicated that CMCNa notably enhanced the inhibitory potency of DNJ on the hydrolysis of starch and the transport of glucose.

2.3. Quantification of DNJ

Due to the high polarity of DNJ, a hydrophilic interaction liquid chromatographic column (HILIC) was used to quantify DNJ. DNJ and miglitol (IS) can be separated by the HILIC column (Fig. 3A). The retention time of DNJ and miglitol are 6.921 min and 3.627 min, respectively. As the electrospray ion source (ESI) and the positive mode were selected for the quantification of DNJ, the analytes produced strong $[M+H]^+$ signals at m/z 164.2 for DNJ and at m/z 208.2 for miglitol (IS). The frag-

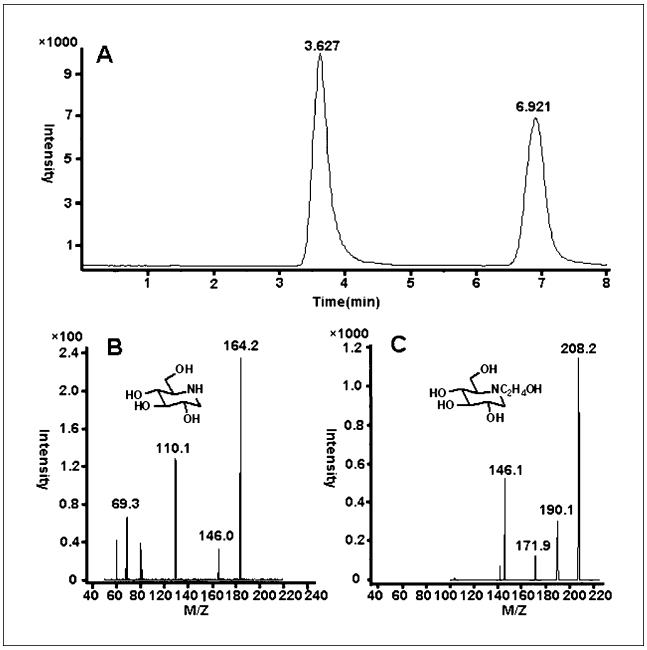


Fig. 3: MRM chromatogram of biological sample with DNJ and DNJ added with miglitol as IS (A), Peak 1 DNJ (6.921 min); Peak 2 miglitol (3.627 min). MS^2 product ions spectra of the protonated molecular ions of DNJ (B) and miglitol (C)

ment ions of the highest intensity were observed at m/z 110.1 for DNJ (Fig. 3B) and at m/z 146.1 for miglitol (IS), respectively (Fig. 3C). Therefore, the transition m/z 164.2 → 110.1 was used for determination of DNJ and m/z 208.2 → 146.1 was chosen for the quantification of IS by MRM. This method was validated based on the Chinese Pharmacopoeia 2010 edition for bioanalytical method validation. The calibration curves showed a good linearity in the range between 10 and 4000 ng/mL, the equation was $y = 0.0022x + 0.0837$, and the correlation coefficient (r^2) was 0.9996. The LLOQ (the lowest concentration of standard curve) was found to be 10 ng/mL. The intra- and inter-day precision, in term of RSD, was less than 10% for all the samples. The intra- and inter-day accuracy ranged from 92.4% to 102.1%. The absolute recovery rate of DNJ from the plasma was greater than 91.8%, and the absolute recovery rate of IS at the level of 250 ng/mL was 95.6%. The stability of DNJ in the rat plasma during the sample storage, preparation and analysis was found to be more than 90.7% of DNJ recovered under all the conditions examined. All the results passed the requirement set by the Standards.

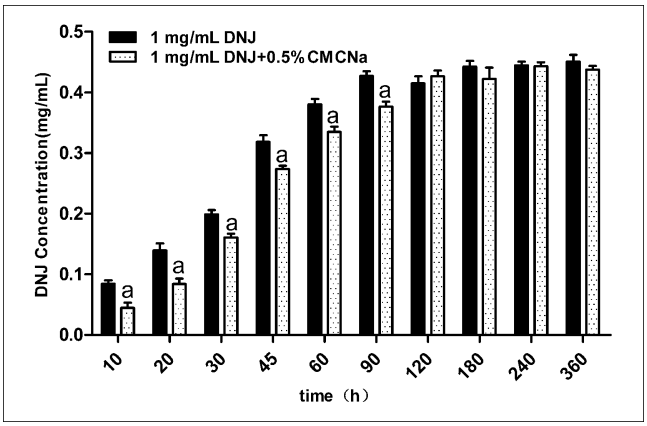


Fig. 4: Absorption profiles of DNJ with and without CMCNa in small intestines ($n = 5$). a represents significant difference ($P < 0.05$) compared with DNJ group

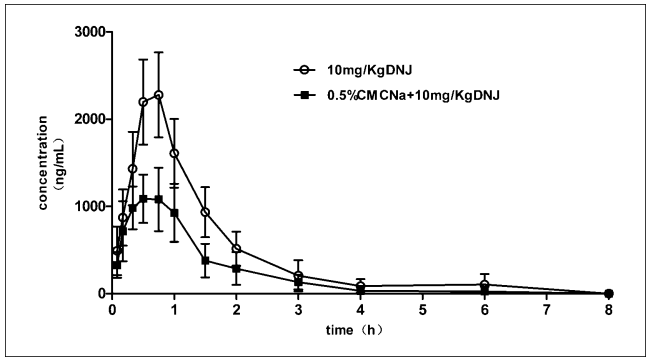


Fig. 5: Mean (± SD) plasma concentration profile over time of 10 mg/mL DNJ and 10 mg/mL DNJ added with 0.5% CMCNa to rats ($n = 10$) after oral administration

2.4. DNJ Absorption characteristics

We studied whether the DNJ absorption characteristics were affected by CMCNa. Fresh small intestines were incubated in Krebs-Henseleit solution to simulate the physiological environment *in vivo*. After the DNJ was injected into the small intestine, the reaction solution outside the small intestine was collected at different time points for the quantification of DNJ. The absorption rate of DNJ with or without CMCNa is shown in Fig. 4. The amount of absorbed DNJ increased over time regardless of the presence of CMCNa, but at the 1.5 h time-point, the amount of adsorbed DNJ combined with CMCNa was remarkably decreased compared with DNJ alone. These result indicated that CMCNa can significantly delay the absorption of DNJ in the small intestine.

2.5. DNJ Pharmacokinetics

Changes in the DNJ absorption characteristics often cause changes of its pharmacokinetics. We therefore studied the DNJ pharmacokinetics with or without CMCNa. We used the validated HPLC-MS/MS method to determine the pharmacokinetics of DNJ with or without 0.5% CMCNa in biological samples. After the blood samples were assayed, the plasma DNJ concentration profiles were calculated overtime (Fig. 5). The pharmacokinetics parameters were calculated by analyzing the DNJ concentration data on non-compartmental model (Table 1). The C_{max} of DNJ decreased from 2566.97 ng/mL to 1186.11 ng/mL by adding CMCNa, and the $AUC_{(0-12h)}$ of DNJ decreased from 3307.65 ng/h/mL to 1672.57 ng/h/mL by adding CMCNa. These results showed that CMCNa indeed markedly changed the pharmacokinetics of DNJ, and the DNJ effect was significantly suppressed with the addition of CMCNa, in the rat model.

Table 1: Main pharmacokinetics parameters of 10 mg/mL DNJ with and without 0.5% CMCNa after oral administration a represents significant difference ($P < 0.05$) compared with the DNJ group

Parameters	Units	DNJ (mean ± SD)	CMCNa + DNJ (mean ± SD)
T_{max}	h	0.65 ± 0.14	0.72 ± 0.26
C_{max}	ng/mL	2566.97 ± 292.50	1186.11 ± 278.06 a
$t_{1/2}$	h	0.93 ± 0.50	0.68 ± 0.21
$AUC_{(0-12h)}$	ngh/mL	3307.65 ± 936.81	1672.52 ± 559.33 a
$AUC(0-\infty)$	ngh/mL	3363.19 ± 934.11	1722.57 ± 566.11 a
CL	Lkg/h	0.81 ± 0.17	0.63 ± 0.14
$MRT_{(0-12h)}$	h	1.334 ± 0.284	1.25 ± 0.3

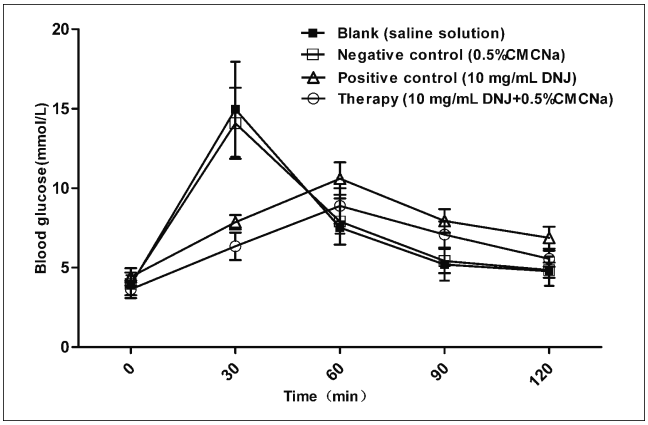


Fig. 6: Effect of DNJ added with CMCNa on blood glucose profiles in KM rats during the oral glucose tolerance test. Values are means ± SD, $n = 10$

2.6. Oral glucose tolerance test

The pharmacodynamics of DNJ with the addition of CMCNa were also studied. The result of the oral glucose tolerance test is shown in Fig. 6 and Table 2. Compared with blank (saline solution), the negative control (0.5% CMCNa) did not change the blood glucose curve, suggesting that CMCNa had no influence on the blood glucose level after starch intake. However, the positive control (10 mg/kg DNJ) and test group (0.5% CMCNa + 10 mg/kg DNJ) showed a profound glucose-lowering effect, with the peak concentration of the blood glucose decreasing notably and being delayed from 30 min to 60 min after starch intake. Compared with the positive control, DNJ combined with CMCNa decreased the maximum level of blood glucose and the $AUC_{(0–120)}$ significantly, suggesting that CMCNa enhanced the inhibitory effect of DNJ.

3. Discussion

Aza sugar molecules including DNJ are an important class of glycosidase inhibitors that have considerable potential as therapeutic agents (Asano 2000). Among the imino sugars, miglitol derived from DNJ has been approved for the treatment of type 2 diabetes. However, since the long-term use of miglitol has undesirable side effects such as flatulence, abdominal cramping, its use may be limited (Sakaguchi and Kasuga 2007). As DNJ was isolated from natural products including mulberries and sericulture products, it is considered as a safe α-glucosidase inhibitor with only few side effects.

Most of the carbohydrates, such as starch and sucrose are complex molecules. The digestion of these carbohydrates relies on a series of enzymatic reactions (Dyer et al. 2002). Starch is firstly digested by the pancreatic α-amylase in duodenum and jejunum, and the major products of starch hydrolysis are maltose, isomaltose, maltotriose, and α-dextrins. As these oligosaccharides are hardly absorbed and transported through the intestinal mucosa, further digestions are essential to fully hydrolyze these molecules into monosaccharides (Gray 1992). This step is mainly executed by α-glucosidase, which is a family of the small intestinal brush-border membrane bound enzymes, including primarily malto-glucoamylase (which can cleave α-1,4 linkages in oligosaccharides of 5–9 glucose molecules long) and sucrose-isomaltase (which acts on α-1,6 linkages in α-dextrins) (Koh et al. 2010). The predominant product of α-glucosidase hydrolysis is D-glucose, which can be actively transported and absorbed into bloodstream by glucosyltransferase and glucose transporter (GLUT)(Drozdowski and Thomson 2006). Therefore, the process leads to increase of plasma glucose level.

Table 2: Effect of DNJ added with CMCNa on blood glucose levels after starch loading a represents significant difference ($P < 0.05$) compared with the blank group, b indicates significant difference ($P < 0.05$) compared with the positive control group

Group	AUC (mmol·min/L)	C_{max} (mmol/L)	T_{max} (min)
Blank	960.6 ± 155.3	14.96 ± 3.00	30
Negative control	954.3 ± 151.5	14.28 ± 2.23	30
Positive control	961.5 ± 85.06	10.60 ± 1.02 a	60
Therapy	807.0 ± 100.1 ab	8.88 ± 1.119 a	60

As α-glucosidase inhibitors can competitively bind to the catalytic site of α-glucosidase to delay the glucose absorption and transport, they have been employed to treat type 2 diabetes mellitus (Bischoff 1995). Acarbose, similar to the oligosaccharides, can reversibly bind to the α-amylase with a higher affinity and stop the oligosaccharides hydrolysis to modulate the blood glucose level (Krentz 2006). Miglitol provides a reversible competitive binding to the brush-border α-glucosidases and reduces glucose transport owing to its similarity to glucose (Sels et al. 1999).

DNJ, as a α-glucosidase inhibitor, has an excellent capability to inhibit α-glucosidase, but its postprandial hypoglycemic activity *in vivo* is moderate (Martin 2007). Miglitol is a DNJ derivative and is also approved for the treatment of diabetes. The structures of these two molecules share many similarities, but their absorption characteristics are quite distinct. The absorption rate of miglitol is slower than that of DNJ, which may be due to their slight structural differences. Its ethanol hydroxyl group may change the lipo-hydro partition coefficient and reduce the affinity for glucosyltransferase and glucose transporter of miglitol, then the absorption rate of miglitol is lower than that of DNJ. Slowing down the absorption of DNJ probably enhances the postprandial hypoglycemic activity *in vivo*. This led us to suppress the absorption to enhance the hypoglycemic activity *in vivo* for the research and development of DNJ as a drug. Adding adjuvants to DNJ may be a suitable strategy to enhance the postprandial hypoglycemic activity of DNJ *in vivo*.

CMCNa, HEC, MC and Carbopol are commonly and widely used as adjuvants for drugs, and no side effects have been seen previously for these molecules. In this study, we compared and evaluated the effect of four different adjuvants to DNJ for inhibitory activities against α-glucosidase. CMCNa, HEC, MC enhanced the inhibitory activity of DNJ against α-glucosidase at the concentration of 0.5%, but Carbopol was excluded as it completely inhibited the activity of α-glucosidase. The effect of the three adjuvants (CMCNa, HEC, MC) may be due to their viscosity, as they decreased the fluidity of the reaction system, and reduced the velocity of the hydrolysis of starch. CMCNa exhibited a significant improvement of inhibitory potency on the hydrolysis of starch and the transport of glucose. This may also due to the viscosity of CMCNa, which probably suppressed the absorption of DNJ to the outside of the intestine, and prolonged the inhibition time of DNJ in the small intestine.

CMCNa's delaying effect to the DNJ absorption should be confirmed *in vivo*, so a HPLC-MS/MS method was established for the quantification of DNJ, which was applied to the studies of absorption characteristics and the pharmacokinetics of DNJ with or without CMCNa. The results indicated that CMCNa significantly slowed down the DNJ absorption rate in the small intestine and significantly suppressed the absorption of DNJ in rats. Since the molecular structure of DNJ is to D-glucose, we speculated that DNJ is likely to be the substrate of the

glucosyltransferase and glucose transporter (Voss et al. 2007), and have a greater chance of being absorbed into the bloodstream except its normal physiological free transport. CMCNa may have some interaction with DNJ, thus decreasing the absorption of DNJ.

As the absorption rate of DNJ was lowered by CMCNa, DNJ's postprandial hypoglycemic activity *in vivo* should be enhanced as it would be assumed that slower absorption of DNJ would enhance the postprandial hypoglycemic activity. The pharmacodynamics study by the oral glucose tolerance test confirmed that CMCNa had a remarkable effect on assisting DNJ's potency to lower blood glucose level. As DNJ can be the substrate for glucose transporter, it may become an inhibitor of glucose transport in the small intestine. Our results showed that CMCNa delayed the absorption of DNJ and prolonged the inhibition time of DNJ against α-glucosidase, and DNJ binding to glucose transporter suppressed the glucose transport in the small intestine. These results proved that CMCNa can improve the pharmacodynamics by changing DNJ absorption characteristics and pharmacokinetics.

4. Experimental

4.1. Reagents

1-Deoxynojirimycin (DNJ) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Miglitol was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The purified DNJ (> 98.0%) was obtained from Keyuan Biotechnology INC (Chengdu, China). HPLC grade methanol, acetonitrile, and acetic acid were purchased from TEDIA Company (Fairfield, OH, USA).Water was distilled and purified using a Milli-Q Water Purification System (Millipore, Bedford, MA, USA). All the other reagents were of analytical grade.

4.2. Measurement of α-glucosidase activity

Carboxymethylcellulose sodium (CMCNa), hydroxyethyl cellulose (HEC), methylcellulose (MC), and Carbopol were chosen as adjuvants in combination with DNJ treatment. The inhibitory effect of DNJ/adjuvant combinations against α-glucosidase was determined using a quantitative assay measuring reaction between α-glucosidase and starch. Adjuvant solution (1 mL) was added into an Eppendorf tube. Then 25 μL DNJ solution and 25 μL rat intestine α-glucosidase (2500 U/mL, 1 U is defined as a nanomole production of glucose per minute) were put into the tube. After vortex mixing for 30 s, 100 μL 0.1% starch solution was added as substrate and incubated at 37 °C for 30 min. The reaction was terminated by 10 μL 1 M HCl. Quantification of glucose was carried out by glucose-oxidase method with minor modifications. 50 μL reaction solution mixed with 150 μL working solution of the glucose kit were loaded into a 96-well microplates. After incubation at 37 °C for 15 min, the absorbance was measured by microplate reader (BIO-RAD, Model 680, CA).

4.3. Everted intestinal sac method

Male Sprague-Dawley rats (250 ± 25 g) were fasted for 16 h before the experiments. The rats were sacrificed by cervical vertebrae disruption. The entire small intestinal was quickly removed by cutting off the upper end of the duodenum and lower end of the ileum, followed by stripping the mesentery carefully. After rinsing the small intestinal with ice-cold saline solution (0.9% w/v), it was cut into several segments 4-cm long each. Each segment was everted and two ends were tied with silk thread. Krebs-Henseleit solution (200 μL) was injected into the everted intestinal sac. The intestinal sac was incubated with agitation for 1 h at 37 °C in 4 mL adjuvant solution with different concentrations containing 1% starch with or without 2.5 μg/mL DNJ. The solution inside and outside the intestinal sac was collected and centrifuged for 10 min at 10,000 rpm. The supernatant was used to determine the concentration of glucose by the glucose-oxidase method.

4.4. Quantification of DNJ

4.4.1. HPLC-MS/MS

A high performance liquid chromatography (HPLC) (Agilent 1200) connected to a triple quadrupole mass spectrometer (Agilent 6410) and equipped with Waters Atlantis Hilic silica (3 μm, 150 mm × 2.1 mm) analytical column (Waters, Milford, USA) was used for the quantification of DNJ. The mobile phase was a mixture of acetonitrile-ammonium formate (20 mM) (82:18, v/v) containing 0.4% formic acid at a fixed flow rate at 0.4 mL/min. The analytical run time was 8 min. The mass spectrometer equipped with an electrospray ionization source (ESI) was operated in the positive ion mode using multiple selected reactions monitoring (MRM). The mass transition was m/z 164.2→110.1 for DNJ. The optimized MS parameters were as follow: gas temperature, 350 °C; MS1 heater, 100 °C; MS2 heater, 100 °C; gas flow, 11 L/min; dwell time, 250 ms; nebulizer pressure, 35 psi; fragmentor voltage, 90 V; collision energy, 12 eV (DNJ).

4.4.2. Sample preparation

An aliquot of 5 μL of miglitol (IS) working solution (1 μg/mL) was added to 20 μL of sample in a 1.5 mL Eppendorf tube. After vortex mixing for 30 s, 400 μL of a mixture of acetonitrile-methanol (3:1, v/v) was added for protein precipitation. Then the mixture was vortex mixed for 2 min and centrifuged for 10 min at 8000 rpm. The supernatant was transferred into a new Eppendorf tube and evaporated to dryness under reduced pressure at 50 °C. The residue was reconstituted in 100 μL of mobile phase and vortex mixed for 5 min. After the centrifugation procedure at 13,000 rpm for 10 min, the HPLC-MS/MS method mentioned above was used for the quantification of DNJ.

4.5. Measurement of the DNJ absorption rate

The small intestine was acquired and processed as described in 'Everted Intestinal Sac Method'. It was cut into several segments at 10-cm long each. After 0.5 mL 1 mg/mL DNJ with or without 0.5% CMCNa was added into the segments of intestine, the two ends were tied with silk threads and the segments of intestine was incubated at 37 °C in 5 mL Krebs-Henseleit solution. Then the solution outside the intestinal was collected at 10, 20, 30, 45, 60, 90, 120, 180, 240, 360 min, after the 'sample preparation' procedure, and 5 μL supernatant was used for the quantification of DNJ.

4.6. Pharmacokinetics of DNJ

Male Sprague-Dawley rats (250 ± 25 g) were obtained from the Academy of Military Medical Science (Beijing, China). The breeding condition was the same as that for KM rats described previously. The rats were randomly divided into two experimental groups. One was administrated with DNJ via the gastric gavage; while the other group with DNJ plus 0.5% CMCNa. The DNJ doses were 10 mg/kg. The animals were fasted for 16 h but with free access to water before the drug administering. Blood samples (0.2 mL) were collected into sterile heparinized tubes at 10, 20, 30 and 45 min, 1, 1.5, 2, 3, 4, 6, 8 and 12 h after dosing. Plasma was prepared by centrifugation at 3000 rpm for 10 min and stored at -80 °C until analysis. After the sample preparation and the quantification of DNJ, their concentration profiles over time were obtained and their pharmacokinetic parameters were calculated from the plasma concentration using the DAS 2.1.1 software (Clinical Drug Evaluating Center, Anhui, China).

4.7. Oral glucose tolerance test

The oral glucose tolerance test was performed in KM rats. The rats were housed in a room with a controlled temperature at 25 °C and a 12 h light-dark cycle, with free access to food and water. The studies were approved by the Animal Ethical Committee of Nankai University, in accordance with Principles of Laboratory Animal Care and Use in Research (Ministry of Health, Beijing, China). Animals were fasted for 16 h. Immediately after starch solution (3 g/kg) was loaded for all the groups, saline solution, adjuvant solution, DNJ solution as well as DNJ/adjuvant combination were administrated via the gastric gavage for each group respectively. Blood samples were collected by cutting the tip of the tail vein at 30, 60, 90, 120 min after starch loading. The plasma glucose levels were assayed by the glucose kit from Biosino Biotechnology and Science (Beijing, China).

4.8. Statistical analysis

All the results were expressed as mean ± S.D. Statistical significance was calculated by Student's *t*-test and one-way analysis of variance (ANOVA) using GraphPad Prism version 5.0 (GraphPad Software Inc., San Diego, CA, USA). Differences were considered significant if $p < 0.05$.

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References

Ahr HJ, Boberg M, Krause HP, Maul W, Muller FO, Ploschke HJ, Weber H, Wunsche C (1989) Pharmacokinetics of acarbose. Part I: Absorption,

concentration in plasma, metabolism and excretion after single administration of acarbose to rats, dogs and man. Arzneimittelforschung 39: 1254–1260.

Asano N (2000) Alkaloidal sugar mimetics: biological activities and therapeutic applications. J Enzyme Inhib 15: 215–234.

Bembi B, Deegan P (2008) Gaucher disease: improving management. Acta Paediatr 97: 81–82.

Bischoff H (1995) The mechanism of alpha-glucosidase inhibition in the management of diabetes. Clin Invest Med 18: 303–311.

Chang J, Wang L, Ma D, Qu X, Guo H, Xu X, Mason P, Bourne N, Moriarty R, Gu B, Guo J, Block T (2009) Novel imino sugar derivatives demonstrate potent antiviral activity against flaviviruses. Antimicrob Agents Chemothes 53: 1501–1508.

Drozdowski LA, Thomson AB (2006) Intestinal sugar transport. World J Gastroenterol 12: 1657–1670.

Dyer J, Merediz EFC, Salmon KSH, Proudman CJ, Edwards GB, Shirazi-Beechey SP (2002) Molecular characterisation of carbohydrate digestion and absorption in equine small intestine. Equine Veterinary 34: 349–358.

Gray GM (1992) Starch digestion and absorption in nonruminants. J Nutr 122: 172–177.

Inoue S, Tsuruoka T, Ito T, Niida T (1968) Structure and synthesis of nojirimycin. Tetrahedron 24: 2125–2144.

Johnston PS, Lebovitz HE, Coniff RF, Simonson DC, Raskin P, Munera CL (1998) Advantages of alpha-glucosidase inhibition as monotherapy in elderly type 2 diabetic patients. J Clin Endocrinol Metab 83: 1515–1522.

Kim JY, Kwon HJ, Jung JY, Kwon HY, Baek JG, Kim YS, Kwon O (2010) Comparison of absorption of 1-deoxynojirimycin from mulberry water extract in rats. J Agric Food Chem 58: 6666–6671.

Kimura T, Nakagawa K, Kubota H, Kojima Y, Goto Y, Yamagishi K, Oita S, Oikawa S, Miyazawa T (2007) Food-grade mulberry powder enriched with 1-deoxynojirimycin suppresses the elevation of postprandial blood glucose in humans. J Agric Food Chem 55: 5869–5874.

Koh LW, Wong LL, Loo YY, Kasapis S, Huang D (2010) Evaluation of different teas against starch digestibility by mammalian glycosidases. J Agric Food Chem 58: 148–154.

Kong WH, Oh SH, Ahn YR, Kim KW, Kim JH, Seo SW (2008) Antiobesity effects and improvement of insulin sensitivity by 1-deoxynojirimycin in animal models. J Agric Food Chem 56: 2613–2619.

Konno K, Ono H, Nakamura M, Tateishi K, Hirayama C, Tamura Y, Hattori M, Koyama A (2006) Mulberry latex rich in antidiabetic sugar-mimic alkaloids forces dieting on caterpillars. P Natl Acad Sci USA 103: 1337–1341.

Krentz AJ (2006) Comparative safety of newer oral antidiabetic drugs. Expert Opin Drug Saf 5: 827–834.

Kuriyama C, Kamiyama O, Ikeda K, Sanae F, Kato A, Adachi I, Imahori T, Takahata H, Okamoto T, Asano N (2008) *In vitro* inhibition of glycogen-degrading enzymes and glycosidases by six-membered sugar mimics and their evaluation in cell cultures. Bioorg Med Chem 16: 7330–7336.

Martin O (2007) Iminosugars: current and future therapeutic applications. Ann Pharmacother Fr 65: 5–13.

Miyahara C, Miyazawa M, Satoh S, Sakai A, Mizusaki S (2004) Inhibitory effects of mulberry leaf extract on postprandial hyperglycemia in normal rats. J Nutr Sci Vitaminol (Tokyo) 50: 161–164.

Monte S, Schentag J, Adelman M, Paladino J (2010) Glucose supply and insulin demand dynamics of antidiabetic agents. J Diabetes Sci Technol 4: 365–381.

Nakagawa K, Kubota H, Kimura T, Yamashita S, Tsuzuki T, Oikawa S, Miyazawa T (2007) Occurrence of orally administered mulberry 1-deoxynojirimycin in rat plasma. J Agric Food Chem 55: 8928–8933.

Newbrun E, Hoover CI, Walker GJ (1983) Inhibition by acarbose, nojirimycin and 1-deoxynojirimycin of glucosyltransferase produced by oral streptococci. Arch Oral Biol 28: 531–536.

Nirogi RV, Kandikere VN, Shukla M, Mudigonda K, Maurya S, Boosi R, Yerramilli A (2006) Liquid chromatographic tandem mass spectrometry method for the quantification of miglitol in human plasma. Arzneimittelforschung 56: 328–336.

Ramachandran A, Snehalatha C (2011) Diabetes prevention programs. Med Clin North Am 95: 353–372, viii.

Sakaguchi K, Kasuga M (2007) [Adverse effects of alpha-glucosidase inhibitors]. Nippon Rinsho 65: 183–187.

Sels JP, Huijberts MS, Wolffenbuttel BH (1999) Miglitol, a new alpha-glucosidase inhibitor. Expert Opin Pharmacother 1: 149–156.

Takiya L, Chawla S (2002) Therapeutic options for the management of type 2 diabetes mellitus. Am J Manag Care 8: 1009–1023.

Tanaka Y, Kato J, Kohara M, Galinski M (2006) Antiviral effects of glycosylation and glucose trimming inhibitors on human parainfluenza virus type 3. Antivir Res 72: 1–9.

Voss AA, Diez-Sampedro A, Hirayama BA, Loo DD, Wright EM (2007) Imino sugars are potent agonists of the human glucose sensor SGLT3. Mol Pharmacol 71: 628–634.

Yagi M, Kouno T, Aoyagi Y, Murai H (1976) The structure of moranoline, a piperidine alkaloid from Morus species. Nippon Nogeikagaku Kaishi 50: 571–573.

Yatsunami K, Ichida M, Onodera S (2008) The relationship between 1-deoxynojirimycin content and alpha-glucosidase inhibitory activity in leaves of 276 mulberry cultivars (Morus spp.) in Kyoto, Japan. J Nat Med 62: 63–66.