Effect of Decrease in Both Postprandial Blood Glucose (PBG) and Fasting Blood Glucose (FBG) Levels in Normal Beagle Dogs with Nateglinide Enteric Coated Granules and Immediate Release Tablets

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Nateglinide is a new quick action/short duration (QRSD) type of oral blood glucose regulator, and nateglinide immediate release tablets are used for patients with mild diabetes under the trade name of Fastic® tablets. In this study, we attempted to determine if it was possible to control both post-prandial blood glucose level (PBG) and fasting blood glucose level (FBG) for moderate or severe diabetes through controlled release of nateglinide. Enteric coated granules were selected for the administration form for controlled release of nateglinide, and three types of enteric coated granules were prepared having dissolution pH values of 5.5, 6.5 and 7.2. The three types of enteric coated granules were each administered separately or the enteric coated granules having an dissolution pH of 6.5 were administered simultaneous to administration of nateglinide immediate release tablets to normal beagle dogs just before feeding followed by measurement of plasma nateglinide concentration, plasma insulin concentration and blood glucose level. In the case of administering enteric coated granules alone (nateglinide: 9 mg/kg), the absorption of nateglinide was confirmed to tend to be delayed as the dissolution pH increased. In the case of an dissolution pH of 5.5, decreases in both PBG and FBG were observed. In the case of dissolution pH values of 6.5 and 7.2, only decrease in FBG was observed. In case of nateglinide immediate release tablets (nateglinide: 9 mg/kg), only decrease in PBG was observed. Decreases in both PBG and FBG were observed in the case of simultaneous administration of dissolution pH 6.5 enteric coated granules and nateglinide immediate release tablets just before feeding (nateglinide: 90 mg/head+60 mg/head). A correlation was observed between plasma nateglinide concentrations and blood glucose levels. On the other hand, there were no correlations observed between changes in plasma insulin concentrations and blood glucose levels. In case of nateglinide immediate release tablets (nateglinide: 150 mg/head), Decreases in both PBG and FBG were observed. However, the nateglinide controlled release formulation is more useful than the nateglinide immediate release tablets from the view point of avoidance of side effect, or of easy control of both PBG and FBG. On the basis of these results, the design of a controlled release formulation that contains nateglinide was suggested to enable control of both PBG and FBG for moderate and severe diabetes patients.

Key words nateglinide; fast-acting blood glucose regulator; post-prandial blood glucose level (PBG); fasting blood glucose level (FBG); controlled release formulation

Ordinary antidiabetics for oral administration are classified into two types. The first type primarily controls postprandial blood glucose level (PBG), while the other type primarily controls fasting blood glucose level (FBG).[1,2] There is currently no oral antidiabetic capable of controlling both PBG and FBG, and such an antidiabetic is believed to be the most useful for the treatment of diabetes.

The D-phenylalanine derivative, nateglinide ((−)-*N*-(*trans*-4-isopropylcyclohexanecarbonyl)-D-phenylalanine) was developed by Ajinomoto for use as an antidiabetic (Fig. 1). Although nateglinide stimulates insulin secretion, it has a different chemical structure from sulfonylureas,[1,3] demonstrates a quick action/short duration type effect,[1,2,4] and its effect is believed to effectively control primarily PBG. Since blood glucose level inhibitory effects dissipate in a short period of time, nateglinide is expected to realize (1) avoidance of a hypoglycemic state, and (2) avoidance of β cell exhaustion following long-term administration and avoidance of secondary failure.[1] Nateglinide immediate release (IR) tablets are currently available commercially in the form of Fastic® tablets, and are used primarily for patients with mild diabetes. On the other hand, it is important to control FBG in patients with moderate and severe diabetes who exhibit elevated FBG levels. If the nateglinide oral controlled release formulation were available that is capable of controlling both PBG and FBG, it could be expected to offer the advantages of (1) improving compliance by reducing the number of administrations per day (Fastic® tablets: 3 times per day), and (2) increasing the number of choices available for treating diabetes in moderate and severe diabetes patients.

Gliclazide is an antidiabetic that is a long acting type stimulator of insulin secretion having a sulfonylurea moiety. In the case of repeated administration of gliclazide to normal rats, the blood glucose lowering action has been reported to weaken during the second administration.[5] Nateglinide is also an insulin secretion stimulator. Consequently, in the case of designing a controlled release formulation that contains nateglinide for the purpose of effectively inhibiting both PBG and FBG, there have been concerns that even if it is

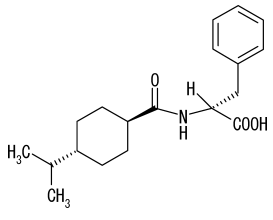

Fig. 1. Chemical Structure of Nateglinide

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possible to control PBG, it may not be possible to adequately control FBG. Furthermore, although controlled release formulations containing metformin have been released commercially, these are able to control FBG but are unable to adequately control PBG.[6)]

For this reason, we felt that it was necessary to confirm the possibility of whether or not nateglinide is able to control both PBG and FBG by conducting an *in vivo* study using nateglinide oral controlled release formulations. Although there are various ways to achieve controlled release formulations such as enteric coated granules, matrices and time-dependent release formulations, this study was conducted while focusing on enteric coated granules.

In this study, we assessed PBG and FBG following administration of nateglinide oral controlled release formulations to normal beagle dogs. A discussion was then made as to whether or not PBG and FBG can be controlled by a controlled release formulation using a quick action/short duration type blood glucose regulator, nateglinide.

Experimental

Materials Nateglinide (Ajinomoto Co. Inc., Japan), lactose mono-hydrate (DMV Japan), hydroxypropylcellulose, low-substituted hydroxypropylcellulose (Shin-etsu Chemical Co. Ltd., Japan), dry methacrylic acid copolymer LD (Röhm GmbH, Germany), methacrylic acid copolymer S (Röhm GmbH, Germany), Hydroxypropylmethylcellulosephtalate 220824 (HPMCP HP-50, Shinetsu Chemical Co. Ltd., Japan), macrogol 6000 (Nihon Oil and Fats Co. Ltd., Japan), talc and ethanol (Wako Pure Chemical, Japan) were used in the study.

Fastic® tablets (Ajinomoto Co. Inc., Japan) were used as an IR tablets *in vivo* study.

Equipment An extrusion granulator (DG-L1, Fuji Paudal, Japan), spherized granulator (Q-230, Fuji Paudal, Japan), fluidized bed granulator (FLO-1, Freund industry Co., Japan) and homogenizer (T25-ST, JANEE & KUNKEL GMBH Co. KG, Germany) were used.

Preparation of Nateglinide Enteric Coated Granules Two hundred and fifty grams of nateglinide, 10 g of hydroxypropylcellulose, and 425 g of lactose mono hydrate were suspended and dissolved in 815 g of water with a homogenizer. After mixing this suspension with 300 g of low-substituted hydroxypropylcellulose, extrusion granulation was conducted. The resulting granules were rounded by a spherical granulator, and then dried in a fluidized bed dryer. Fractions of 500—1400 μm granules were obtained by grading, and then used for coating.

Table 1 shows the compositions of the enteric coating solutions. The resulting core granules were coated with the coating solutions using a fluidized bed coating machine (FLO-1, Freund Ind., Japan). Three types of enteric coated granules were obtained consisting of enteric coated granules A (hydroxypropylmethylcellulosephtalate 220824), enteric coated granules B (dry methacrylic acid copolymer LD), enteric coated granules C (methacrylic acid copolymer S).

Dissolution Profiles of Nateglinide Enteric Coated Granules The dissolution profiles of the resulting granules were evaluated (JP13, paddle method, 50 rpm, test fluid: 900 ml, nateglinide: 90 mg/vessel) with a dissolution tester (NTR-VS6P, Toyama sangyo Co. Ltd., Japan). The test fluids consisted of JP1 fluid (JP13, Disintegration test fluid No. 1) containing 0.6 w/v% polysorbate 80 for pH=1.2, four-time diluted McIlvaine buffer (pH=4.0) containing 0.5 w/v% polysorbate 80 for pH=4.0, and Clark–Lubs buffer for pH=5.5—7.6.

Dissolution rates were determined with a reversed phase HPLC system consisting of an L-6000 constant flow pump and a L-4000 UV detecter operating at 210 nm (Hitachi Corp., Japan). Separations were performed with a reversed phase C-18 column (4.5×150 mm, GL Science, Japan). The mobile phase consisted of acetonitrile–pH=2.5 phosphate buffer (55 : 45, v/v). Nateglinide eluted at about 10 min at 40 °C (at a flow rate of 1.5 ml/min).

Plasma Nateglinide Concentration,[7)] Plasma Insulin Concentration and Blood Glucose Level Nateglinide preparations were administered to normal male beagle dogs (body weight: *ca.* 10 kg) just before feeding. One hundred and fifty grams of dry DS meal suspended in 600 g of hot water was forcibly administered to the beagle dogs with a syringe. Feeding was conducted within 12 min.

In case of oral administration of enteric coated granules (nateglinide: 9 mg/kg), only feeding, IR tablets (nateglinide: *ca.* 9 mg/kg), blood samples were taken before and at 15, 30, 45, 60, 120, 180, 240, 360, 480 min (enteric coated granules: $n=3$, only feeding: $n=6$).

In case of oral administration of both IR tablets and enteric coated granules B, blood samples were taken before and at 15, 30, 45, 60, 120, 240, 360, 540, 720, 1440 min after oral administration for IR tablets only (nateglinide: 60, 150 mg), IR tablets (nateglinide: 60 mg)+enteric coated granules B (nateglinide: 30 mg or 60 mg). Each point and vertical bar represent mean±S.E.M. ($n=6$). In case of oral administration of both IR tablets (nateglinide: 60 mg)+enteric coated granules B (nateglinide: 90 mg), blood samples were taken before and at 15, 30, 45, 60, 120, 180, 240, 360, 480, 540, 720, 1440 min after oral administration. Each point and vertical bar represent mean±S.E.M. ($n=6$), except at 180, 480, 540, 720, 1440 min ($n=3$).

Blood was sampled from a leg vein. Whole blood was centrifuged at 1700 ***g*** for 15 min at 5 °C and plasma was collected for analysis. A 50 μl aliquot of internal standard solution was spiked into 0.5 ml plasma in an Eppendorf tube followed by the addition of 0.5 ml of 0.05 M pH=6.0 phosphate buffer. The mixture was vortex-mixed for 10 s and applied to a Sep-Pak Vac tC18 cartridge which was pre-equilibrated with 5 ml of 0.05 M pH=6.0 phosphate buffer. The cartridge was washed with 2 ml of water and finally eluted with 2 ml of ethanol. The elute was evaporated to dryness *in vacuo* at 30 °C. The residue was dissolved in 0.2 ml of mobile phase and 20 μl of this solution was used for the HPLC sample.

Plasma nateglinide concentration was determined with a two-column switching HPLC system consisting of a 600E multi solvent pump system, 515 HPLC pump (Waters, Japan), 2487 UV detector (Waters, Japan) operating at 210 nm, and SPV-N-6A column switching apparatus (GL Science, Japan). Separations were performed with an Inertsil ODS-3 reversed phase C-18 column (4.0×20 mm, GL Science, Japan) and L-column ODS (4.6×250 mm, Kagakubushitsukenkyukikou, Japan).

Three types of mobile phases were used consisting of acetonitrile : pH=6.6 0.05 mol/l phosphate buffer=3 : 7, v/v (mobile phase A), acetonitrile : pH=6.6 0.05 mol/l phosphate buffer=45 : 55, v/v (mobile phase B), and acetonitrile : pH=6.6 0.05 mol/l phosphate buffer=6 : 4, v/v (mobile phase C). The time table of the column switching pattern is shown in Table 2. At a flow rate of 1.0 ml/min, nateglinide eluted at about 7.5 min at 40 °C.

Determination of plasma insulin concentration was conducted using a kit for assay of insulin in plasma (Morinaga Seikagakukenkyusho Co. Ltd., Japan). Blood glucose level was determined with the Fuji DRICHEM 3500S (FUJIFILM Co., Japan).

Statistical analyses were performed using the Student's *t*-test.

Results and Discussion

Dissolution Profiles of Enteric Coated Granules Nateglinide core granules were coated using the coating solutions shown in Table 1 to prepare three types of nateglinide enteric coated granules.

An evaluation was first made of the relationship between the coated amount of enteric coating material (dry methacrylic acid copolymer LD, Eudragit L100-55) and acid resistance (value of dissolution rate at 120 min in JP1 fluid containing 0.6 w/v% Polysorbate 80) through coating using methacrylic acid copolymer LD coating solution. Nateglinide

Table 1. Composition of Each Enteric-Coating Solution [w/w%]

	Enteric coated granules		
	A	B	C
Eudragit L100-55		7.0	
Eudragit S100			7.0
HPMCP HP-50	7.0		
Macrogol 6000	0.7	0.7	0.7
Talc	1.0	3.5	3.5
Ethanol	73.0	70.0	70.0
Water	18.3	18.8	18.8

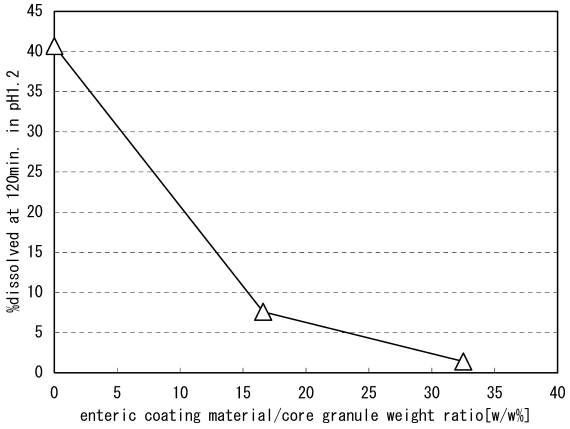

Fig. 2. Relationship between an Amount of Enteric Coating Material and Enteric Property

Enteric coating material: dry methacrylyc acid copolymer LD.

Table 2. Column Switching Program[7)]

Time [min]	600E pump system	515 pump	Column switching[a)]
0.0—0.7	Mobile phase A	Mobile phase B	1
0.7—2.5	Mobile phase A	Stop	2
2.5—8.0	Mobile phase C	Mobile phase B	1
8.0—20.0	Mobile phase A	Mobile phase B	1

a) 1: The mobile phase A and C passed through a 600E pump system, injector and pre column. The mobile phase B passed through a 515 pump, main column and detector. 2: The mobile phase A passed through a 600E pump system, injector, pre column, main column and detector. A 515 pump stopped.

Table 3. Enteric Property of the Obtained Granules

Enteric coated granules	% dissolved in JP1 fluid[a)] at 120 min [%]
A	0.9±0.3
B	1.4±0.3
C	0.5±0.0

a) JP1 containing 0.6 w/w% polysorbate 80. All data are expressed means±S.D. (n=3).

is poorly water soluble drug. Polysorbate 80 was added to acid pH testing fluid to satisfy sink condition. Those results are shown in Fig. 2.

The 120 min dissolution rate at pH 1.2 decreased as the coated amount increased, and acid resistance was obtained when coated to about 15 w/w% or more. Namely, the 120 min dissolution rate at pH 1.2 was found to be 10% or less. This indicates that the surfaces of the core granules are covered with an enteric coating having adequate acid resistance as a result of coating the enteric coating material at 15 w/w%. Enteric coated granules B were obtained by coating with dry methacrylic acid copolymer LD at 32.5 w/w%.

With reference to the results described above, enteric coated granules A and C were obtained by coating the same core granules with hydroxypropylmethyl-cellulose phthalate 220824 (HPMCP HP-50) and methacrylic acid copolymer S (Eudragit S100) at 24.0 w/w% and 33.9 w/w%, respectively, using the coating solutions shown in Table 1. As shown in Table 3, the 120 min dissolution rates at pH 1.2 of enteric coated granules A, B and C were all 10% or less and confirmed to demonstrate sufficient acid resistance.

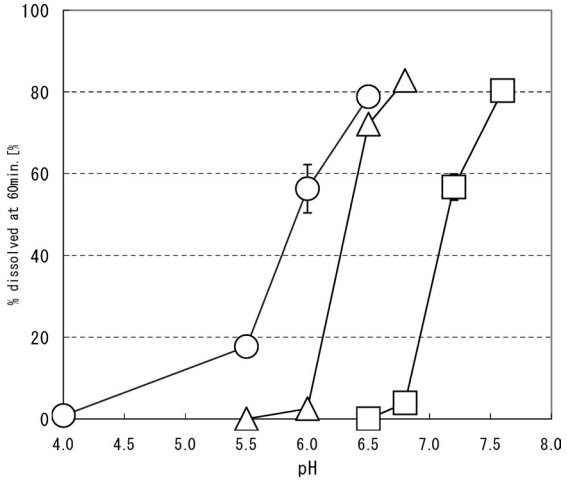

Fig. 3. pH Dissolution Relationship for 3 Types of Enteric Coated Granules

JPXIII paddle method (50 rpm), n=3, nateglinide: 90 mg/vessel, mean±S.D. Medium: pH=4.0: McIlvaine buffer 4 times diluted with water (0.5 w/v% polysorbate 80), pH=5.5—7.6: Clark Lubs buffer (KH_2PO_4+NaOH). ○: enteric coated granules A, △: enteric coated granules B, □: enteric coated granules C.

Moreover, dissolution behavior in the neutral pH region was also evaluated for the resulting enteric coated granules (Fig. 3). Here, the dissolution behavior of each the enteric coated granules was classified based on the approach of dissolution pH as advocated in refs. 8, 9, 10, 11. In this study, dissolution pH is defined as the pH at which the 60 min dissolution rate reaches 10% or more. Enteric coated granules A, B and C were confirmed to each have different dissolution pH, demonstrating values of 5.5, 6.5 and 7.2, respectively. The enteric coating materials used for enteric coated granules A, B and C have been reported to dissolve at pH 5.0, 6.0 and 7.0, respectively.[12,13)] The trends of the dissolution pH values of the resulting enteric coated granules agreed with the trends of the pH values at which the enteric coating materials dissolve. Furthermore, the dissolution rate of nateglinide IR tablets (Fastic tablets) under the same conditions is nearly 100%. On the basis of the above results, enteric coated granules A, B and C were confirmed to elute more slowly than nateglinide IR tablets, and the their degrees of controlled release were each found to be different, with their dissolution rates becoming slower in the order of enteric coated granules A, B and finally C.

Plasma Nateglinide Concentration, Plasma Insulin Concentration and Blood Glucose Level after Oral Administration of Enteric Coated Granules to Normal Beagle Dogs Enteric coated granules A, B and C were orally administered (nateglinide: 9 mg/kg) to normal beagle dogs just before feeding followed by measurement of plasma nateglinide concentrations, plasma insulin concentrations and blood glucose levels. Results are also shown for a control (feeding only) and IR tablets (nateglinide: 90 mg/body, *ca.* 9 mg/kg) used as controls.

Plasma nateglinide concentration profiles are shown in Fig. 4a. The release of nateglinide is inhibited as the dissolution pH increases, eventually leading to delayed absorption. The C_{max} values and *AUC* values of the enteric coated granules were lower than those of the IR tablets. However, an increasing trend was observed in plasma nateglinide concentrations of enteric coated granules B starting at 5 h after admin-

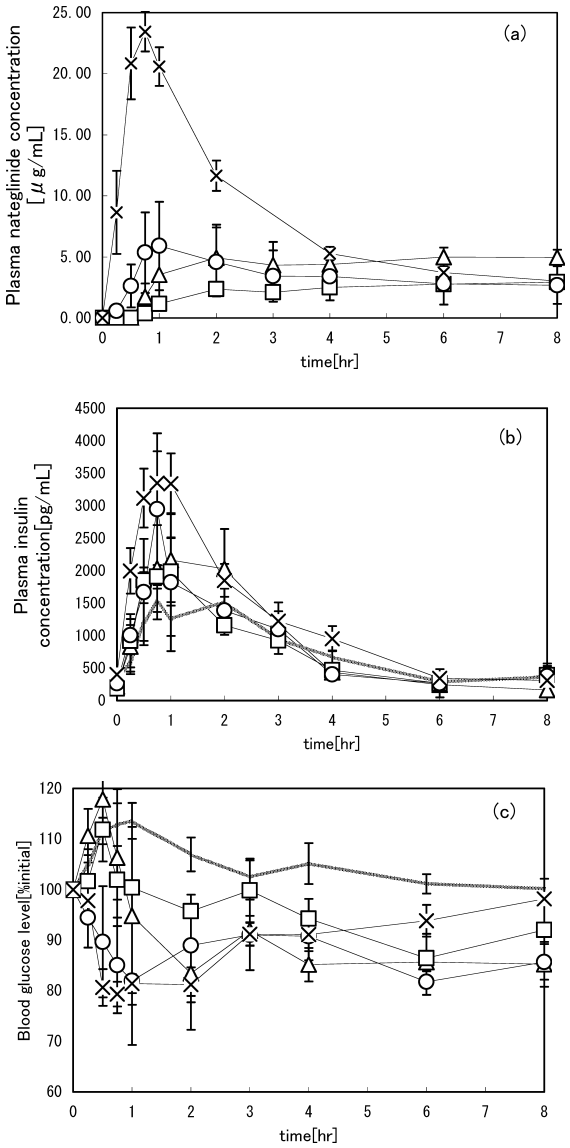

Fig. 4. Plasma Nateglinide Concentration Profiles (a), Plasma Insulin Concentration Profiles (b), Blood Glucose Level (% initial) (c), after Oral Administration of Enteric Coated Granules A (○), B (△), C (□) (Nateglinide: 9 mg/kg) and Immediate Release Tablets (×) (Nateglinide: *ca.* 9 mg/kg) in Beagle Dogs Just before Feeding, or Only Feeding (—)

Each point and vertical bar represent mean±S.E.M. (enteric coated granules: $n=3$, immediate release tablets and only feeding: $n=6$).

istration as compared with that of the IR tablets.

Plasma insulin concentration profiles are shown in Fig. 4b. The respective insulin C_{max} values consisted of 1521± 385 pg/ml (T_{max}: 2 h) for the control (feeding only), 3349± 488 pg/ml (T_{max}: 0.75 h) for the IR tablets, 2947±1169 pg/ml (T_{max}: 0.75 h) for enteric coated granules A, 2163±337 pg/ml (T_{max}: 1 h) for enteric coated granules B, and 1985± 524 pg/ml (T_{max}: 1 h) for enteric coated granules C. There were significant differences observed ($p<0.05$, Student's *t*-test) for plasma insulin concentrations from 0.25 to 1 h between the IR tablets and the control. And there were no significant differences observed for plasma insulin concentrations starting at 3 h after administration between the enteric coated granules dose groups or between the enteric coated granules dose groups and the control (Student's *t*-test). In addition, there was no correlation observed between plasma nateglinide concentration and plasma insulin concentration.

Blood glucose level profiles are shown in Fig. 4c. In the case of enteric coated granules B (dissolution pH: 6.5) and enteric coated granules C (dissolution pH: 7.2), FBG decreased to a maximum of about 83% and about 86%, respectively, as compared with blood glucose levels immediately before administration. In the case of enteric coated granules A (dissolution pH: 5.5), both FBG and PBG decreased, with both decreasing to a maximum of about 82% as compared with blood glucose levels immediately before administration. In case of the IR tablets, FBG at 8 h after administration did not decrease, although PBG decreased to a maximum of about 79% as compared with blood glucose levels immediately before administration.

Although there appears to be a correlation between blood glucose level and plasma nateglinide concentration, there were no correlation between plasma insulin concentration and blood glucose level.

On the basis of the above results, it was suggested that enteric coated granules have the ability of decrease in FBG, although IR tablets do not, and that only FBG or both PBG and FBG can be decreased by controlled release of a quick action/short duration type of blood glucose regulator, nateglinide.

Plasma Nateglinide Concentration, Plasma Insulin Concentration and Blood Glucose Level after Oral Administration of Both Enteric Coated Granules and IR Tablets After deciding to focus on enteric coated granules B having a dissolution pH of 6.5 which effectively lowered FBG without hardly any decrease in PBG, enteric coated granules B and nateglinide IR tablets (nateglinide: 60 mg) were simultaneously administered orally to normal beagle dogs just before feeding to evaluate the effects on PBG and FBG. Results are also shown for a control (feeding only) and nateglinide IR tablets only (nateglinide: 60 mg, 150 mg) used as controls.

Plasma nateglinide concentration profiles are shown in Fig. 5a. The nateglinide C_{max} values were 15.63±1.08 ng/ml (T_{max}: 0.75 h), 34.80±5.15 ng/ml (T_{max}: 0.5 h) for the IR tablets (nateglinide: 60 mg, 150 mg), 12.59±1.83 ng/ml (T_{max}: 1 h) for the IR tablets (nateglinide: 60 mg)+enteric coated granules B (nateglinide: 30 mg), 16.69±0.53 ng/ml (T_{max}: 0.75 h) for the IR tablets (nateglinide: 60 mg)+enteric coated granules B (nateglinide: 60 mg) and 15.60± 2.08 ng/ml (T_{max}: 1 h) for the IR tablets (nateglinide: 60 mg)+enteric coated granules B (nateglinide: 90 mg), respectively. There were no significant differences of the C_{max} values observed between the IR tablets (nateglinide: 60 mg) and the IR tablets (nateglinide: 60 mg)+enteric coated granules groups (Student's *t*-test). This is because C_{max} values are only dependent on the IR component. On the other hand, plasma nateglinide concentrations from 3 to 12 h after administration demonstrated an increasing trend accompanying an increasing amounts of nateglinide in the controlled release component (enteric coated granules B). There were significant differences observed at 4, 6, 12 h between the IR tablets (nateglinide: 60 mg) and the IR tablets (nateglinide: 60 mg)+ enteric coated granules B (nateglinide: 30 mg), and at 2, 4, 6, 9, 12, 24 h between the IR tablets (nateglinide: 60 mg) and the IR tablets (nateglinide: 60 mg)+enteric coated granules B (nateglinide: 60 mg), and at 2, 4, 6, 9, 12, 24 h between the

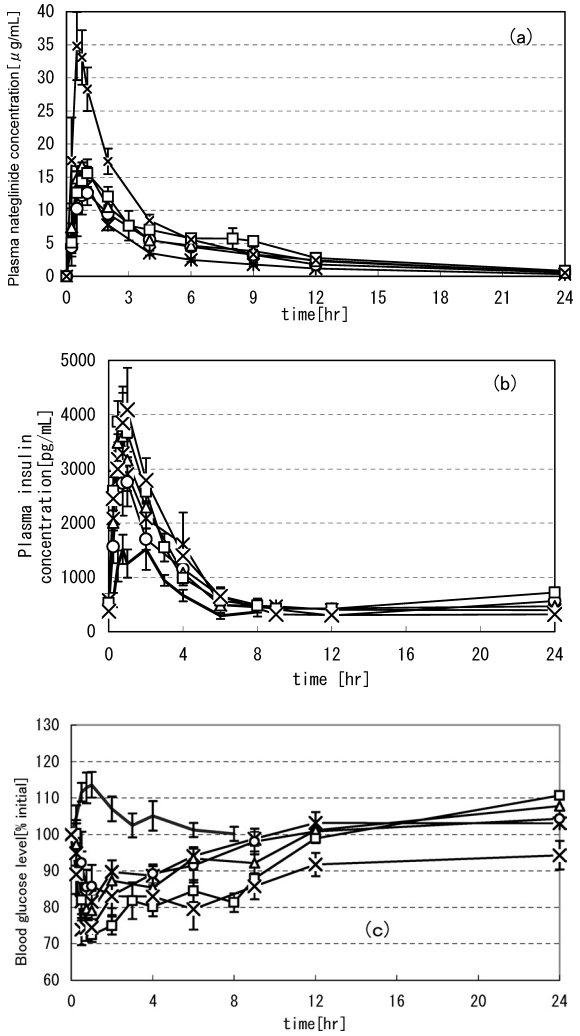

Fig. 5. Plasma Nateglinide Concentration Profiles (a), Plasma Insulin Concentration Profiles (b), Blood Glucose Level (c) after Oral Administration of Both Enteric Coated Granules B and Immediate Release Tablets in Fasted Beagle Dogs Just before Feeding, or Only Feeding

Each point and vertical bar represent mean±S.E.M. —: only feeding, *: immediate release tablets (nateglinide: 60 mg), ×: immediate release tablets (nateglinide: 150 mg), ○: immediate release tablets (nateglinide: 60 mg)+enteric coated granules B (nateglinide: 30 mg), △: immediate release tablets (nateglinide: 60 mg)+enteric coated granules B (nateglinide: 60 mg), □: immediate release tablets (nateglinide: 60 mg)+enteric coated granules B (nateglinide: 90 mg).

IR tablets (nateglinide: 60 mg) and the IR tablets (nateglinide: 60 mg)+enteric coated granules B (nateglinide: 90 mg). On the other hand, there were significant differences of plasma nateglinide concentration from 0.25 to 9 h observed between the IR tablets (nateglinide: 60 mg) and the IR tablets (nateglinide: 150 mg) ($p<0.05$, Student's *t*-test).

Plasma insulin concentration profiles are shown in Fig. 5b. The insulin C_{max} values were 1521±385 pg/ml (T_{max}: 2 h) for the control, 3287±413 pg/ml (T_{max}: 0.75 h), 4089±775 pg/ml (T_{max}: 1 h) for IR tablets (nateglinide: 60 mg, 150 mg), 2758±599 pg/ml (T_{max}: 0.75 h) for IR tablets (nateglinide: 60 mg)+enteric coated granules B (nateglinide: 30 mg), 3600±446 pg/ml (T_{max}: 0.75 h) for IR tablets (nateglinide: 60 mg)+enteric coated granules B (nateglinide: 60 mg), and 3871±378 pg/ml (T_{max}: 0.5 h) for IR tablets (nateglinide: 60 mg)+enteric coated granules B (nateglinide: 90 mg), respectively.

There were significant differences of plasma insulin concentration observed are at 0.25, 0.5, 0.75 h between the control (feeding only) and IR tablets (nateglinide: 60 mg), and at 0.25, 0.5, 1, 6 h between the control (feeding only) IR tablets (nateglinide: 60 mg)+enteric coated granules B (nateglinide: 30 mg), and at 0.25, 0.5, 1, 6 h between the control (feeding only) and IR tablets (nateglinide: 60 mg)+enteric coated granules B (nateglinide: 60 mg), and at 0.25, 0.5, 0.75, 1, 2, 4, 6 h between the control (feeding only) and IR tablets (nateglinide: 60 mg)+enteric coated granules B (nateglinide: 90 mg) ($p<0.05$, Student's *t*-test). On the other hand, in the case of comparing the groups administered enteric coated granules+IR tablets with the groups administered IR tablets (60 mg, 150 mg) only, there were no significant differences observed (Student's *t*-test) with the exception of the IR tablets (60 mg)+enteric coated granules (90 mg) at 24 h after administration. This significant difference observed at 24 h after administration is believed to be an artifact.

Blood glucose level profiles are shown in Fig. 5c. In the case of comparing enteric coated granules+IR tablets with nateglinide IR tablets (nateglinide: 60 mg), there were significant differences in blood glucose levels at 1, 2, 4, 6, 9 h after administration in the IR tablets (nateglinide: 60 mg)+enteric coated granules (nateglinide: 90 mg) group ($p<0.05$, Student's *t*-test).

Based on the results of Figs. 5a and c, there is believed to be a correlation between blood glucose levels and plasma nateglinide concentrations.

Although decreases in blood glucose levels only continued for about 6 h in the case of IR tablets (nateglinide: 60 mg), decreases continued for up to about 9 h in the case of simultaneous administration with enteric coated granules (IR tablets (nateglinide: 60 mg)+enteric coated granules (nateglinide: 90 mg) group).

In case of IR tablets (nateglinide: 150 mg), the decrease in both PBG and FBG was observed. There were significant differences of blood glucose level observed at 6, 9, 12, 24 h between the IR tablets (nateglinide: 150 mg) and the IR tablets (nateglinide: 60 mg) ($p<0.05$, Student's *t*-test).

Not only the combination of the IR tablets (nateglinide: 60 mg) and enteric coated granules (nateglinide: 90 mg), but also the IR tablets (nateglinide: 150 mg) decreased both PBG and FBG. However, nateglinide controlled release formulation is believed to be more useful than the IR tablets (nateglinide: 150 mg) according to the reasons as follows.

(1) The IR tablets (nateglinide: 150 mg) has higher C_{max} of plasma nateglinide concentration than that of the combination of the IR tablets (nateglinide: 60 mg) and enteric coated granules (nateglinide: 90 mg). The IR tablets (nateglinide: 150 mg) may have side effect (hypoglycemic state) easier than the combination of the IR tablets (nateglinide: 60 mg) and enteric coated granules (nateglinide: 90 mg).

(2) It is easier to control both PBG and FBG with the combination of the IR tablets (nateglinide: 60 mg) and enteric coated granules (nateglinide: 90 mg) than with the IR tablets (nateglinide: 150 mg), because the combination can adjust the balance of IR part and controlled release part.

On the basis of these results, it was indicated that it is possible to lower both PBG and FBG by controlled release of nateglinide. It was also indicated that it is possible to control both PBG and FBG by using a combined IR and controlled release formulation containing nateglinide.

It was initially believed in this study that it would be difficult to lower fasting blood glucose levels even if the release of an insulin secretion stimulator like nateglinide was able to be controlled. However, our study confirmed that it is possible to lower fasting blood glucose levels while continuing to lower postprandial blood glucose levels by controlling the release of nateglinide. Although the cause of this finding is currently unclear, the following possible reasons can be considered.

1) Nateglinide and sulfonylureas (SU) type blood glucose regulator bind to SU receptors on pancreas β cells. As a result, Ca^{2+} channels open and extracellular Ca^{2+} flows into the β cells resulting in secretion of insulin.[1)]

2) In the case of SU type compounds, although basal insulin secretion is maintained since there is comparatively strong affinity for SU receptors, first-phase insulin secretion due to Ca^{2+} influx does not occur.[1)]

3) On the other hand, in the case of a quick action/short duration blood glucose regulator like nateglinide, since the drug rapidly dissociates from the SU receptors,[1)] there is a correlation between plasma drug concentration and blood glucose levels, which is thought to enable decreases in both PBG and FBG. Furthermore, further studies will be required in the future to assess the blood glucose lowering effects during continuous administration of a nateglinide controlled release formulation so as to lower postprandial blood glucose and fasting blood glucose levels.

A correlation was observed between plasma nateglinide concentrations and blood glucose levels. However, there were no well-defined correlations observed between plasma insulin concentrations and blood glucose levels similar as to when only enteric coated granules were administered. This is believed to be due to having sampled blood from a vein in the leg. Kawamori *et al.* and other researchers have pointed out that plasma insulin concentrations of peripheral veins do not serve as an indicator of insulin secretion from the pancreas based on the results of insulin kinetics and metabolism studies using normal fasting dogs.[14—16)] In addition, venous plasma insulin concentrations after having passed through the liver have been indicated as being 50% or less of concentrations in the portal vein.[17,18)] In other words, the control of blood glucose levels is thought to take place based on insulin concentration in the portal vein and not in the peripheral blood. It is thought that a correlation between plasma insulin concentrations and blood glucose levels would be observed if it were possible to measure plasma insulin concentration in the portal vein.

Conclusion

This study was conducted to determine whether or not it is possible to control both PBG and FBG by controlled release of nateglinide, a quick action/short duration type of oral blood glucose regulator. Enteric coated granules were selected as the drug form for controlled release of nateglinide, and three types of enteric coated granules were formulated having dissolution pH values of 5.5, 6.5 and 7.2, respectively. The three types of enteric coated granules separately or enteric coated granules having a dissolution pH of 6.5 and nateglinide IR tablets simultaneously were administered to normal beagle dogs just before feeding followed by measurement of plasma nateglinide concentration, plasma insulin concentration and blood glucose level. In the case of administration of enteric coated granules alone, the absorption of nateglinide was confirmed to have a tendency to be delayed accompanying increases in the dissolution pH. Decreases in both PBG and FBG were observed in the case of enteric coated granules having a dissolution pH of 5.5. Although FBG was observed to decrease in the case of enteric coated granules having a dissolution pH of 6.5 or 7.2, there were hardly any decreases in PBG. A correlation was observed between plasma nateglinide concentration and blood glucose level. On the other hand, there was no correlation observed between plasma insulin concentration and blood glucose level. Decreases in both PBG and FBG were observed in the case of simultaneous administration of dissolution pH 6.5 enteric coated granules and IR tablets just before feeding.

On the basis of these results, the design of a controlled release formulation that contains nateglinide is believed to enable control of both PBG and FBG for moderate and severe diabetes patients.

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