Development of Nateglinide Oral Controlled Release Formulation to Achieve Ideal Control of Blood Glucose Level for Type 2 Diabetes

January 2016

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Development of Nateglinide Oral Controlled Release Formulation to Achieve Ideal Control of Blood Glucose Level for Type 2 Diabetes

A Dissertation Submitted to

the Graduate School of Life and Environmental Sciences,

the University of Tsukuba

in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy in Biotechnology

(Doctoral Program in Bioindustrial Sciences)

Chisato MAKINO

Contents

Abbreviations

PBG: Post prandial blood glucose level

FBG: Fasting blood glucose level

SU: Sulfonylureas

POP: Proof of principle

JP: Japanese pharmacopoeia

JP1 fluid: Japanese pharmacopoeia 16, disintegration test fluid No.1

JP2 fluid: Japanese pharmacopoeia 16, disintegration test fluid No.2

C_{max}: Maximum plasma concentration of administered drug

T_{max}: The time after administration when the maximum plasma concentration is reached

PPARγ: Peroxisome proliferator-activated receptor γ

GLP-1: Glucagon-like peptide-1

DPP- IV: Dipeptidyl peptidase-4

SGLT2: Sodium/glucose co-transporter 2

α-GI: α -Glucosidase inhibitor

HbA1c: Hemoglobin A1c

JDS: The Japan diabetes society

NGSP: National glycohemoglobin standardization program

NG: No good

DDS: Drug delivery system

HPMCP: Hydroxypropylmethylcellulose phthalate

HPC: Hydroxypropylcellulose

HPMC: Hydroxypropylmethylcellulose

Mg-St: Magnesium stearate

QASD: Quick action/short duration

NDA: New drug application

N.S.: Not significant

AUC: Area under the plasma concentration-time curve

b.i.d. (bis in die): Administered twice a day

SD: Standard deviation

SEM: Standard Error

Chapter 1

Background

Importance of Controlling Blood Glucose Level for Diabetes

There are 2 kinds of diabetes[1)]. The one is “Diabetes mellitus type 1”, the other is “Diabetes mellitus type 2”. Diabetes mellitus type 1 is a disease caused by the lack of insulin. Insulin must be administered for type 1 by injection or inhalation. Diabetes mellitus type 2 is due to lack of insulin release, insulin resistance by cells, and so on. Treatments for type 2 are,

(1) using agents that increase the amount of insulin secreted by the pancreas

(2) using agents that increase the sensitivity of target organs to insulin

(3) using agents that decrease the rate at which glucose is absorbed from the gastrointestinal tract.

Some drugs are used often in combination.

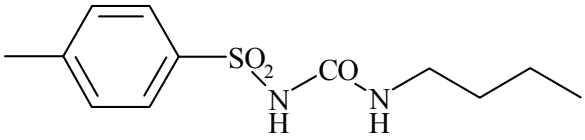
Ordinary oral antidiabetics for type 2 are classified into two types[1-19)]. The first type primarily controls PBG, while the other type primarily controls FBG[2, 3)]. There is currently no oral antidiabetic capable of controlling both PBG and FBG, and such an antidiabetic is believed to be the most useful for the treatment of diabetes. Table 1-1 shows anti-diabetic oral preparations for type 2 diabetes on the market.

Table1-1: Typical anti-diabetic oral preparations for type 2 diabetes on the market[4-19].

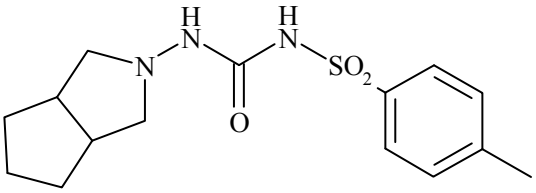
Drug substance	Type	Drug product	Maker
Gliclazide	sulfonylureas	Glimicron ®	Dainippon Sumitomo Pharm.
Glibenclamide		Euglucon®	Chugai Pharmaceutical
Glimepiride		Amaryl®	Sanofi
Nateglinide	meglitinides	Fastic®	Ajinomoto Pharmaceutical
Repaglinide		Surepost®	Dainippon Sumitomo Pharm.
Mitiglinide		Glufast®	Kissei
Pioglitazone	thiazolidinediones	Actos®	Takeda Pharmaceutical
Metformin	biguanides	Metgluco®	Dainippon Sumitomo Pharm.
Voglibose	alpha glucosidase inhibitors	Basen®	Takeda Pharmaceutical
Acarbose		Glucobay®	Bayer
Sitagliptin phosphate	DPP -IV inhibitors	Januvia®	MSD
Tofogliflozin	SGLT-2 inhibitors	Deberza®	Kowa
Ipragliflozin		Suglat®	Astellas
Canagliflozin		Canaglu®	Tanabe-Mitsubishi
Empagliflozin		Jardiance®	Boehringer Ingelheim, Japan
Sergliflozin etabonate		under developing	

Increasing insulin secretion from the pancreas has been the major area targeted to treat type 2 diabetes. SU agents increase insulin secretion[1]. They primarily lower FBG by increasing the release of insulin from the pancreas. The first generations of these drugs include tolbutamide, while the2nd drugs include gliclazide (Glimicron®)[4], Glibenclamide (Euglucon®)[5], and the 3rd drugs include glimepiride (Amaryl®)[6]. Sulfonylureas have the risk of causing hypoglycemia.

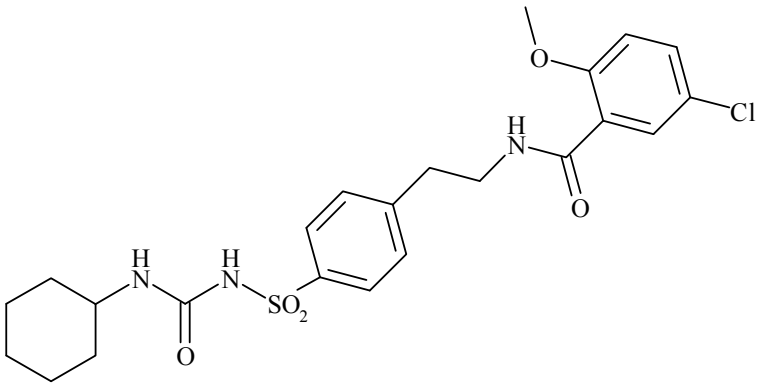
tolbutamide



gliclazide



glibenclamide



glimepiride

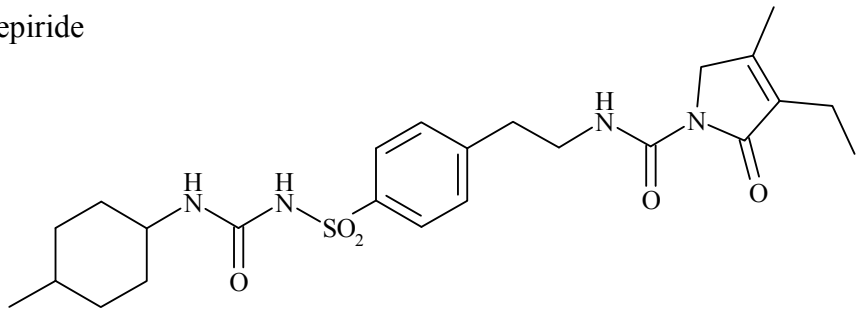
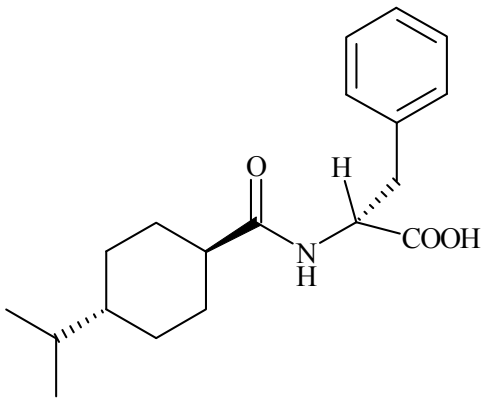


Figure 1-1: Chemical structure of sulfonylureas.

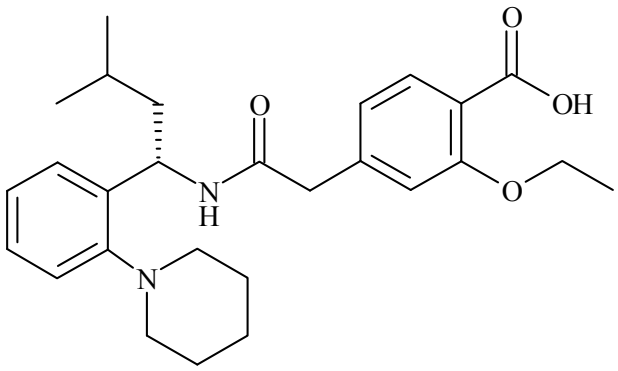
Representative examples were indicated.

Meglitinides help the pancreas insulin secretion and are often called "short-acting type insulin secretagogues". Meglitinides primarily decrease PBG by increasing the release of insulin from the pancreas. Meglitinides include nateglinide[7], repaglinide[8] and mitiglinide[9]. These drugs have to be taken just before meal to show post prandial blood glucose levels lowering effect. In this study, nateglinide was used as an active ingredient for controlled release formulation design.

nateglinide



repaglinide



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mitiglinide

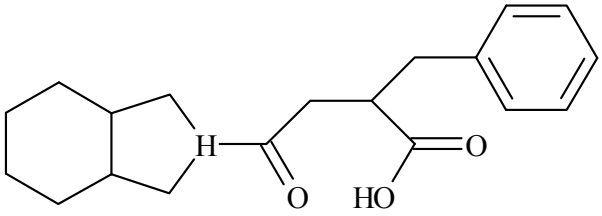


Figure 1-2: Chemical structure of meglitinides.

Representative examples were indicated.

Thiazolidinediones are known as "glitazones" which bind to PPARγ, a type of nuclear regulatory proteins involved in transcription of genes regulating glucose and fat metabolism. Thiazolidinediones primarily decrease FBG. They include pioglitazone[10].

pioglitazone

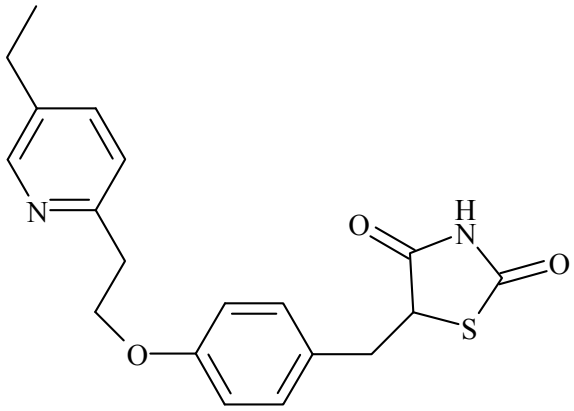


Figure 1-3: Chemical structure of thiazolidinediones.

Representative examples were indicated.

Biguanides reduce hepatic glucose output and increase the uptake of glucose by the periphery, including skeletal muscle. They primarily decrease FBG, and include metformin[11]. Metformin has become the most commonly used agent for type 2 diabetes.

metformin

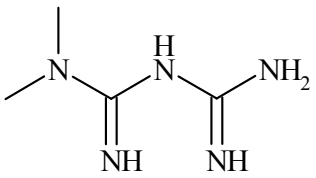
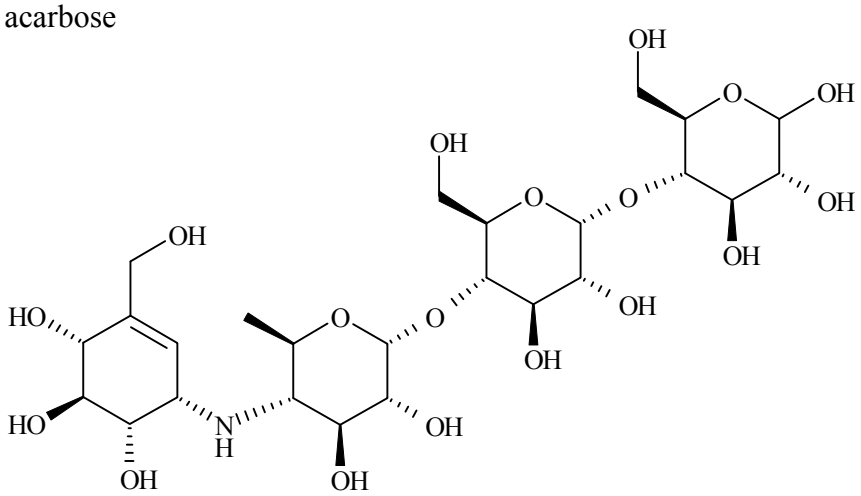


Figure 1-4: Chemical structure of biguanides.

Representative examples were indicated.

Alpha-glucosidase inhibitors do not have a direct effect on insulin secretion or sensitivity. These agents slow the digestion of carbohydrates in the small intestine, so that glucose derived from the carbohydrates in a meal is absorbed more slowly. They primarily decrease PBG. They include voglibose[12] and acarbose[13]. They have to be taken just before a meal to show PBG lowering effect.

acarbose



voglibose

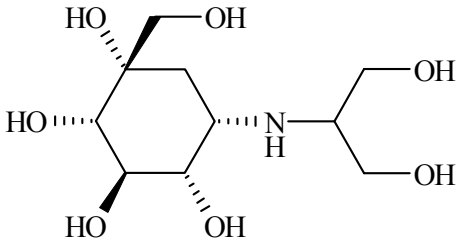


Figure 1-5: Chemical structure of alpha glycosidase inhibitors.

Representative examples were indicated.

GLP-1 works on glucose-mediated insulin secretion. It is broken down by an enzyme called DPP- IV. So DPP-IV inhibitors promote insulin secretion. They include sitagliptin phosphate[14]. They fundamentally control both PBG and FBG, if the blood glucose level is high. However further clinical research is necessary.

sitagliptin

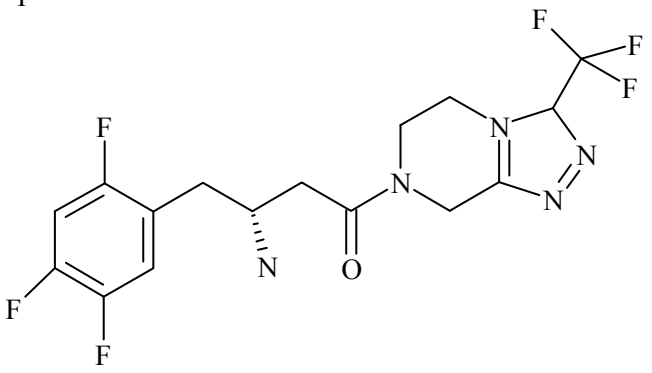
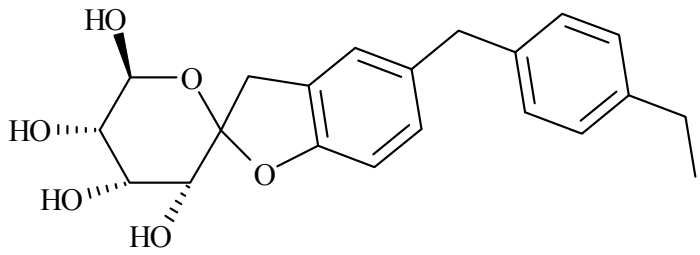


Figure 1-6: Chemical structure of DPP-IV inhibitors.

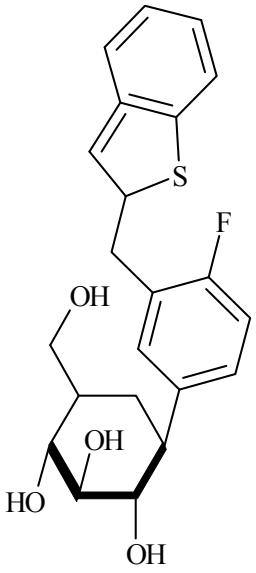
Representative examples were indicated.

SGLT2 is a member of the sodium glucose cotransporter family which are sodium-dependent glucose transport proteins. SGLT2 is the major co-transporter involved in glucose the reabsorption in the kidney. When inhibiting SGLT2, the reabsorption of glucose in the kidney is restricted, consequently blood glucose level decreases. Some drugs have been in the market and others are currently undergoing clinical trials.

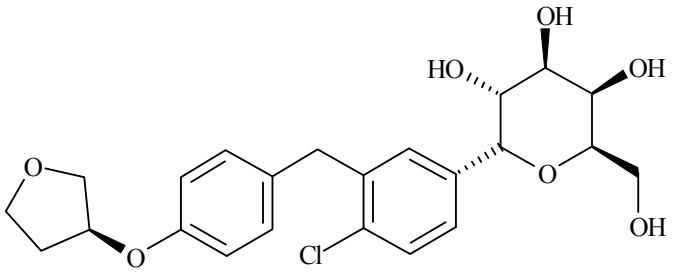
tofogliflozin[15)]



ipragliflozin[16)]



empagliflozin[17)]



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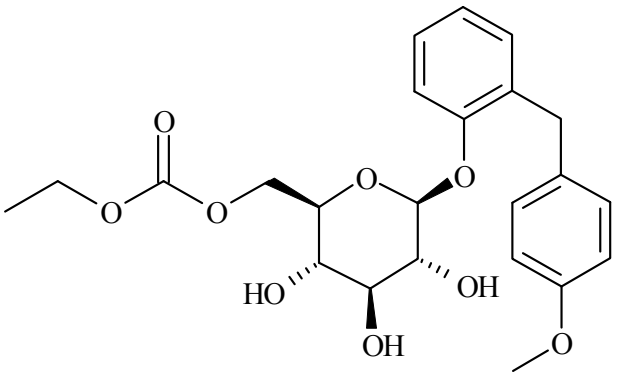
sergliflozin [18)]



canagliflozin[19)]

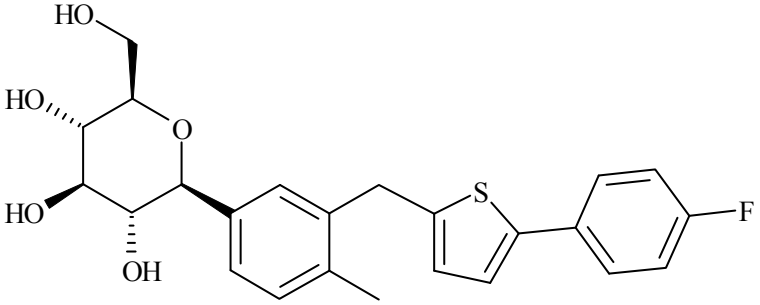


Figure 1-7: Chemical structure of SGLT-2 inhibitors.

Representative examples were indicated.

Rosiglitazone/metformin (Avandamet®)[20)], pioglitazone/glimepiride (Duetact®)[21)] and pioglitazone/metformin (Actosplusmet®)[22)] are new combination drug products that are on the market to treat diabetes. The benefit with these combination drugs is to lead to better compliance, because there are fewer tablets to take. However, the above drug products cannot effectively/timely control both PBG and FBG.

It is important to control the blood glucose level so as to prevent complications, such as microangiopathy, atherosclerosis. Evaluation items and control levels are shown in Table 1-2. HbA1c values are indexes that reflect average blood glucose levels of diabetes. It is the first selection index of diabetic control and patient's self-management. However, circadian variation of blood glucose levels is not able to be estimated by HbA1c values. Furthermore, there are other factors that change HbA1c values. Not only HbA1c values but FBG, PBG (after the meal in 2 hr), blood glucose level profile should be considered so as to estimate control level of diabetes[1)].

Table 1-2: Evaluation item and control level[1)].

Control level / Evaluation item	Excellent	Good	Fair	Poor
HbA1c (%) (JDS)	Less than 5.8	5.8-6.5	6.5-7.9 (Insufficient) 7.0-8.0 (defect)	8.0 or more
HbA1c (%) (International, NGSP)	Less than 6.2	6.2-6.9	6.9-7.4 (Insufficient) 7.4-8.4 (defect)	8.4 or more
FBG (mg/dL)	80-110	110-130	130-160	160 or more
PBG (mg/dL) (after the meal in 2 hr)	80-140	140-180	180-220	220 or more

NGSP value (%) = JDS (%) × 1.02 + 0.25

Generally, the treatment plan for type 2 diabetes as shown in the Figure 1-8 is conducted[23]. If it is impossible to decrease blood glucose levels by control of amount of meal/activity, oral preparation for diabetes is necessary. If it is impossible with the oral preparation, insulin therapy is necessary.

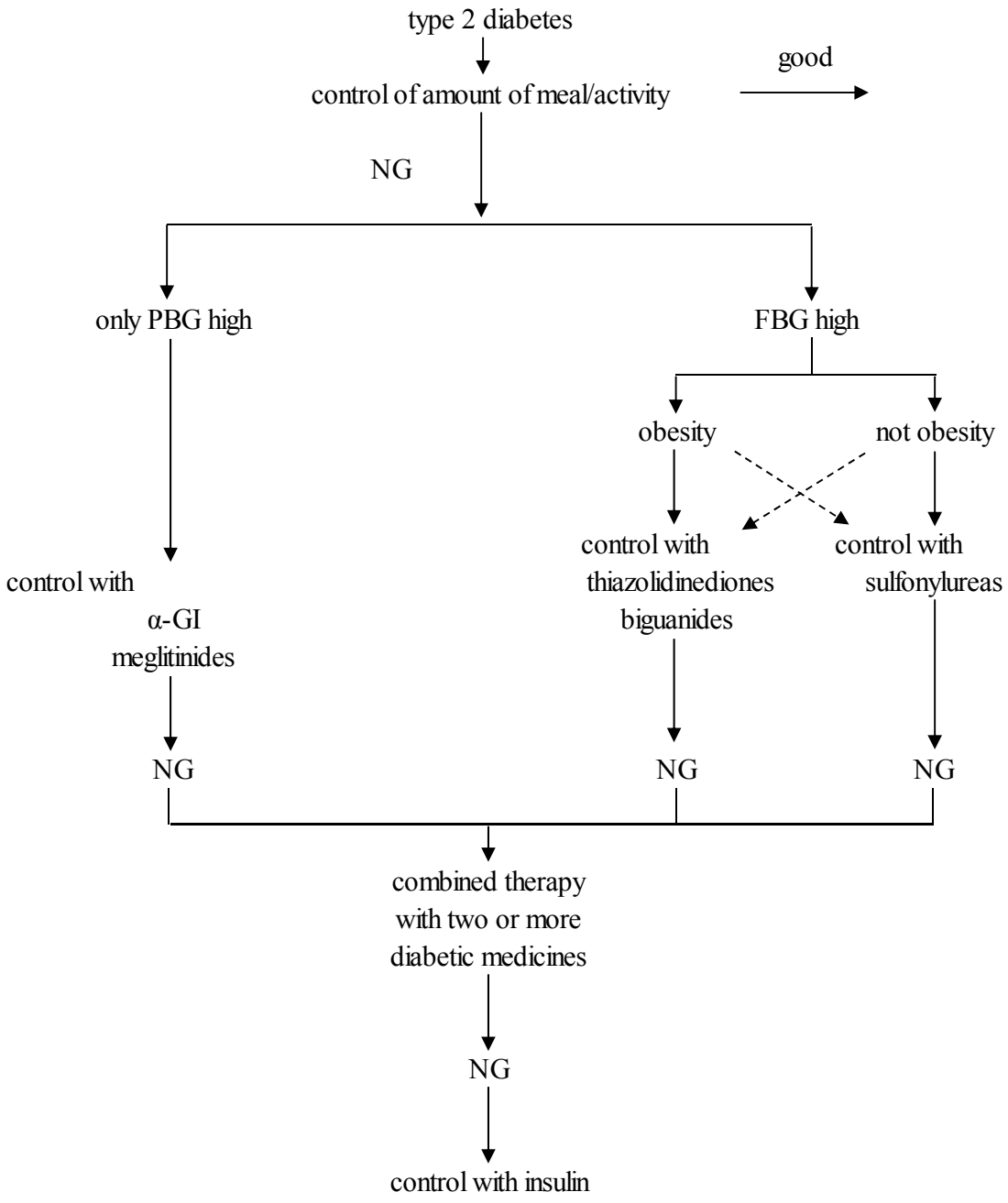


Figure 1-8: Treatment of diabetes[23].

Ordinary antidiabetics for oral administration are classified into two types. The first type primarily controls PBG, while the other type primarily controls FBG[1-3]. It is important to control FBG in patients with moderate and severe diabetes who exhibit elevated FBG levels. However, there is currently no oral antidiabetic capable of controlling both PBG and FBG sufficiently/timely. The oral anti-diabetics agents mentioned above show different blood glucose levels (Figure 1-9).

The author thought that control of a blood glucose level might be possible with controlled release formulation containing an insulin secretagogue, nateglinide.

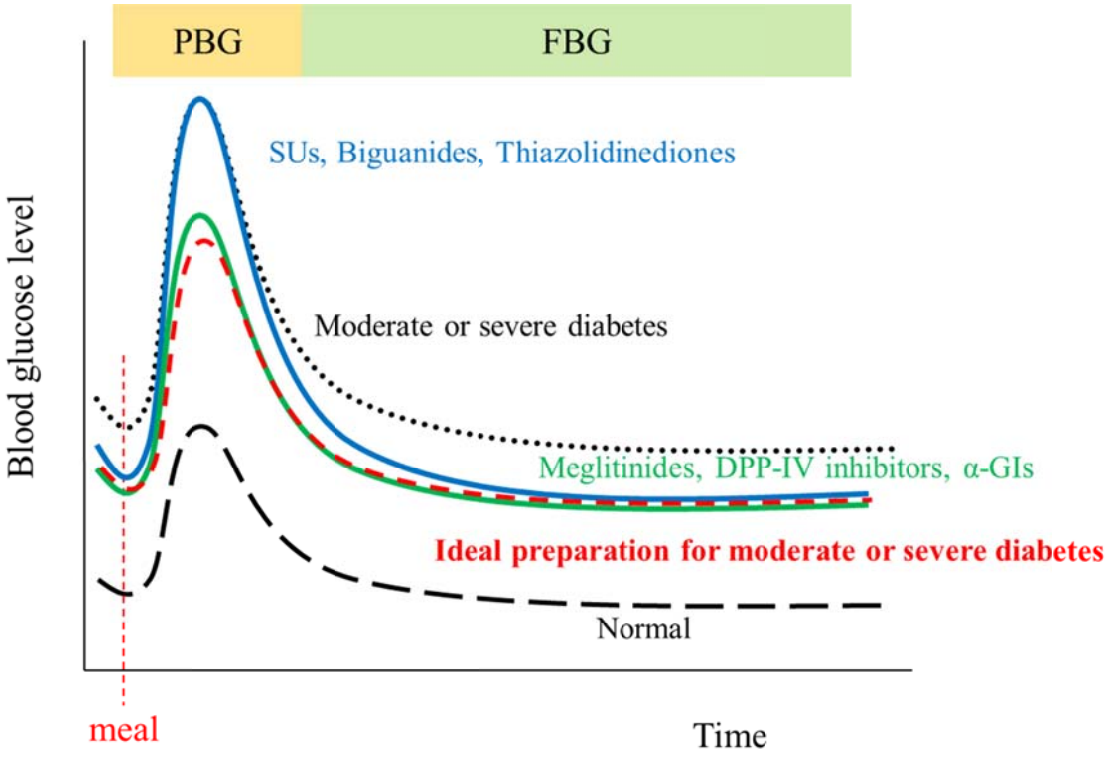


Figure 1-9: Pattern diagram of blood glucose levels.

DDS Technology for Oral Administration

When considering oral DDS technology, it is important to understand gastro-intestinal transit time and pH in human (Figure 1-10). DDS technologies as below are known[24], and show in each of the following paragraphs in detail.

○ pH Dependent technology

Material that is dissolved by triggering pH is used.

Dosage form: Enteric coated tablets, Enteric coated granules,
Enteric matrix tablets, Enteric matrix granules

○ Time dependent technology

Material that is dissolved slowly, or not dissolved is used.

Dosage form: Coated tablets, Coated granules,
Matrix tablets, Matrix granules

○ Others

Material that is dissolved/decomposed by flora in the gut is used.

Dosage form: Tablets, Granules

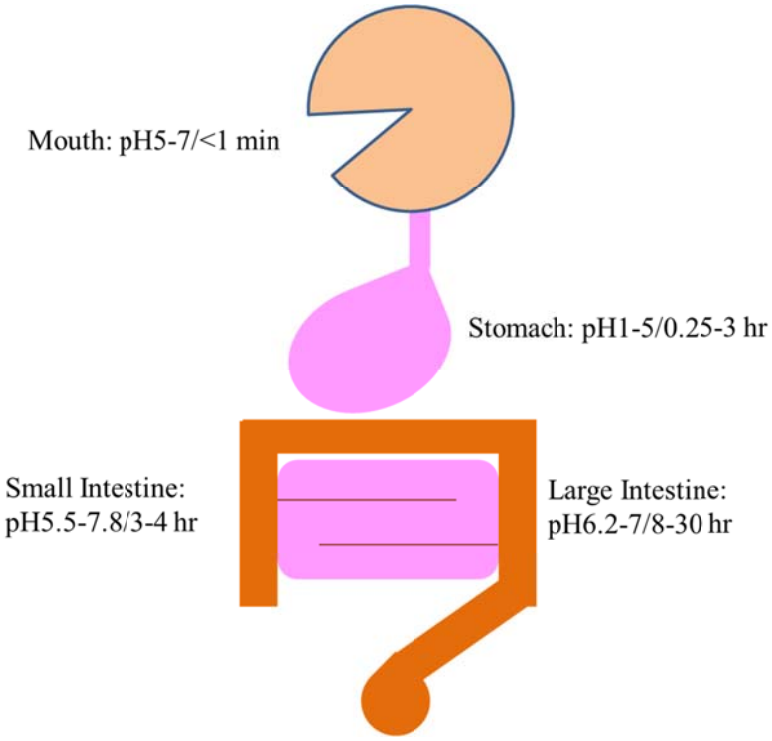


Figure 1-10: pH profile and transit times in human gastro intestinal tract. Drawn based on an information described in an e-learning program by Evonik Industries: "https://e-lab.eudragit.com/".

Regarding pH dependent technology, enteric materials are generally used[24-26]. Coating or granulating or tableting is conducted with the materials. The materials are anionic polymers, such as poly acrylic polymer derivatives and anionic poly saccharides derivatives. Typical enteric materials are shown in Table 1-3. The obtained enteric formulation shows pH dependent release profile *in vitro*. In an acid pH region, a drug is not released. This property is called "enteric property", or "acid resistance". In neutral pH region, the drug is released. The release behavior shows as "retard" or "prolonged". Enteric coated tablets show retarded release profile *in vivo*. On the other hand, enteric coated granules show prolonged release *in vivo*, when administered after meal[27].

Table1-3: Typical enteric coating materials.

Generic Name	Trade Name / Grade	Maker
dry methacrylic acid copolymer LD / methacrylic acid copolymer LD	Eudragit®L100-55 / Eudragit®L30D-55	Röhm GmbH
methacrylic acid copolymer S	Eudragit®S100	Röhm GmbH
methacrylic acid copolymer L	Eudragit®L100	Röhm GmbH
hydroxypropylmethylcellulose phthalate	HPMCP	Shinetsu Chemical
hydroxypropylmethylcellulose acetate succinate	AQOAT®	Shinetsu Chemical

<Enteric coated granules>

Enteric coated granules are obtained by the coating with enteric coating material on core granules. Core granules refer to the original granules to be coated. Core granules can be manufactured by obtaining granules that contain the drug and have a shape suitable for coating, for example using an extrusion granulation method and high speed stirring granulation method. With highly plasticized coating film, the obtained enteric coated granules are able to be compressed to prepare tablet formulation[26].

<Enteric coated tablets>

Enteric coated tablets are obtained by coating with an enteric coating material on core tablets. Core tablets refer to the original tablets to be coated. Core tablets can be manufactured with a tableting machine. Coating methods include a fluidized bed coating method and a tumbling coating method for granules. On the other hand, the methods include a pan coating method for tablets.

<Enteric matrix (granules, tablets)>

An enteric matrix is obtained by mixing enteric material and active ingredient, and the mixture is compressed. The form includes a granule or a tablet. Details are

described in <Enteric coated granules>, <Enteric coated tablets>.

Regarding time dependent technology, poor-water soluble materials are generally used[24,25]. Coating or granulating or tableting is conducted with the materials. The materials are non-ionic polymers, such as poly acrylic polymer derivatives and poly saccharides derivatives. Typical materials are shown in Table 1-4. The obtained time dependent release formulation shows the time dependent release. The release behavior shows "prolonged". About a formulation form, it is similar to the contents described in <Enteric coated granules>, <Enteric coated tablets>.

Table1-4: Typical time dependent release coating materials.

Generic Name	Trade Name / Grade	Maker
aminoalkyl methacrylate copolymer RS	Eudragit®RSPO	Röhm GmbH
ethylcellulose	EC	Dow chemical

Degradable/non degradable formulation is obtained by dispersing a drug in a "gel structure" comprising of poor-water soluble materials / water sellable materials / water soluble polymers[24]. They are classified into two types. One is a degradable (erosion) type, and another is a non-degradable (non-erosion) type. Typical matix materials are shown in Table 1-5. The erosion type matrix formulation is obtained with water soluble polymers. On the other hand, non-degradable matrix formulation is obtained with poor-water soluble materials. The obtained matrix formulation shows a pH dependent release, a time dependent release or a timed release profiles *in vitro*.

Matrix granules can be manufactured by obtaining granules that contain the drug, for example using an extrusion granulation method and a high speed stirring

granulation method. Matrix tablets can be manufactured with a tableting machine.

Table 1-5: Typical matrix materials.

	Generic Name	Trade Name / Grade	Maker
degradable	hydroxypropylcellulose	HPC	Nisso
	hydroxypropylmethylcellulose	HPMC	Shinetsu Chemical
	dry methacrylic acid copolymer LD / methacrylic acid copolymer LD	Eudragit®L100-55 / Eudragit®L30D-55	Röhm GmbH
	methacrylic acid copolymer S	Eudragit®S100	Röhm GmbH
	methacrylic acid copolymer L	Eudragit®L100	Röhm GmbH
	hydroxypropylmethylcellulose phthalate	HPMCP	Shinetsu Chemical
	hydroxypropylmethylcellulose acetate succinate	AQOAT®	Shinetsu Chemical
non degradable	aminoalkyl methacrylate copolymer RS	Eudragit®RSPO	Röhm GmbH
	ethylcellulose	EC	Dow chemical
	hydrogenated oil	Caster wax	NOF

Next, a modified release formulation containing both immediate release portion and controlled release portion is described[24-27]. Regarding the modified release formulation containing both immediate release granules and enteric coated granules, the modified release granules are obtained by mixing both immediate release granules and enteric coated granules[27]. Capsules are also obtained by filling the above mentioned granules into a capsule shell. With enteric coated granules comprising of highly plasticized enteric coating film[26], a modified release tablet is able to be obtained. Figure 1-11 shows the modified release tablet containing both immediate release granules and compressionable enteric coated granules. This formulation shows both an immediate release profile and a pH dependent release profile *in vitro*. This is obtained by tableting after mixing both immediate release granules and enteric coated granules with lubricants. As the enteric coating film is highly plasticized, acid resistance of enteric coated granules was maintained even after tableting.

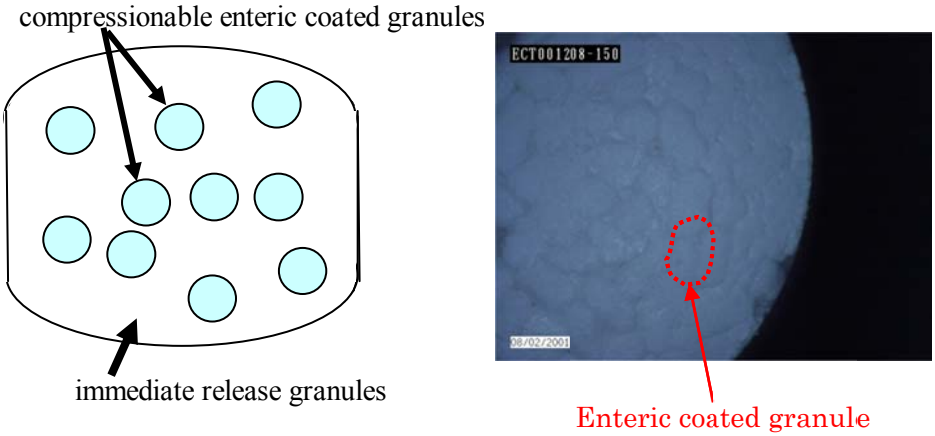


Figure 1-11: The section (left) and the surface (right) of controlled release tablet containing both compressionable enteric coated granules and immediate release granules.

The territory surrounded with a red dotted line indicates enteric-coated granule.

Then, a modified release formulation containing both immediate release granules and time dependent release granules is described. Modified release granules are obtained by mixing both immediate release granules and time dependent release granules. Capsule drug products are also obtained by filling the above mentioned granules into a capsule shell[28]. Furthermore, modified release formulation containing both immediate release granules and matrix tablet is described. With a matrix tablet, a modified release formulation is able to be obtained. Figure 1-12 shows the modified release tablet containing both immediate release granules and a matrix tablet. This tablet is called a "dry coated tablet". A special tableting machine is necessary to produce the dry coated tablet that has both "granules" feeding equipment and "core tablet" feeding equipment. This formulation shows an immediate release profile, then a

time dependent release profile *in vitro*.

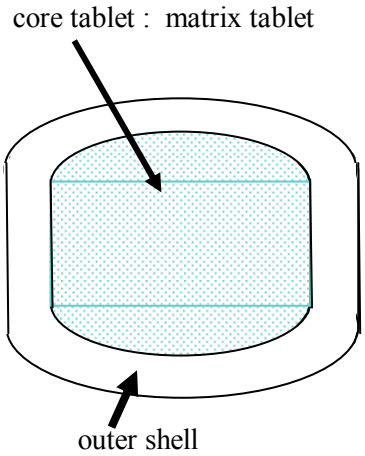


Figure 1-12: The section of controlled release tablet containing both a matrix tablet and immediate release granules.

Immediate release granules form an outer shell.

The modified release formulation containing both an immediate release portion and a controlled release portion using nateglinide is expected to control both PBG and FBG (Figure 1-13).

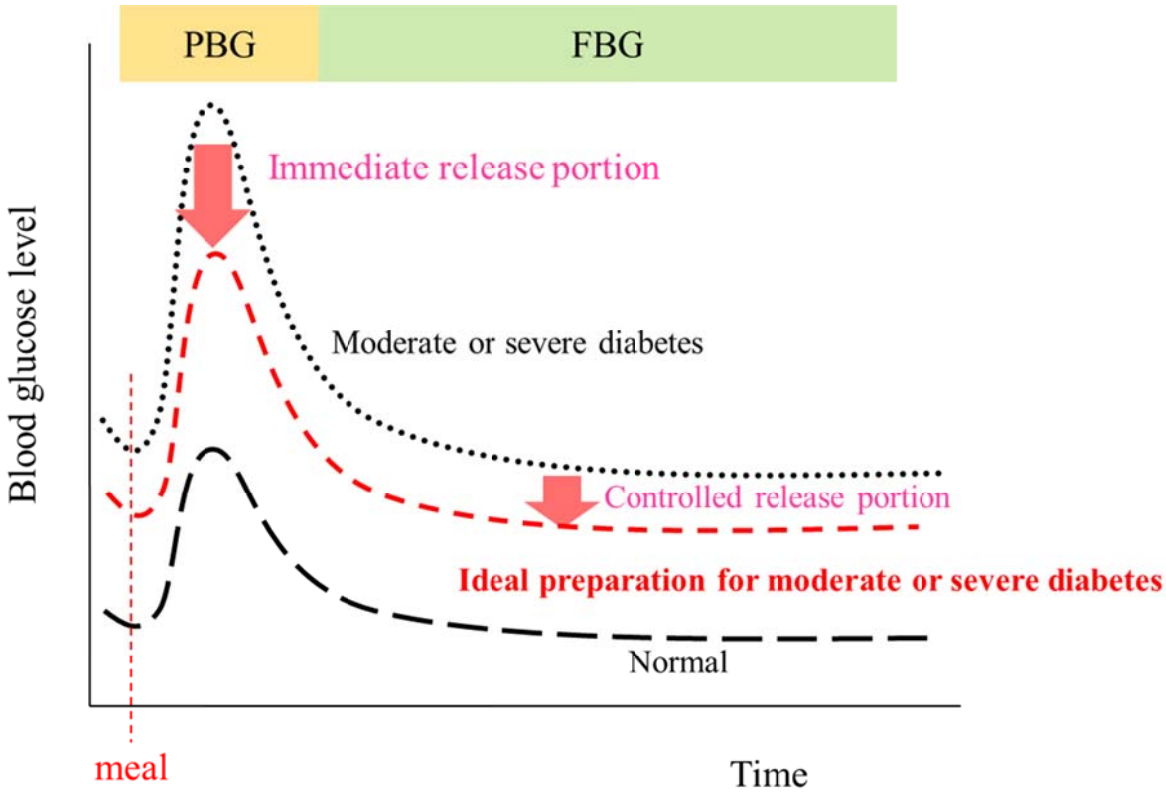


Figure 1-13: Pattern diagram of blood glucose levels.

Immediate release portion decreases PBG.

Controlled release portion decreases FBG.

Concern in Multiple Administration of Insulin Secretagogue

It is thought that control of a blood glucose level might be possible with the controlled release formulation containing an insulin secretagogue, nateglinide, that demonstrates a quick action/short duration type effect[3]. If the nateglinide oral controlled release formulations were available that were 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; nateglinide immediate release tablet), and (2) increasing the number of choices available for treating diabetes in moderate and severe diabetes patients.

Nateglinide stimulates insulin secretion. It has a different chemical structure from sulfonylureas[3], 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 [3].

Gliclazide is an anti-diabetic agent 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 effect has been reported to weaken during the second administration[3] (Figure 1-14). 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 possible to control PBG, it may not be possible to adequately control FBG.

On the other hand, both nateglinide and SU agents bind on the SU receptor. However, it was reported that nateglinide dissociates rapidly more than SU dose[29]. Then the author expected that it is possible to decrease both PBG and FBG with

multiple-administering nateglinide controlled release formulation.

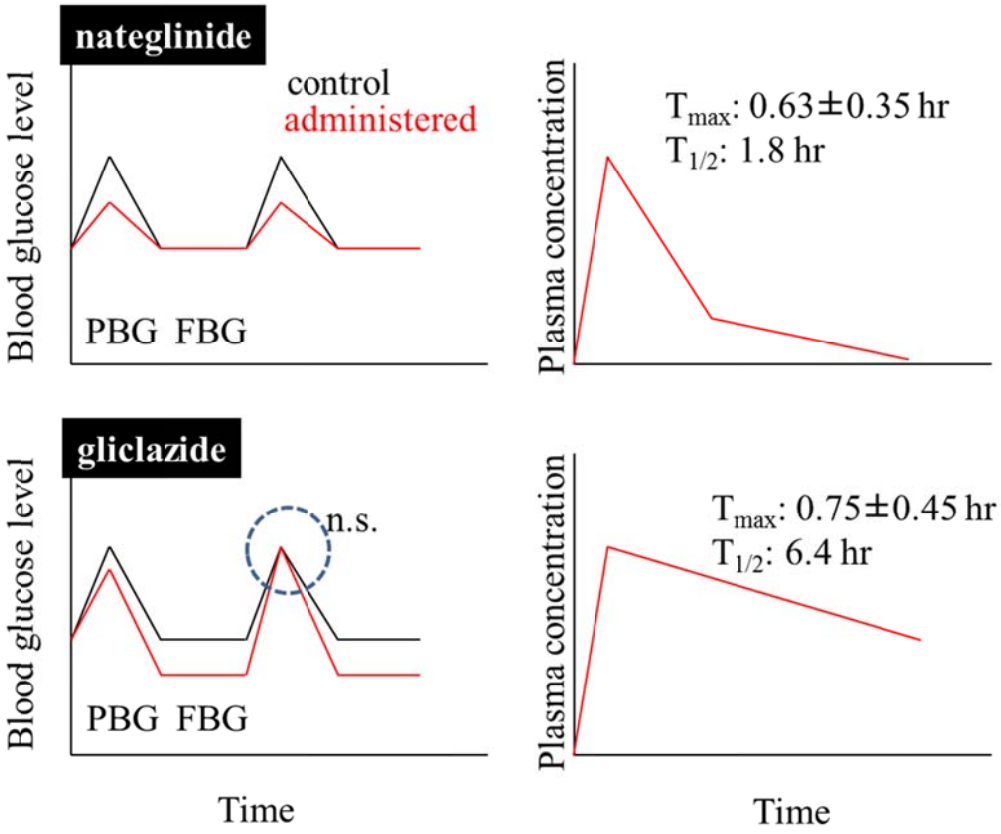


Figure 1-14: Concern in multiple administration of insulin secretagogue.

(altered with reference 3)

In the case of repeated administration of gliclazide to normal rats, the blood glucose lowering effect has been reported to weaken during the second administration.

Chapter 2

Effect of Decrease in both PBG and FBG in Normal Beagle Dogs with Controlled Release Form of Nateglinide

2.1. Design of Nateglinide Enteric Coated Granules and Evaluation of Dissolution Profile

Summary

The D-phenylalanine derivative, nateglinide ((-)-*N*-(*trans*-4-isopropylcyclo-hexanecarbonyl)-D-phenylalanine, Figure 1-2) is a new QASD type of oral blood glucose regulator. In this chapter 2, the author attempted to determine if it was possible to control both PBG and FBG in normal beagle dogs with 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 by fluidized bed coating. The obtained granules had good acid resistance and dissolution pH values of 5.5, 6.5 and 7.2 respectively.

Material and Methods

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), Hydroxypropylmethylcellulosephtalate220824 (HPMCP HP-50, Shinetsu Chemical Co. Ltd., Japan), macrogol6000 (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 immediate release tablets.

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.

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 μm - 1400 μm granules were obtained by grading, and then used for coating.

Table 2-1 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 (hydroxypropylmethylcellulosephtalate220824), enteric coated granules B (dry methacrylic acid copolymer LD), enteric coated granules C (methacrylic acid copolymer S).

Table 2-1: 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	−	−
macrogol6000	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

The dissolution profiles of the resulting granules were evaluated (JP16, 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 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^{o}C (at a flow rate of 1.5 mL/min).

Results

Nateglinide core granules were coated using the coating solutions shown in Table 2-1 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 minutes in JP1 fluid containing 0.6 w/v% Polysorbate 80) through coating using methacrylic acid copolymer LD coating solution (Figure 2-1). The 120 minute 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 minute dissolution rate at pH 1.2 was found to be 10% or less. 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 hydroxyproprylmethylcellulose phthalate 220824 and methacrylic copolymer S at 24.0 w/w% and 33.9 w/w%, respectively. As shown in Table 2-2, the 120 minute 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.

Moreover, dissolution behavior in the neutral pH region was also evaluated for the resulting enteric coated granules (Figure 2-2). Here, the dissolution behavior of each the enteric coated granules was classified based on the approach of dissolution pH[27]. In this study, dissolution pH is defined as the pH at which the 60 minute 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.

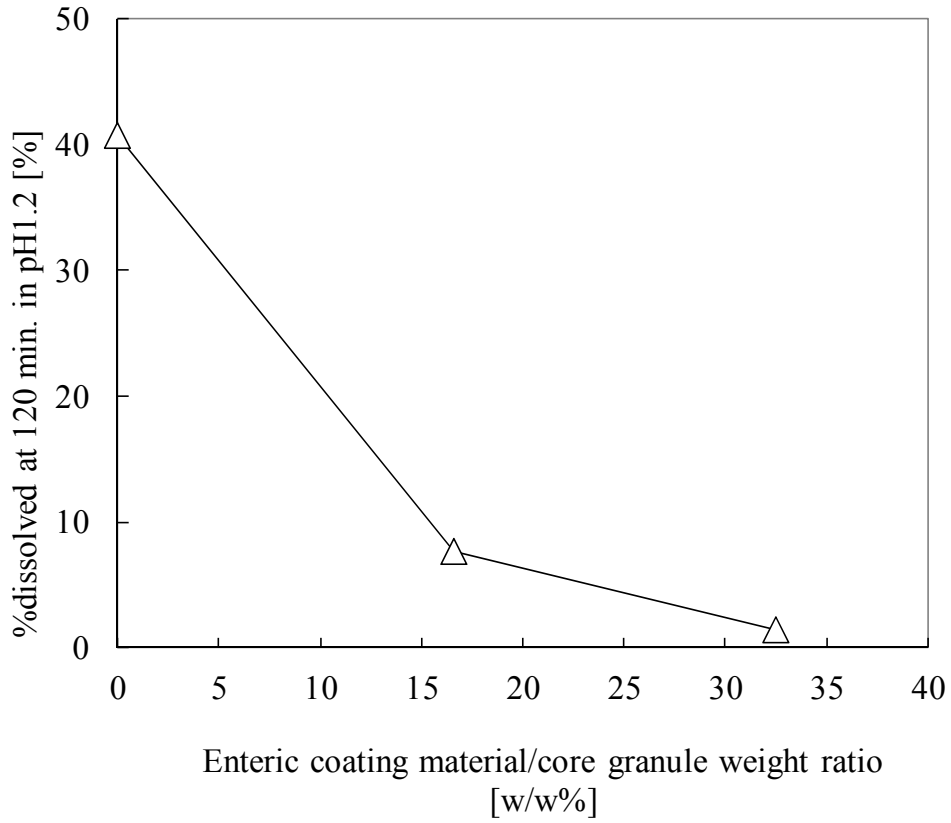


Figure 2-1: Relationship between an amount of enteric coating material and enteric property.

Dry methacrylyc acid copolymer LD was used as an enteric coating material.

JPXIII paddle method (50 rpm), n=3, nateglinide: 90 mg/vessel,

Medium: JP1 fluid containing 0.6 w/w% polysorbate 80.

Table 2-2: Enteric property of the obtained granules.

*: JP1 fluid containing 0.6 w/w% polysorbate 80, mean ± SD (n=3).

enteric coated granules	%dissolved in JP1fluid* at 120min [%]
A	0.9±0.3
B	1.4±0.3
C	0.5±0.0

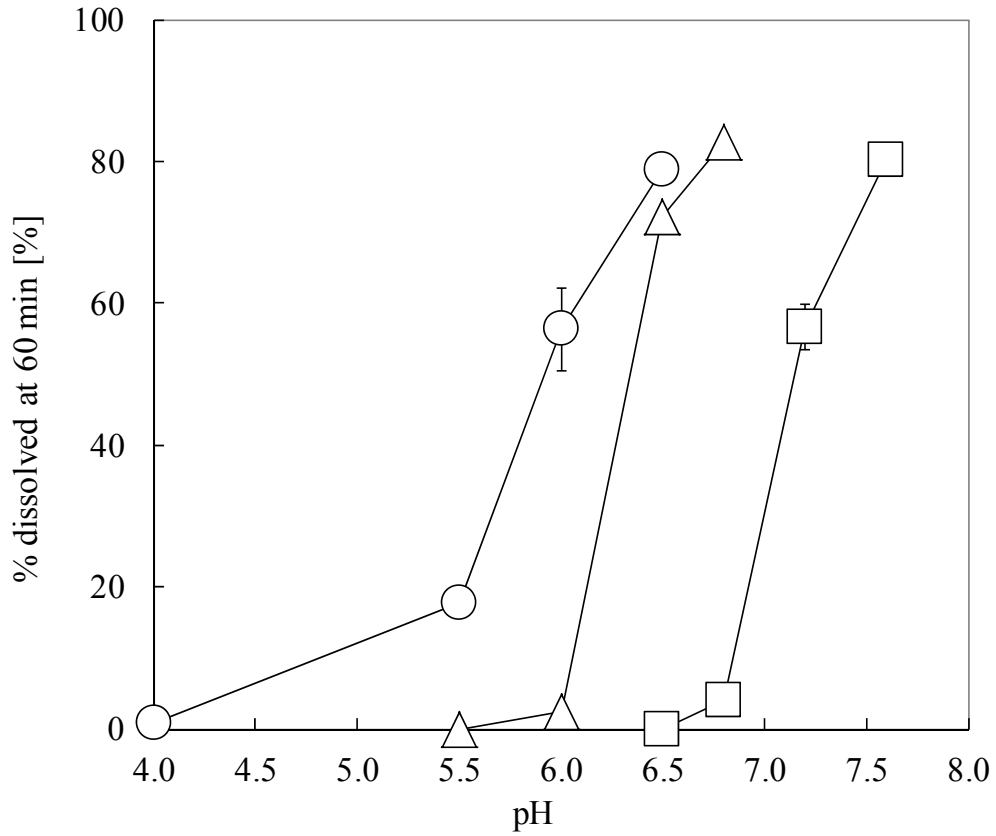


Figure 2-2: pH Dissolution relationship for 3 types of enteric coated granules.

JPXIII paddle method (50 rpm), n=3, nateglinide: 90 mg/vessel, mean ± SD, 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, △: B, □: C.

Discussion

Nateglinide is poorly water soluble drug. Polysorbate 80 was added to acid pH testing fluid to satisfy sink condition. Figure 2-1 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%.

Dissolution behavior in the neutral pH region was also evaluated for the resulting enteric coated granules. Here, the dissolution behavior of each the enteric coated granules was classified based on the approach of dissolution pH as advocated in reference 27. In this study, dissolution pH is defined as the pH at which the 60 minute 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[30]. 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 immediate release 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 immediate release 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. When these enteric coated granules are administered with food, controlled release of nateglinide is expected.

2.2. Nateglinide Plasma Concentration Profile, Insulin Plasma Concentration Profile and Blood Glucose Level When Administering Nateglinide Enteric Coated Granules just before Feeding in Normal Beagle Dogs

Summary

The obtained three types of enteric coated granules were each administered separately 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.

Materials and Methods

The three types of enteric coated granules were obtained in 2.1. Fastic ® tablets (nateglinide: 30mg/tablet, Ajinomoto Co. Inc., Japan) were used as an immediate release tablets *in vivo* study. Prior to being conducted, *in vivo* studies were assessed according to the guidelines of Ethical Committee in INA Research Co., Ltd. (Japan). The guideline was established based on "Doubutsu no aigo oyobi kanri ni kannsuru houritsu (Law about protection of an animal and management)" (October 1st, 1973, No. 105), "Jikkendoubutsu no shiyou oyobi hokan nado ni kansuru kijyun (The standard

about the breeding of an experimental animal and the safekeeping, etc.)" (March 1st, Sourihu No. 6), “Jikkendoubutsu gaidorain no sakutei ni tsuite (About decision of an animal experiment guideline)" (November 5th, 1980, Sougakusho No. 1513), "Daigaku nado ni okeru doubutsujikken ni tsuite (About the animal experiment that is conducted in a university" (May 25th, 1987, Bungakujyou No. 141). 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, immediate release 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). Blood was sampled from a leg vein. Whole blood was centrifuged at 1700 *g* for 15 min at 5^{o}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 mol/L 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 mol/L 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^{o}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)[31]. 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 2-3. At a flow rate of 1.0 mL/min, nateglinide eluted at about 7.5 min at 40°C[31].

Table 2- 3 : Column switching program[31].

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: Mobile Phase A and C passed through a 600E pump system, injector and pre column.
Mobile Phase B passed through a 515 pump, main column and detector.
2: Mobile Phase A passed through a 600E pump system, injector, pre column, main column and detector.
A 515 pump stopped.

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 by using the Student's *t*-test.

Results

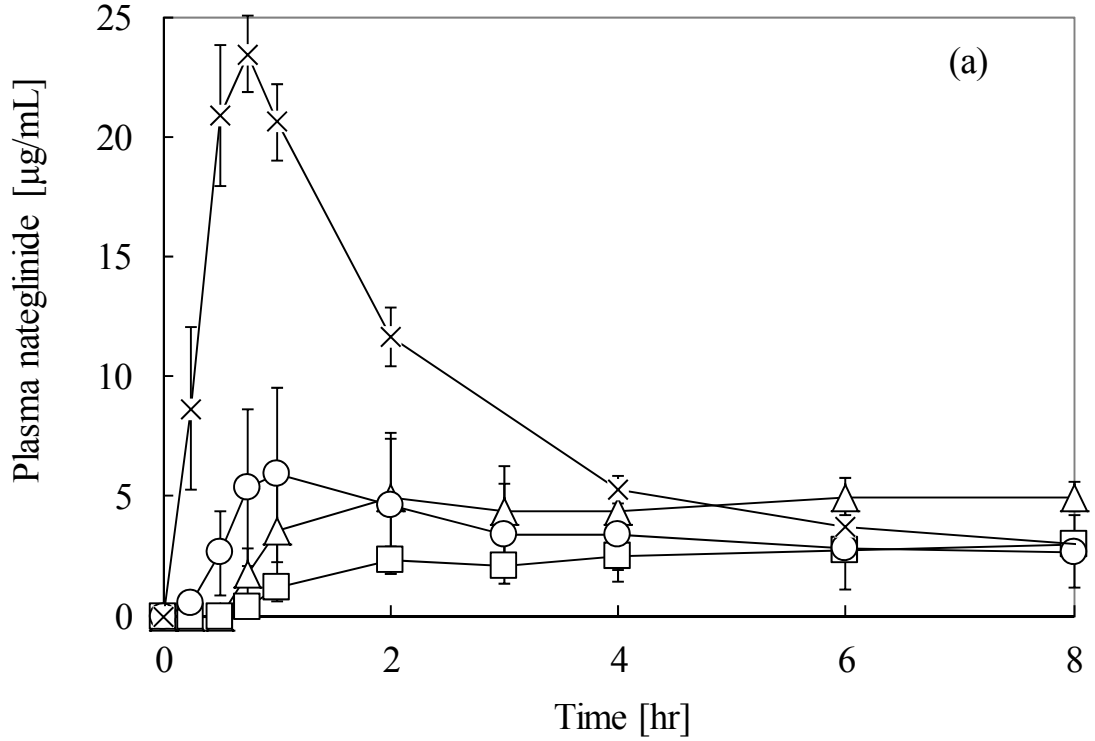
Enteric coated granules A, B and C were orally administered (nateglinide: 9 mg/kg) to normal beagle dogs just before feeding followed by measurement of plasma nateglinide concentrations, plasma insulin concentrations and blood glucose levels. Results are also shown for a control (feeding only) and immediate release tablets (nateglinide: 90 mg/body, ca. 9 mg/kg) used as controls.

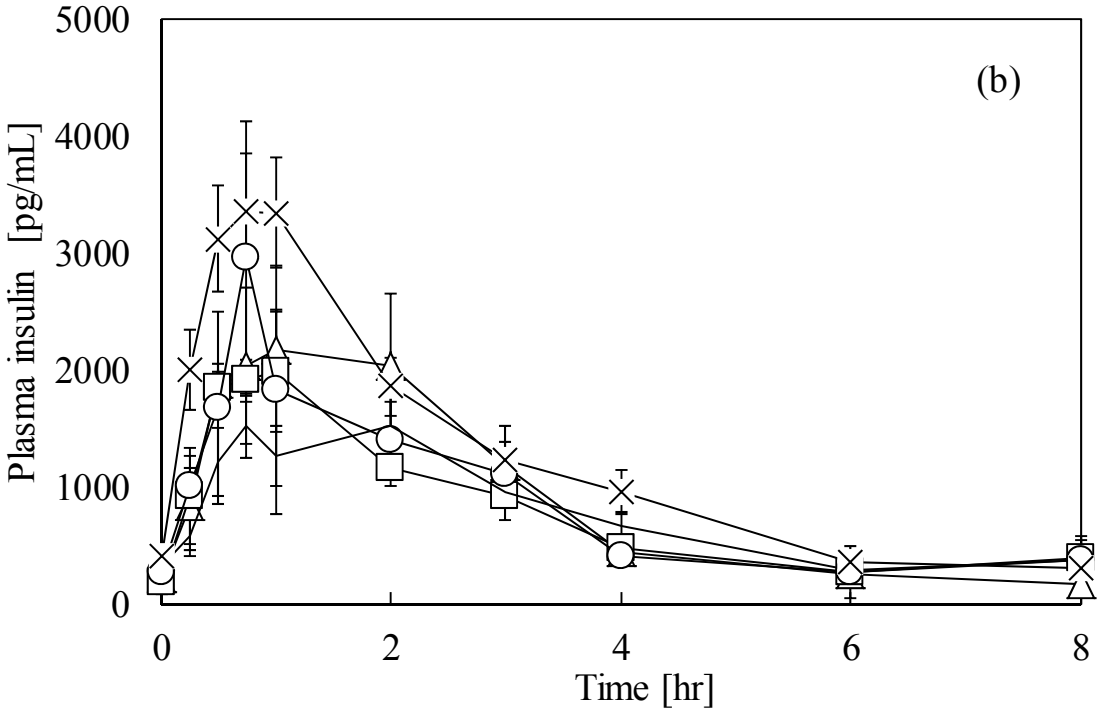
Plasma nateglinide concentration profiles are shown in Figure 2-3 (a). The release of nateglinide is inhibited as the dissolution pH increases, eventually leading to delayed absorption. The C_{max} values and AUC values of the enteric coated granules were lower than those of the immediate release tablets. However, an increasing trend was observed in plasma nateglinide concentrations of enteric coated granules B starting at 5 hr after administration as compared with that of the immediate release tablets.

Plasma insulin concentration profiles are shown in Figure 2-3 (b). The respective insulin C_{max} values consisted of 1521±385 pg/mL (T_{max}: 2 hr) for the control (feeding only), 3349±488 pg/mL (T_{max}: 0.75 hr) for the immediate release tablets , 2947±1169 pg/mL (T_{max}: 0.75 hr) for enteric coated granules A, 2163±337 pg/mL (T_{max}: 1 hr) for enteric coated granules B, and 1985±524 pg/mL (T_{max}: 1 hr) for enteric coated granules C. There were significant differences observed ($p<0.05$, Student's *t*-test) for plasma insulin concentrations from 0.25 hr to 1hr between the immediate release tablets and the control. And there were no significant differences observed for plasma insulin concentrations starting at 3 hr 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 Figure 2-3 (c). 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 immediate release tablets, FBG at 8 hr after administration did not decrease, although PBG decreased to a maximum of about 79% as compared with blood glucose levels immediately before administration.


(a)
Plasma nateglinide [μg/mL]
25
20
15
10
5
0
0
2
4
6
8
Time [hr]


(b)
Plasma insulin [pg/mL]
5000
4000
3000
2000
1000
0
0
2
4
6
8
Time [hr]

(continued)

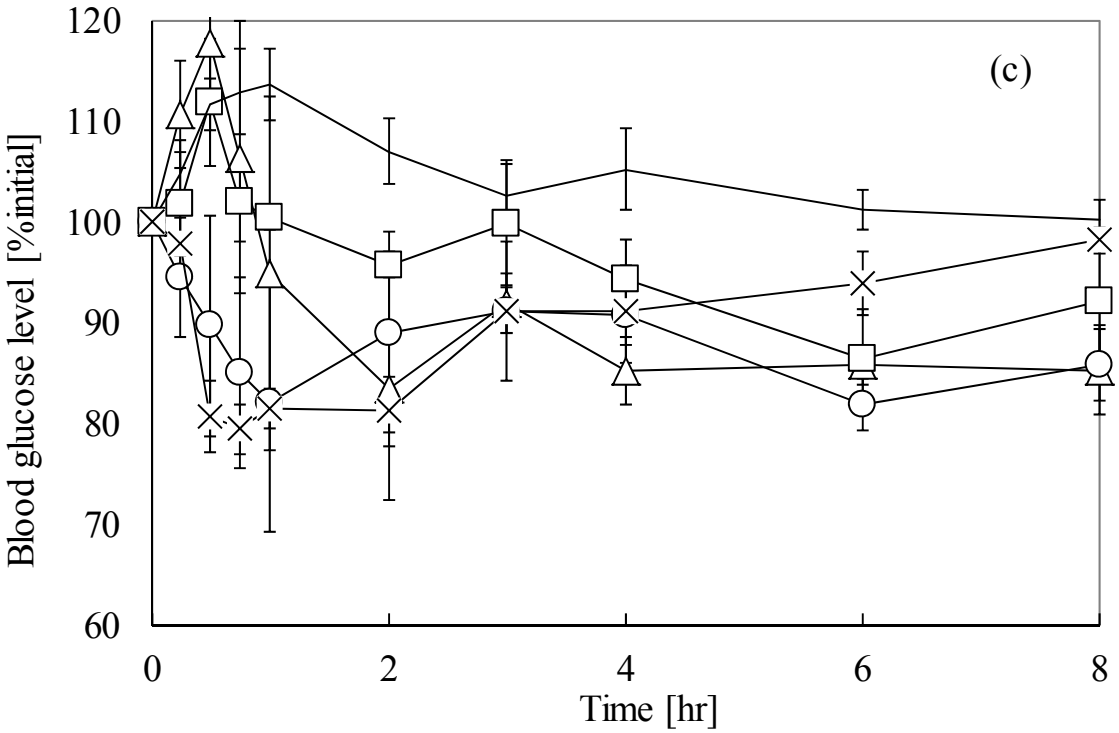


Figure 2-3: Plasma nateglinide concentration profile (a), plasma insulin concentration profile (b), blood glucose level (%initial) (c), after oral administration of enteric coated granules A (○), B (△), C (□) (nateglinide: 9 mg/kg) and immediate release tablets (×) (nateglinide: ca.9 mg/kg) in beagle dogs just before feeding, or only feeding (-).

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

Discussion

The obtained three types of enteric coated granules were each administered separately 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: 9mg/kg), the absorption of nateglinide was confirmed to tend to be delayed as the dissolution pH increased. This means nateglinide dissolved from enteric coated granules slowly *in vivo*.

There was no correlation observed between plasma nateglinide concentration and plasma insulin concentration. It is not obvious why there was no correlation although nateglinide was an insulin secretagogue. Further study is required.

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 : 9mg/kg), only decrease in PBG was observed. 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 immediate release 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.

2.3. Nateglinide Plasma Concentration Profile, Insulin Plasma Concentration Profile and Blood Glucose Level When Administering both Nateglinide Immediate Release Tablets and Enteric Coated Granules just before Feeding in Normal Beagle Dogs

Summary

The obtained 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.

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. In case of nateglinide immediate release tablets (nateglinide : 9 mg/kg), only decrease in PBG was observed.

On the basis of these results, it is confirmed to decrease both PBG and FBG in normal beagle dogs with controlled release of nateglinide. Nateglinide controlled

release formulation is considered to enable control of both PBG and FBG for moderate and severe diabetes patients.

Materials and Methods

Enteric coated granules B were obtained in 2.1. Fastic ® tablets (nateglinide: 30mg/tablet, Ajinomoto Co. Inc., Japan) were used as immediate release tablets *in vivo* study.

Prior to being conducted, protocols of in vivo studies were assessed according to the guidelines of Ethical Committee in INA Research Co., Ltd. (Japan). The guideline was established based on "Doubutsu no aigo oyobi kanri ni kannsuru houritsu (Law about protection of an animal and management)" (October 1st, 1973, No. 105), "Jikkendoubutsu no shiyou oyobi hokan nado ni kansuru kijyun (The standard about the breeding of an experimental animal and the safekeeping, etc.)" (March 1st, Sourihu No.6), “Jikkendoubutsu gaidorain no sakutei ni tsuite (About decision of an animal experiment guideline)" (November 5th, 1980, Sougakusho No.1513), "Daigaku nado ni okeru doubutsujikken ni tsuite (About the animal experiment that is conducted in a university" (May 25th, 1987, Bungakujyou No.141). 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 both immediate release 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 immediate release tablets only (nateglinide: 60, 150 mg), immediate release tablets (nateglinide: 60 mg) + enteric

coated granules B (nateglinide: 30 mg or 60 mg). Each point and vertical bar represent mean ± SEM (n=6). In case of oral administration of both immediate release 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 ± SEM (n=6), except at 180, 480, 540, 720, 1440 min (n=3). Blood sampling, determination of plasma nateglinide concentration, determination of plasma insulin concentration and statistical analysis were conducted according to 2.2.

Results

After deciding to focus on enteric coated granules B having a dissolution pH of 6.5 which effectively lowered FBG with hardly any decrease in PBG, enteric coated granules B and nateglinide immediate release 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 immediate release tablets only (nateglinide: 60 mg, 150 mg) used as controls.

Plasma nateglinide concentration profiles are shown in Figure 2-4 (a). The nateglinide C_{max} values were 15.63 ± 1.08 μg/mL (T_{max}: 0.75 hr) , 34.80 ± 5.15 μg/mL (T_{max}: 0.5 hr) for the immediate release tablets (nateglinide: 60 mg, 150 mg), 12.59 ± 1.83 μg/mL (T_{max}: 1 hr) for the immediate release tablets (nateglinide: 60 mg) + enteric coated granules B (nateglinide: 30 mg), 16.69 ± 0.53 μg/mL (T_{max}: 0.75 hr) for the immediate release tablets (nateglinide: 60 mg) + enteric coated granules B (nateglinide: 60 mg) and 15.60 ± 2.08 μg/mL (T_{max}: 1 hr) for the immediate release

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 immediate release tablets (nateglinide: 60 mg) and the immediate release tablets (nateglinide: 60 mg) + enteric coated granules groups (Student's *t*-test). This is because C_{max} values are only dependent on the immediate release component. On the other hand, plasma nateglinide concentrations from 3 hr to 12 hr 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 hr between the immediate release tablets (nateglinide: 60 mg) and the immediate release tablets (nateglinide: 60 mg) + enteric coated granules B (nateglinide: 30 mg) , and at 2, 4, 6, 9, 12, 24 hr between the immediate release tablets (nateglinide: 60 mg) and the immediate release tablets (nateglinide: 60 mg) + enteric coated granules B (nateglinide: 60 mg), and at 2, 4, 6, 9, 12, 24 hr between the immediate release tablets (nateglinide: 60 mg) and the immediate release 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 hr to 9 hr observed between the immediate release tablets (nateglinide: 60 mg) and the immediate release tablets (nateglinide: 150 mg) ($p<0.05$, Student's *t*-test).

Plasma insulin concentration profiles are shown in Figure 2-4 (b). The insulin C_{max} values were 1521 ± 385 pg/mL (T_{max}: 2 hr) for the control, 3287 ± 413 pg/mL (T_{max}: 0.75 hr), 4089 ± 775 pg/mL (T_{max}: 1 hr) for immediate release tablets (nateglinide: 60 mg, 150 mg), 2758 ± 599 pg/mL (T_{max}: 0.75 hr) for immediate release tablets (nateglinide: 60 mg) + enteric coated granules B (nateglinide: 30 mg), 3600 ± 446

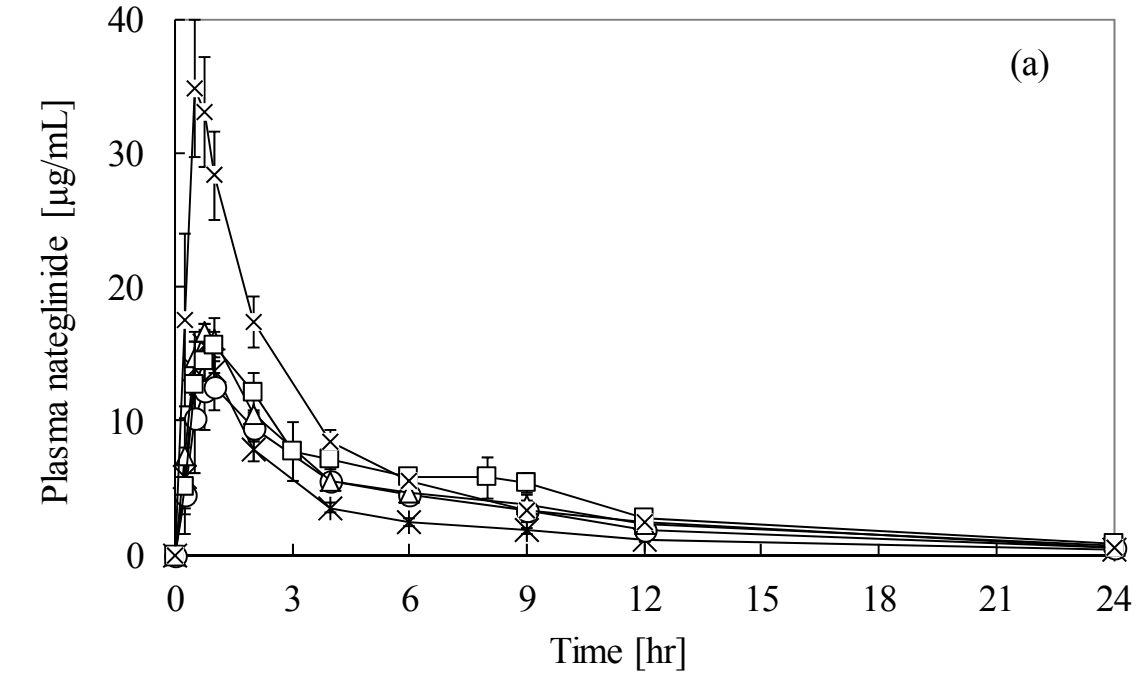
pg/mL (T_{max}: 0.75 hr) for immediate release tablets (nateglinide: 60 mg) + enteric coated granules B (nateglinide: 60 mg), and 3871 ± 378 pg/mL (T_{max}: 0.5 h) for immediate release tablets (nateglinide: 60 mg) + enteric coated granules B (nateglinide: 90 mg), respectively. There were significant differences of plasma insulin concentration observed are at 0.25, 0.5, 0.75 hr between the control (feeding only) and immediate release tablets (nateglinide: 60 mg), and at 0.25, 0.5, 1, 6 hr between the control (feeding only) immediate release tablets (nateglinide: 60 mg) + enteric coated granules B (nateglinide: 30 mg) , and at 0.25, 0.5, 1, 6 hr between the control (feeding only) and immediate release tablets (nateglinide: 60 mg) + enteric coated granules B (nateglinide: 60 mg), and at 0.25, 0.5, 0.75, 1, 2, 4, 6 hr between the control (feeding only) and immediate release 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 + immediate release tablets with the groups administered immediate release tablets (60 mg, 150 mg) only, there were no significant differences observed (Student's t-test) with the exception of the immediate release tablets (60 mg) + enteric coated granules (90 mg) at 24 hr after administration. This significant difference observed at 24 hr after administration is thought to be an artifact.

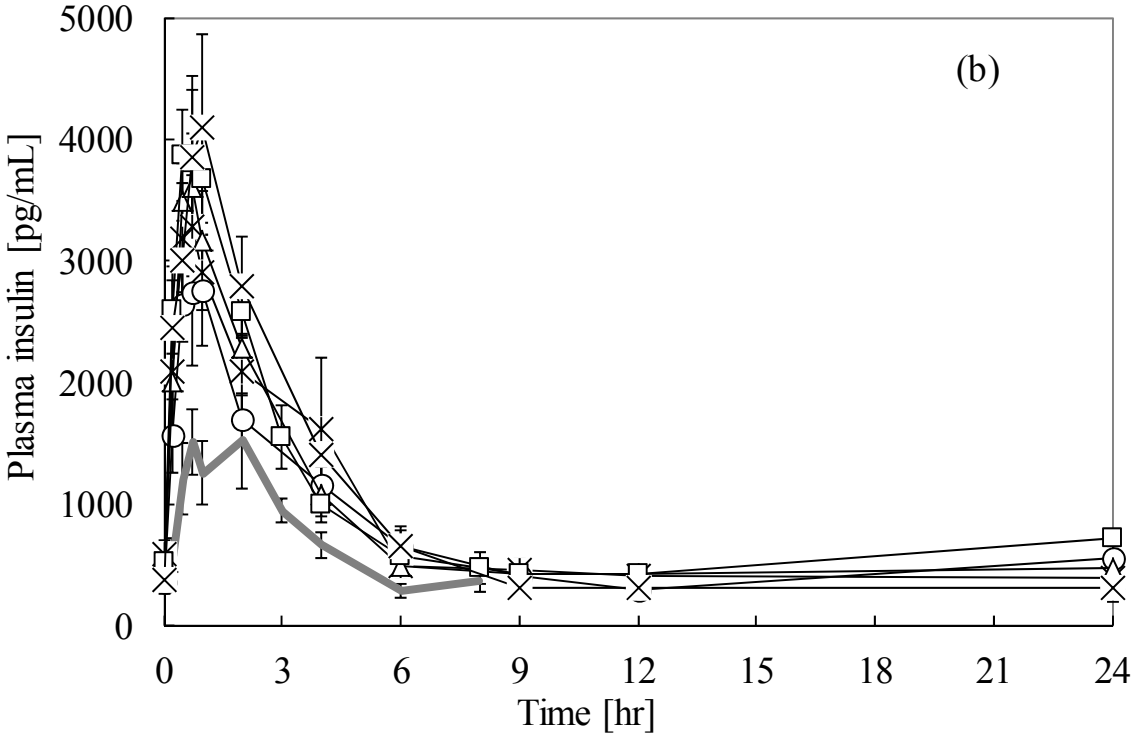
Blood glucose level profiles are shown in Figure 2-4 (c). In the case of comparing enteric coated granules + immediate relase tablets with nateglinide immediate release tablets (nateglinide: 60 mg), there were significant differences in blood glucose levels at 1, 2, 4, 6, 9 hr after administration in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

Based on the results of Figures 2-4 (a) and 2-4 (c), there is thought to be a

correlation between blood glucose levels and plasma nateglinide concentrations. Although decreases in blood glucose levels only continued for about 6 hr in the case of immediate release tablets (nateglinide: 60 mg), decreases continued for up to about 9 hr in the case of simultaneous administration with enteric coated granules (immediate release tablets (nateglinide: 60 mg) + enteric coated granules (nateglinide: 90 mg) group).

In case of immediate release 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 hr between the immediate release tablets (nateglinide: 150 mg) and the immediate release tablets (nateglinide: 60 mg) ($p<0.05$, Student's *t*-test).


(a)
Plasma nateglinide [μg/mL]
40
30
20
10
0
0
3
6
9
12
15
18
21
24
Time [hr]


(b)
Plasma insulin [pg/mL]
5000
4000
3000
2000
1000
0
0
3
6
9
12
15
18
21
24
Time [hr]

(continued)

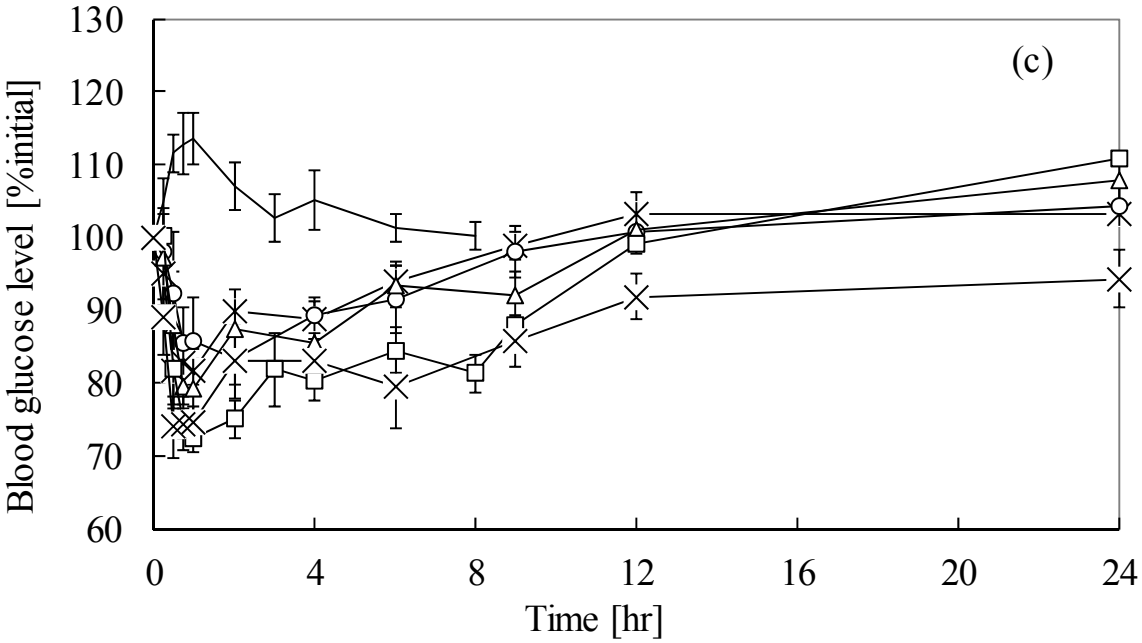


Figure 2-4: Plasma nateglinide concentration profile (a), plasma insulin concentration profile (b), blood glucose level (c) after oral administration of both enteric coated granules B and immediate release tablets in fasted beagle dogs just before feeding, or only feeding.

Each point and vertical bar represent mean ± SEM.

－: 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).

Discussion

The obtained 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.

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. In case of nateglinide immediate release tablets (nateglinide: 9 mg/kg), only decrease in PBG was observed.

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

(1) The immediate release tablets (nateglinide: 150 mg) has higher C_{max} of plasma nateglinide concentration than that of the combination of the immediate release tablets (nateglinide: 60 mg) and enteric coated granules (nateglinide: 90 mg).

The immediate release tablets (nateglinide: 150 mg) may have side effect (hypoglycemic state) easier than the combination of the immediate release 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 immediate release tablets (nateglinide: 60 mg) and enteric coated granules (nateglinide: 90 mg) than with the immediate release tablets (nateglinide: 150 mg), because the combination can adjust the balance of immediate release 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 immediate release 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 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[3].
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[3].

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[3)], 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[32-34)]. 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[35, 36)]. 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.

On the basis of these results, it is confirmed to decrease both PBG and FBG in normal beagle dogs with controlled release of nateglinide. Nateglinide controlled

release formulation is considered to enable control of both PBG and FBG for moderate and severe diabetes patients.

Chapter 3

Research for Dosage Form of Nateglinide Controlled Release Formulation

3.1. Design of Nateglinide Controlled Release Formulation Containing Immediate Release Granules and Enteric Coated Granules, and Evaluation of Dissolution Profile

Summary

The author designed a single unit type controlled release tablet containing nateglinide to decrease both PBG and FBG in normal beagle dogs. The tablet contains 60 mg of nateglinide in an immediate release portion, and 90 mg of nateglinide in a controlled release portion. Compressionable enteric coated granules were selected as the controlled release portion to primarily decrease FBG, and they were prepared by an aqueous coating with Eudragit®. Three types of nateglinide controlled release tablets were obtained, and their weights were 418.1 – 425.1 mg/tablet containing the above compressionable enteric coated granules. Even after tableting, the dissolution behavior of enteric coated granules was maintained approximately. From the view point of dissolution behavior, it was expected that it was able to control both PBG and FBG.

Materials and Methods

Nateglinide (Ajinomoto Co. Inc., Japan), lactose mono-hydrate (DMV, Japan), hydroxypropylcellulose (Nihon Soda, Japan), low-substituted hydroxypropyl cellulose (Shin-etsu Chemical Co. Ltd., Japan), magnesium stearate (Mg-St, Taiheikagakusangyo, Japan), methacrylic acid copolymer LD (Eudragit®L30D-55, Röhm GmbH, Germany), poly (methyl acrylate-co-methyl methacrylate- co-methacrylic acid) (Eudragit® FS30D, Röhm GmbH, Germany), ethylacrylate methylmethacrylate copolymer dispersion (Eudragit® NE30D, Röhm GmbH,

Germany), croscarmellose sodium (Ac-Di-Sol®, Asahikasei Co. Ltd., Japan), triethyl citrate (CBC, Japan), polysorbate 80 (Wako Pure Chemical Co. Ltd., Japan), glycelyl monostearate (Higuchi Shoukai, Co. Ltd., Japan), sodium hydroxide (JIS K8576, reagent grade, Junseikagaku, Japan), citric acid (JIS K8283, reagent grade, Junseikagaku, Japan) were used in the study.

A high shear mixer (FS-10JD, Fukae Powtec, Japan), an extrusion granulator (DG-L1, Fuji Paudal, Japan), a cutter mill (New Speed Mill (ND-10), Okadaseiko, Japan), a spherized granulator (Q-230, Fuji Paudal, Japan), a fluidized bed granulator/dryer (FLO-1, Freund Industry Co., Japan), a V-blender (VFS, Tsutsuirikakagaku, Japan), a tableting machine (HT-AP15SSII, Hata Machinery, Japan), and a microfluidizer (Nanomizer Inc., Japan) were used.

Three-hundred-seventy-five grams of nateglinide, 637.5 g of lactose monohydrate and 450.0 g of low-substituted hydroxypropyl cellulose were mixed in a high shear mixer for 10 min. Subsequently, 1,035 g of a binding solution of 15 g of hydroxypropyl cellulose in water was added, and granulation was conducted for 2.5 min (agitator: 400 rpm, chopper: 3600 rpm). The total amount of the resulting product was uniformly granulated with a cutter mill (2000 rpm, screen: 1×10 mm), and dried with a fluidized bed drier (FLO-1) (inlet air temperature: 80°C). The obtained granules were screened through a sieve of 850 μm. The granular product remaining on the sieve of 850 μm was forcibly passed through the sieve, and both products were mixed to form immediate release granules.

Composition of core granules is shown in Table 3-1. Five-hundred grams of nateglinide, 5 g of hydroxypropylcellulose, and 10 g of polysorbate 80 were suspended and dissolved in 800 g of water with a microfluidizer (pressure: 1200 kgf/cm^2). After

mixing this suspension with the mixture of 22.1 g of lactose monohydrate and 252.8 g of croscarmellose sodium, extrusion granulation was conducted (diameter: 1.0 mmφ). The resulting granules were rounded by a spherical granulator (rotor agitation: 450 rpm), and then dried in a fluidized bed dryer (inlet temperature: 80°C). Fractions of 850 μm - 1400 μm granules were obtained by grading, and then they were used for coating. Table 3-2 shows the compositions of the used enteric coating suspensions. The resulting core granules were coated with the coating solutions using a fluidized bed coating machine (outlet temperature during coating : 26 – 31°C (Enteric Coated Granules (a)), 18 - 26°C (Enteric Coated Granules (b)), 20 – 26°C (Enteric Coated Granules (c)). After drying, these enteric coated granules were annealed at 35°C overnight. Particle diameter was evaluated by sieving method. Loss on drying test was conducted at 80°C with an infrared moisture determination balance (AD-230, KETT ELECTRIC LABORATORY, Japan).

Table 3-1: Composition of core granules for enteric coating.

nateglinide	90.0
lactose monohydrate	4.0
croscarmellose sodium	45.5
hydroxypropylcellulose	0.9
polysorbate 80	1.8

*: The composition when nateglinide is 90 mg.

Table 3-2: Composition of enteric coating suspension.

	Enteric Coated Granules					
	(a)		(b)		(c)	
	weight [g]	solid content [g]	weight [g]	solid content [g]	weight [g]	solid content [g]
methacrylic acid copolymer LD	208.0	62.40	70.0	21.00		
poly(methyl acrylate-co-methyl methacrylate- co- methacrylic acid)			630.0	189.00	700.0	210.00
2 w/w% NaOH aq.	3.5	0.07				
ethylacrylate methylmethacrylate copolymer dispersion	104.0	31.20				
20 w/w% citric acid aq.	1.0	0.20				
Glycelyl monostearate	2.9	2.90	6.3	6.30	6.3	6.30
Polysorbate 80			1.1	1.10	1.1	1.10
33 w/w% Polysorbate 80 aq.	1.5	0.50				
Triethyl citrate	9.8	9.80	10.3	10.30	10.5	10.50
water	624.0		1812.3		1400.0	
total	954.7	107.07	2530.0	227.70	2117.9	227.90

The obtained enteric coated granules and immediate release granules were mixed with a V-blender (38 rpm, 10 min). Then the obtained granules and magnesium stearate were mixed (38 rpm, 5 min). The granular product was tableted to obtain the nateglinide controlled release tablets (target hardness: ca. 30 N). Hardness of the obtained tablets was evaluated with tablet tester (TS-50N, Okada Seiko, Japan).

The dissolution behaviors of nateglinide preparations were evaluated (JP15, paddle method, 50 rpm, test fluid: 900 mL, nateglinide: 90 or 150 mg/vessel) with a dissolution tester（NTR-VS6P, Toyama Sangyo Co. Ltd., Japan). Each test fluid was JP1 fluid (JP15, Dissolution Test Fluid No.1) containing 0.6 w/v% (nateglinide: 90 mg/vessel), 1.2 w/v% (nateglinide: 150 mg/vessel) of polysorbate 80 for pH=1.2, and Clark-Lubs buffer for pH=5.5-6.8. Dissolution rates were determined with a reversed phase HPLC system consisting of an L-6000 constant flow pump and an L-4000 UV detector 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).

Results

In order to decrease FBG with nateglinide, it is necessary to conduct a sustained release of nateglinide. When administering enteric coated granules with meal, it was reported that a sustained release of the drug was achieved. Moreover, it was reported that nateglinide was only partially absorbed in the stomach, but was well absorbed in the whole area of the intestines, according to the in situ experiment in rats using a ligated loop method[37]. As in the above mentioned results, focusing the enteric coated granule, the author aimed to design compressionable enteric coated granules as a controlled release portion to primarily decrease FBG, and a single unit type nateglinide controlled release tablet containing the enteric coated granules.

Nateglinide core granules were coated using the coating suspensions shown in Table 3-2 to prepare 3 types of compressionable enteric coated granules. The formulations were designed as shown in Reference 26. It was thought to be necessary to coat 15 w/w% or more of enteric coating material on core granules to obtain acid resistance (see 2.1.). Therefore, 15 w/w% or more of enteric coating materials were coated on the core granules (Enteric Coated Granules (a): 22 w/w% of methacrylic acid copolymer LD (Eudragit®L30D-55), Enteric Coated Granules (b): 31.4 w/w% of methacrylic acid copolymer LD + poly (methyl acrylate-co-methyl methacrylate- co-methacrylic acid) (Eudragit®FS30D) (1:9 dry substance weight ratio), Enteric Coated Granules (c): 31.6 w/w% of poly (methyl acrylate-co-methyl methacrylate- co-methacrylic acid)). The loss on drying values were 3.6 w/w% (Enteric Coated Granules (a)), 3.4 w/w% (Enteric Coated Granules (b)), 3.2 w/w% (Enteric Coated Granules (c))

respectively. The average diameter of the obtained compressionable enteric coated granules was about 1 mm.

Three types of nateglinide controlled release tablets were prepared with the composition shown in Table 3-3 (Tablet A, B, C, concave shape, diameter: 10 mmφ). The amounts of nateglinide in both an immediate release portion and a controlled release portion were decided according to Chapter 2 (the immediate release portion: 60 mg of nateglinide, the controlled release portion: 90 mg of nateglinide). The average diameter of used immediate release granules was about 250 μm.

Table 3-3: Composition of nateglinide controlled release tablet containing compressionable enteric coated granules.

natglinide controlled release tablet	immediate release granules (nateglinide: 60 mg) [mg]	Enteric Coated Granules (nateglinide: 90 mg) [mg]		Mg-St [mg]	total weight [mg]
Tablet A	226.4	(a)	192.3	6.4	425.1
Tablet B	226.4	(b)	187.5	4.2	418.1
Tablet C	226.4	(c)	187.9	4.2	418.5

The acid resistance of the compressionable enteric coated granules was evaluated in JP1 fluid (Table 3-4). The dissolution rates at 120 min in JP1 fluid of Enteric Coated Granules (a), (b) and (c) were 6.4%, 0.4%, 0.4% respectively, demonstrating sufficient acid resistance. Then, the dissolution behavior in neutral pH region was also evaluated (Figure 3-1). 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 have a different dissolution pH, demonstrating values of 6.0, 6.5 and 6.8,

respectively. The dissolution rate of nateglinide became slower in the order of Enteric Coated Granules (a), (b) and finally (c). The dissolution rate of nateglinide from immediate release tablet (Fastic® tablet) or of immediate release granules is nearly 100% in both an acid and neutral pH.

In JP1 fluid, dissolution rates at 120 min of the obtained Tablets A, B and C were around 40%. Dissolution behaviors were also evaluated for the obtained Tablet A, B and C (Table 3-5, Figure 3-2).

Table 3-4: Dissolution rate at 120 min of compressionable enteric coated granules in JP1 fluid*.

	%dissolved in JP1fluid* at 120 min [%]
Enteric Coated Granules（a）	6.4 ± 0.0
Enteric Coated Granules（b）	0.4 ± 0.0
Enteric Coated Granules（c）	0.4 ± 0.2

nateglinide: 90 mg/vessel, n=3, mean ± SD.

*: JP1 fluid containing 0.6 w/w% polysorbate 80.

Table 3-5: Dissolution rate at 120 min of nateglinide controlled release tablets in JP1 fluid*.

	%dissolved in JP1fluid* at 120 min [%]
Tablet A	43.3 ± 1.3
Tablet B	40.0 ± 1.8
Tablet C	37.3 ± 1.5

nateglinide: 150 mg/vessel, n=3, mean ± SD.

*: JP1 fluid containing 1.2 w/w% polysorbate 80.

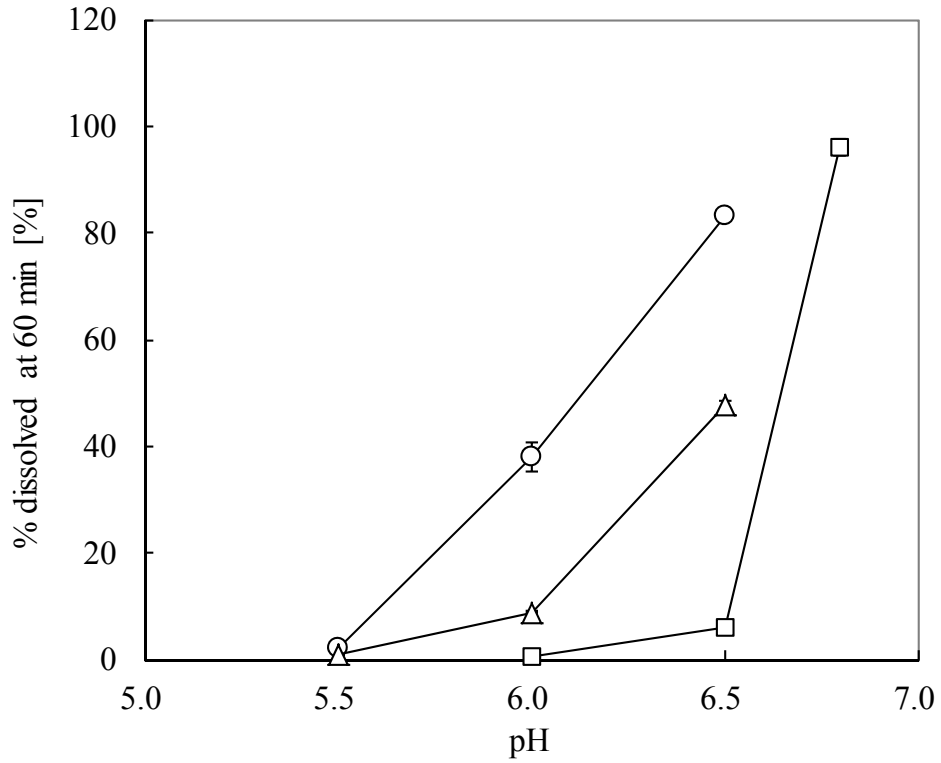


Figure 3-1: pH Relationship of dissolution rate at 60 min of enteric coated granules.

JP15 paddle method (50 rpm), nateglinide: 90 mg/vessel, n=3, mean ± SD,

medium : Clark-Lubs buffer (KH_2PO_4 + NaOH).

○ : Enteric Coated Granules (a).

△ : Enteric Coated Granules (b).

□ : Enteric Coated Granules (c).

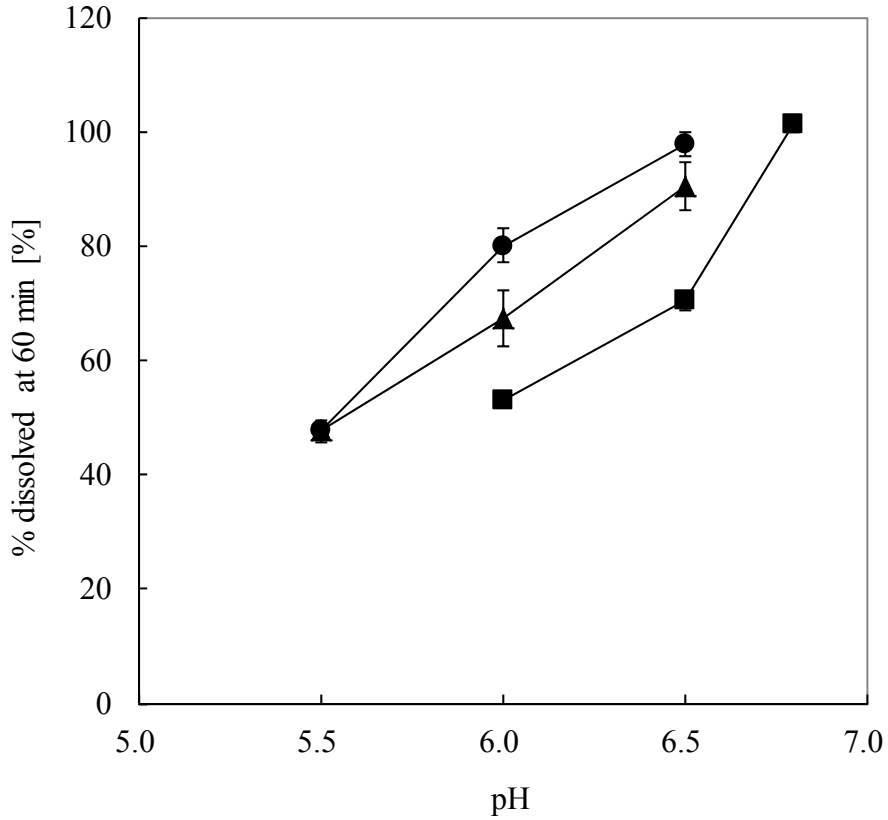


Figure 3-2: pH Relationship of dissolution rate at 60 min of nateglinide controlled release tablets.

JP15 paddle method (50 rpm), nateglinide: 150 mg/vessel, n=3, mean ± SD, medium: Clark-Lubs buffer (KH_2PO_4 + NaOH).

● : Tablet A.

▲ : Tablet B.

■ : Tablet C.

immediate release portion : 60 mg of nateglinide.

controlled release portion : 90 mg of nateglinide.

Discussion

Enteric Coated Granules (a), (b) and (c) were confirmed to have acid resistance and to have a different dissolution pH, demonstrating values of 6.0, 6.5 and 6.8, respectively. The dissolution rate of nateglinide became slower in the order of Enteric Coated Granules (a), (b) and finally (c). Furthermore it was possible to control the dissolution pH value of enteric coated granules by mixing more than 2 kinds of enteric coating materials. The dissolution rate of nateglinide from immediate release tablet (Fastic® tablet) or of immediate release granules is nearly 100% in both an acid and neutral pH. It is suggested that these compressionable enteric coated granules effectively lowered FBG with hardly any decrease in PBG according to previous study.

A nateglinide controlled release tablet was designed, comprised of both immediate release granules and compressionable enteric coated granules. The tablet contained 60 mg of nateglinide in the immediate release portion and 90 mg of nateglinide in the controlled release portion. Three types of nateglinide controlled release tablets were obtained, and their weights were 418.1 – 425.1 mg/tablet. The hardness values of the obtained tablets were about 30 N. These values are lower than those of conventional film coated tablets in the market, but are similar to those of orally disintegrated tablets[38]. Further process studies are required to make the above tablets commercially produced for the market.

Dissolution behaviors were also evaluated for the obtained Tablet A, B and C (Table 3-5, Figure 3-2). In JP1 fluid, dissolution rates at 120 min of the obtained Tablets A, B and C were around 40%. This means that only nateglinide contained in the immediate release portion was dissolved under this dissolution testing condition (60 mg/150 mg = 40 %), and that acid resistance of enteric coated granules was maintained even after

tableting[26]. In neutral pH (pH = 5.5 - 6.8), although the little change in pH relationship of dissolution rate was observed when compared with those shown in Figure 3-1, the dissolution behavior of enteric coated granules was maintained approximately even after tableting. It is thought that further process control studies are required for enteric coating process and tableting process in order to precisely control the dissolution behavior. The content uniformity of both immediate release granules and enteric coated granules in the obtained tablets was not evaluated. However, it was thought that both immediate release granules and enteric coated granules were mixed uniformly according to the deviations of dissolution rates (Table 3-5).

As the above results, it was expected that it was able to control both PBG and FBG using this nateglinide controlled release tablet in beagle dogs.

3.2. Design of Nateglinide Controlled Release Formulation Containing Immediate Release Granules and Erosion Matrix Tablet, and Evaluation of Dissolution Profile

Summary

The author designed a single unit type tablet formulation containing nateglinide to decrease both PBG and FBG in normal beagle dogs. The tablet comprises both a core tablet (an erosion matrix tablet: a controlled release portion (nateglinide: 90 mg)) and an outer shell (an immediate release portion (nateglinide: 60 mg)). The weight, the diameter and the hardness of the obtained tablet were 416.1 mg, 10 mmφ, about 60 N, respectively.

The dissolution study of the obtained tablet in pH 1.2 or 6.8 showed that the nateglinide in the immediate release portion dissolved in almost 30 min, and that 30 min after the dissolution test started, the nateglinide in the controlled release portion had dissolved slowly. As the above results, it was expected that it was able to decrease both PBG and FBG using this nateglinide controlled release tablets in beagle dogs.

Materials and Methods

Nateglinide (Ajinomoto Co. Inc., Japan), hydroxypropylcellulose (Nihon Soda, Japan), lactose mono-hydrate (DMV Japan), hydroxypropylmethylcellulose2910 (HPMC2910), hydroxypropylmethylcellulose2208 (HPMC2208), low-substituted hydroxypropylcellulose (Shin-etsu Chemical Co. Ltd., Japan), microcrystalline cellulose (Asahikasei, Japan), macrogol 6000 (Nihon Oil and Fats Co. Ltd., Japan), magnesium stearate (Mg-St, Taiheikagakusangyo, Japan), were used in the study.

A high shear mixer (MINI/FS-10JD, Fukae Powtec, Japan), a fluidized bed granulator (FLO-1, Freund Industry Co., Japan), a rotating cutter type mill (New speed mill, Okadaseiko, Japan), a tableting machine (HT-AP, Hata Machinery, Japan), and a pan type coating machine (Freund Industry Co., Japan) were used. Hardness of the obtained tablets was evaluated with a tablet tester (TS-50N, Okada Seiko, Japan).

Three-hundred-and-seventy-five grams of nateglinide, 637.5 g of lactose monohydrate and 450.0 g of hydroxypropylcellulose having a low degree of substitution were mixed with a high shear mixer （FS-10JD） for 10 min. Subsequently, 1,035 g of a binding solution of 15 g of hydroxypropylcellulose in water was added, and granulation was conducted for 2.5 min. The total amount of the resulting granular product was uniformly granulated with a new speed mill, and dried with a fluidized bed drier. The obtained granules were screened through a sieve of 850 μm. The granular product remaining on the sieve of 850 μm was forcibly passed through the sieve, and both products were mixed to form immediate release granules.

The compositions are shown in Table 3-6. In the case of Erosion Matrix Tablet A, nateglinide and hydroxypropylmethylcellulose were charged in a high shear mixer (MINI), and mixed. In the case of Erosion Matrix Tablets B and C, nateglinide, hydroxypropylmethylcellulose and microcrystalline cellulose were charged in a high shear mixer (MINI), and mixed. Then, water was added, and granulation was conducted for 1.5 min. The resulting granular product was dried on a shelf, and screened using a sieve with an opening of 850 μm. The thus-obtained granular product was mixed with magnesium stearate, and the mixture was tabletted to obtain erosion matrix tablets using a 7.5 mmφ punch. In the case of Erosion Matrix Tablet C, the resulting erosion matrix tablets were coated using a coating solution formed by

dissolving 50.0 g of hydroxypropylmethyl cellulose 2910 (HPMC2910) and 10.0 g of macrogol 6000 in 1,440 g of water (12.5% of hydroxypropylmethyl cellulose based on the weight of the erosion matrix tablet was coated).

Table 3-6: Composition of erosion matrix tablets.

	ingredients	Erosion Matrix Tablet		
		A	B	C
core tablet	nateglinide	90.0	90.0	90.0
	hydroxypropylmethylcellulose 2910	45.1		22.5
	hydroxypropylmethylcellulose 2208		22.5	
	microcrystalline cellulose		37.5	37.5
	magnesium stearate	2.8	2.3	3.0
coating film	hydroxypropylmethylcellulose 2910			19.2
	macrogol 6000			3.9
	total [mg]	137.8	152.3	176.1

Nateglinide controlled release tablets containing an erosion matrix tablet were prepared using the HPMC2910 coated erosion matrix tablets (Erosion Matrix Tablet C) and the immediate release granules (Figure 3-3, Table 3-7, tablet diameter: 10 mmφ).

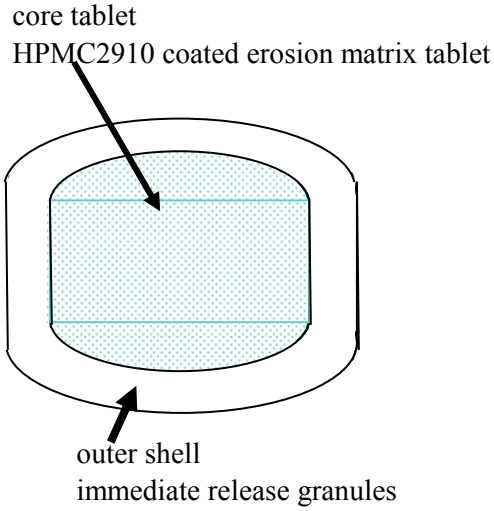


Figure 3-3: The section of nateglinide controlled release tablet containing both immediate release granules and an erosion matrix tablet.

Immediate release granules form an outer shell. Core tablet is HPMC2910-coated erosion matrix tablet.

Table 3-7: Composition of the tablet containing Erosion Matrix Tablet C [mg].

part	component	weight [mg]
core tablet	Erosion Matrix Tablet C	176.1
outer shell	immediate release granules	236.4
	Magnesium stearate	3.6
	total	416.1

The dissolution profiles of nateglinide preparations were evaluated (JP15, paddle method, 50 rpm, test fluid: 900 mL, nateglinide: 90 or 150 mg/vessel) with a dissolution tester (NTR-VS6P, Toyama Sangyo Co. Ltd., Japan). Each test fluid was JP1 fluid (JP15, Dissolution Test Fluid No.1) containing 0.6 w/v% (nateglinide: 90 mg/vessel), 1.2 w/v% (nateglinide: 150 mg/vessel) polysorbate 80 for pH=1.2, and Clark-Lubs buffer for pH=6.8 (JP2 fluid: JP15, Disintegration Test Fluid No.2).

Dissolution rates were determined with a reversed phase HPLC system consisting of an L-6000 constant flow pump and an L-4000 UV detector 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 an acetonitrile – pH=2.5 phosphate buffer (55:45 v/v). Nateglinide eluted in about 10 min at 40^{o}C (at a flow rate of 1.5 mL/min).

Results

Three types of nateglinide erosion matrix tablets as controlled release portions were prepared according to Table 3-6 (Erosion Matrix Tablet A, B, C). The amount of nateglinide in one erosion matrix tablet was determined to be 90 mg according to the results of Chapter 2. Tablet hardness values of the Erosion Matrix Tablet A and B were about 50 N. The value of the Erosion Matrix Tablet C was about 300 N, due to coating with HPMC2910.

Dissolution profile of the obtained erosion matrix tablets was evaluated in JP1 fluid containing 0.6 w/v% Polysorbate 80 or JP2 fluid. Nateglinide is a poorly water-soluble drug. Polysorbate 80 was added to acid pH testing fluid to satisfy the sink condition. The results are shown in Figure 3-4 and 3-5.

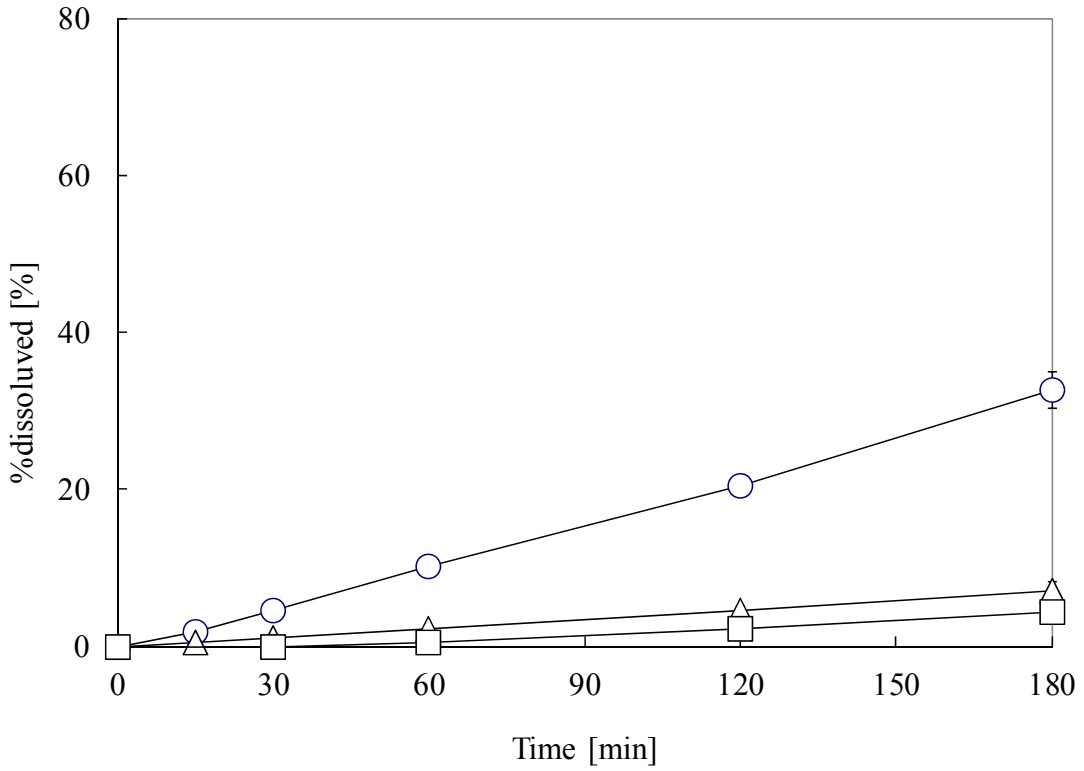


Figure 3-4: Dissolution profile of nateglinide erosion matrix tablets in JP1 fluid containing 0.6w/v% polysorbate 80.

nateglinide: 90 mg/vessel, mean ± SD, n=3.

○ : Erosion Matrix Tablet A.

△ : Erosion Matrix Tablet B.

□ : Erosion Matrix Tablet C.

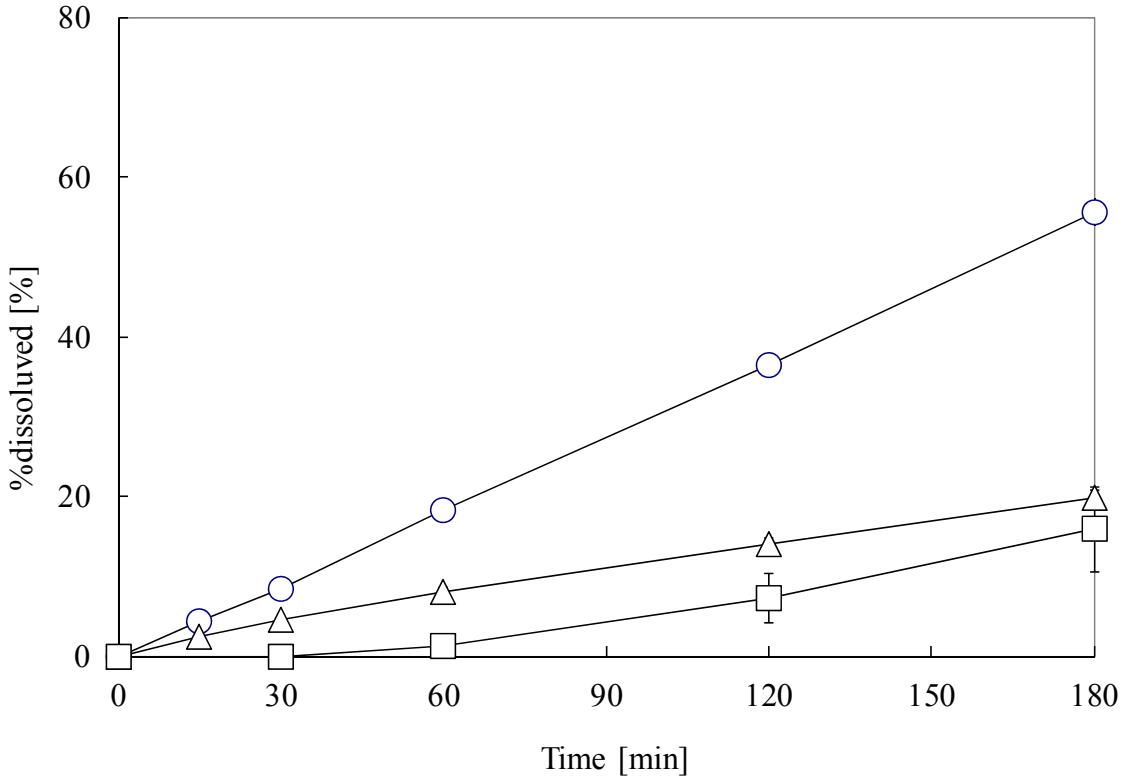


Figure 3-5: Dissolution profile of nateglinide erosion matrix tablets in JP2 fluid.

nateglinide: 90 mg/vessel , mean ± SD, n=3.

○ : Erosion Matrix Tablet A.

△ : Erosion Matrix Tablet B.

□ : Erosion Matrix Tablet C.

The dissolution rate at pH 1.2 decreased in the order of nateglinide Erosion Matrix Tablet A, B and finally C. The dissolution rate at pH 6.8 also decreased in the order of nateglinide Erosion Matrix Tablet A, B and finally C. In the case of Tablet C, the lag time of dissolution was observed. It was assumed to be due to the HPMC2910 coating.

The dissolution rate of nateglinide immediate release tablets (Fastic® tablets) under the same conditions is nearly 100% within 30 min.

After deciding to focus on Erosion Matrix Tablet C (HPMC2910 coated erosion matrix tablet), which was expected to effectively lower FBG with hardly any decrease in PBG, a nateglinide controlled release tablet was designed with both Erosion Matrix Tablet C (90 mg of nateglinide) and immediate release granules (60 mg of nateglinide). The amount of nateglinide in the immediate release portion and in the controlled release portion was determined according to the results of Chapter 2. The diameter of the obtained tablet was 10 mm, and the hardness values of the tablets were about 60 N originating in the outer shell of the tablet.

Dissolution profiles of the obtained nateglinide controlled release tablets containing Erosion Matrix Tablet C were evaluated in JP1 fluid containing 1.2 w/v% Polysorbate 80 or JP2 fluid. The results are shown in Figure 3-6. About 35% of the total dose was dissolved in 30 min at both pH 1.2 and 6.8. This indicated that most of the nateglinide contained in the immediate release portion was dissolved in 30 min. The dissolution rate of nateglinide immediate release tablets (Fastic® tablets) under the same conditions is nearly 100% within 30 min. Thirty minutes after the test started, nateglinide was dissolved slowly at both pH1.2 and 6.8. These behaviors were the same as those of Erosion Matrix Tablet C.

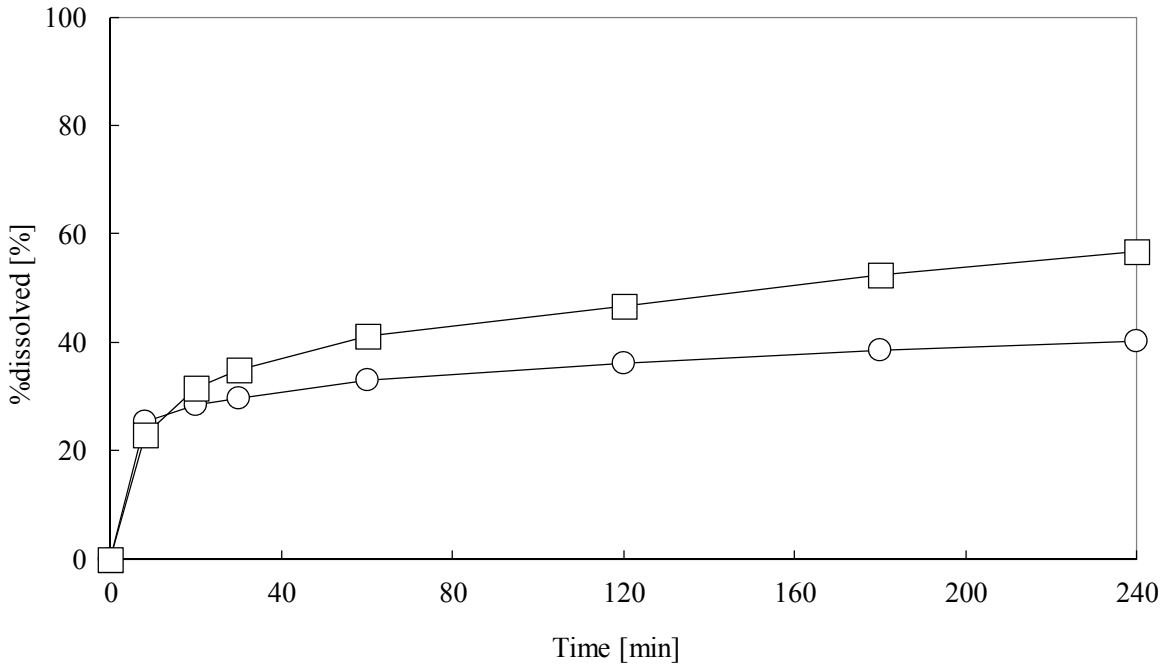


Figure 3-6: Dissolution profile of the obtained nateglinide controlled release tablets containing an erosion matrix tablet.

JP15 paddle method (50 rpm), n=3, nateglinide: 150 mg/vessel, mean ± SD, medium: JP1 fluid containing 1.2 w/v% polysorbate 80 (○), JP2 fluid (□).

Discussion

A single unit type tablet formulation containing nateglinide was designed to decrease both PBG and FBG in normal beagle dogs, using an erosion matrix tablet as a controlled release portion. The tablet comprised both a core tablet (an erosion matrix tablet: a controlled release portion) and an outer shell (an immediate release portion), and contained 60 mg of nateglinide in the immediate release portion and 90 mg of nateglinide in the controlled release portion. The weight, the diameter and the hardness of the obtained tablet were 416.1 mg, 10 mmφ and about 60 N, respectively. The tablet hardness values are similar to those of conventional film coated tablets in the market.

In a dissolution study of the obtained tablet in pH1.2 or 6.8, nateglinide in the immediate release portion was almost dissolved in 30 min. Thirty minutes after the test starts, nateglinide in the controlled release portion was dissolved slowly. It was expected that it was able to control both PBG and FBG using this nateglinide controlled release tablets in beagle dogs.

Although further process studies are required to make the above tablets commercially produced for the market, from the view point of manufacturing robustness, the tablet containing an erosion matrix tablet is thought preferable to that containing enteric coated granules.

Chapter 4

Multiple Administration Study with Nateglinide Controlled Release Formulation

4.1. Single Administration Study with Nateglinide Controlled Release Formulation Containing Immediate Release Granules and Erosion Matrix Tablet, and Evaluation of Nateglinide Plasma Concentration Profile

Summary

The author designed a single unit type tablet that comprised both a core tablet (an erosion matrix tablet: a controlled release portion (nateglinide: 90 mg)) and an outer shell (an immediate release portion (nateglinide: 60 mg)). The weight was 416.1 mg/tablet. An *in vivo* single oral administration study with this tablet in normal beagle dogs showed the bioavailability value of the obtained nateglinide controlled release tablets containing erosion matrix tablet against nateglinide immediate release tablets was 73.6%, although the value of a nateglinide controlled release tablets containing enteric coated granules was 57.2-60.8%. From the view point of nateglinide plasma concentration profiles, it is expected to decrease both PBG and FBG.

Materials and Methods

Nateglinide controlled release tablets (immediate release portion: 60 mg of nateglinide, controlled release portion: 90 mg of nateglinide), obtained in Chapter 3 were used.

Prior to being conducted, protocols of *in vivo* studies were assessed according to the guidelines of Ethical Committee in INA Research Co., Ltd. (Japan). The guideline was established based on "Doubutsu no aigo oyobi kanri ni kannsuru houritsu (Law about protection of an animal and management)" (October 1st, 1973, No. 105),

"Jikkendoubutsu no shiyou oyobi hokan nado ni kansuru kijyun (The standard about the breeding of an experimental animal and the safekeeping, etc.)" (March 1st, Sourihu No.6), “Jikkendoubutsu gaidorain no sakutei ni tsuite (About decision of an animal experiment guideline)" (November 5th, 1980, Sougakusho No.1513), "Daigaku nado ni okeru doubutsujikken ni tsuite (About the animal experiment that is conducted in a university" (May 25th, 1987, Bungakujyou No.141). One tablet was 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. Blood samples were taken before and at 15, 30, 45, 60, 120, 240, 360, 540, 720 and 1440 min (n=3). Determination of plasma nateglinide concentration is described in reference 31.

Results

Plasma nateglinide concentration profiles are shown in Figure 4-1. Results are also shown for nateglinide immediate release tablets only (nateglinide: 60 mg, 150 mg) used as controls. The nateglinide C_{max} values were 13.10 ± 3.05 μg/mL (T_{max}: 0.75 hr) for nateglinide controlled release tablets, 15.63 ± 1.08 μg/mL (T_{max}: 0.75 hr) for the immediate release tablets (nateglinide: 60 mg), and 34.80 ± 5.15 μg/mL (T_{max}: 0.5 hr) for the immediate release tablets (nateglinide: 150 mg). There were no significant differences of the C_{max} values observed between the immediate release tablets (nateglinide: 60 mg) and the nateglinide controlled release tablets (Student's *t*-test). This is because the C_{max} values are based on the immediate release portion. This result confirms the above dissolution results. On the other hand, plasma nateglinide

concentrations from 4 hr to 12 hr after administration demonstrated an increasing trend due to the nateglinide erosion matrix tablet, and there was a significant difference of plasma nateglinide concentrations at 6 hr after administration between the immediate release tablets (nateglinide: 60 mg) and the nateglinide controlled release tablets (Student's *t*-test, $p<0.05$). The bioavailability value of the nateglinide controlled release tablets containing an erosion matrix tablet against nateglinide immediate release tablets was 73.6%, although the value was 57.2-60.8% in the case of the nateglinide controlled release tablet containing enteric coated granules (Chapter 3).

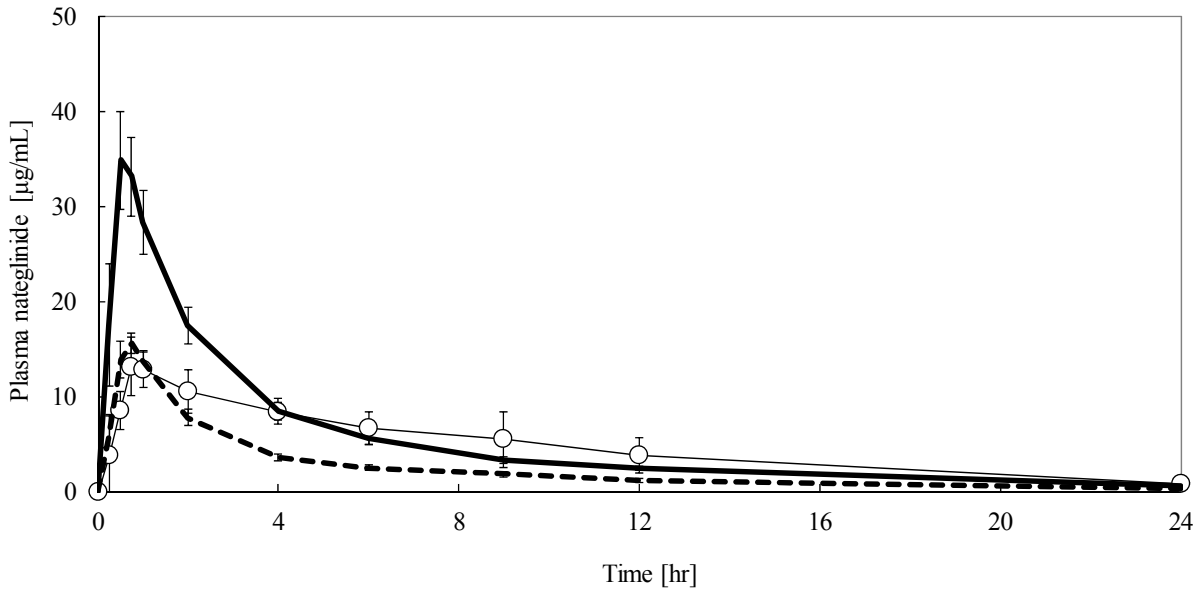


Figure 4-1: Plasma nateglinide concentration profile after an oral administration of nateglinide preparations in fasted beagle dogs just before feeding.

Each point and vertical bar represents mean ± SEM.

▬ ▬ ▬ : immediate release tablets (nateglinide: 60 mg), n=6.

▬▬ : immediate release tablets (nateglinide: 150 mg), n=6.

○ : nateglinide controlled release tablet containing an erosion matrix tablet, n=3.

Discussion

A single unit type nateglinide controlled release tablet was designed to decrease both PBG and FBG. The tablet comprises both an erosion matrix tablet as a controlled release portion (nateglinide: 90 mg) and an immediate release granules as an immediate release portion (nateglinide: 60 mg). The weight, the diameter and the hardness of the obtained tablet were 416.1 mg, 10 mmφ and about 60 N, respectively.

It was considered possible to decrease both PBG and FBG with this nateglinide controlled release tablet according to the plasma nateglinide concentration profiles. And it was possible to improve bioavailability, as compared with nateglinide controlled release tablet containing compressionable enteric coated granules. This means that the bioavailability value of the controlled release portion was improved by using an erosion matrix tablet. It is not obvious why the bioavailability value of administration of the tablet containing the erosion matrix tablet was higher than that of administration of the tablet containing enteric coated granules. Further study is necessary.

It is assumed that not only the nateglinide controlled release tablets containing an erosion matrix tablet (immediate release portion: 60 mg of nateglinide, controlled release portion: 90 mg of nateglinide), but also the immediate release tablets (nateglinide: 150 mg) might decrease both PBG and FBG according to nateglinide plasma concentration profile. However, this nateglinide controlled release tablet is believed to be more useful than the immediate release tablets (nateglinide: 150 mg), because the ability to control both PBG and FBG without side effect, is easier.

4.2. Multiple Administration Study with Nateglinide Controlled Release Formulation Containing Immediate Release Granules and Erosion Matrix Tablet, and Evaluation of Nateglinide Plasma Concentration Profile, Blood Glucose Level and Change in Fasting Blood Glucose Level

Summary

An *in vivo* multiple oral administration study (*b.i.d.* (interval: 12 hr), 8 days) with nateglinide controlled release tablet in normal beagle dogs was conducted. That showed the reproducibility of nateglinide absorption, and decreases in both PBG and FBG even in the 8^{th} days. The ability to decrease the blood glucose level did not weaken during a multiple administration. Furthermore, decrease in FBG at 9:00 A.M. was also observed. On the basis of the above results, nateglinide controlled release tablet was suggested to enable control of both PBG and FBG for moderate and severe diabetes patients.

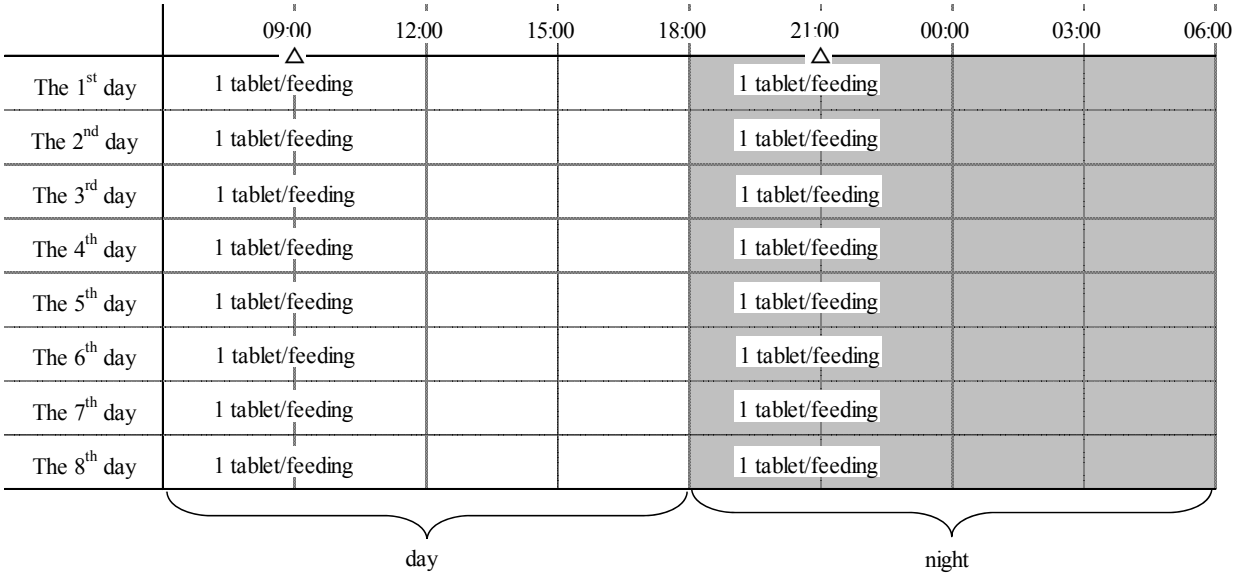
Materials and Methods

Nateglinide controlled release tablets containing an erosion matrix tablet (immediate release portion: 60 mg of nateglinide, controlled release portion: 90 mg of nateglinide), obtained in Chapter 3 were used.

Prior to being conducted, protocols of *in vivo* studies were assessed according to the guidelines of Ethical Committee in INA Research Co., Ltd. (Japan). The guideline was established based on "Doubutsu no aigo oyobi kanri ni kannsuru houritsu (Law about protection of an animal and management)" (October 1st, 1973, No. 105),

"Jikkendoubutsu no shiyou oyobi hokan nado ni kansuru kijyun (The standard about the breeding of an experimental animal and the safekeeping, etc.)" (March 1st, Sourihu No.6), “Jikkendoubutsu gaidorain no sakutei ni tsuite (About decision of an animal experiment guideline)" (November 5th, 1980, Sougakusho No.1513), "Daigaku nado ni okeru doubutsujikken ni tsuite (About the animal experiment that is conducted in a university" (May 25th, 1987, Bungakujyou No.141). One tablet was 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. An 8-day multiple administration study with the nateglinide controlled release tablets was conducted according to Table 4-1. Blood samples were taken at 9:00, 9:15, 9:30, 9:45, 10:00, 11:00, 13:00, 15:00, 18:00, 21:00, 21:15, 21:30, 21:45, 22:00 and 23:00 on the 1st day, 01:00, 05:00, 06:00 and 09:00 on the 2nd day, 09:00 on the 3rd, 4th, 5th, 6th and 7th day, 9:00, 9:15, 9:30, 9:45, 10:00, 11:00, 13:00, 15:00, 18:00, 21:00, 21:15, 21:30, 21:45, 22:00 and 23:00 on the 8th day, 01:00, 05:00, 06:00 and 09:00 on the 9th day after the 1st oral administration (n=3). Plasma nateglinide concentration was evaluated for samples of the 1st, 2nd, 8th and 9th day. Blood glucose level was evaluated for all blood samples.

Table 4-1 : Multiple administration study protocol.



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 portion 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 mol/L pH=6.0 phosphate buffer. The mixture was vortex-mixed for 10 s and applied to a Sep-Pak Vac tC18 cartridge that was pre-equilibrated with 5 mL of 0.05 mol/L 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[31] 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 timetable of the column switching pattern is shown in Table 4-2. At a flow rate of 1.0 mL/min, nateglinide eluted in about 7.5 min at 40^{o}C. Blood glucose level was determined with the Fuji DRICHEM 3500S (FUJIFILM Co., Japan).

Table 4-2: Column switching program.

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: Mobile Phase A and C passed through a 600E pump system, injector and pre column.
Mobile Phase B passed through a 515 pump, main column and detector.
2: Mobile Phase A passed through a 600E pump system, injector, pre column, main column and detector.
A 515 pump stopped.

Results

Plasma nateglinide concentration profiles during *in vivo* multiple oral administration (*b.i.d.* (interval: 12 hr), 8 days) of nateglinide controlled release tablet containing an erosion matrix tablet are shown in Figure 4-2. The nateglinide C_{max} values were 12.65 ± 0.92 μg/mL (T_{max}: 2 hr (11:00, the 1st day)), 19.54 ± 1.25 μg/mL (T_{max}: 13 hr (22:00, the 1st day)), 16.12 ± 2.84 μg/mL (T_{max}: 2 hr (11:00, the 8th day)), and 16.63 ± 1.72 μg/mL (T_{max}: 12.75 hr (21:45, the 8th day)). There were no significant difference of the C_{max} values observed among 13 hr (22:00, the 1st day) and 2 hr (11:00, the 8th day) and 12.75 hr (21:45, the 8th day) (Student's *t*-test). However, there were significant differences of the C_{max} values observed between 2 hr (11:00, the 1st day) and 13 hr (22:00, the 1st day) (Student's *t*-test, $P<0.05$). C_{max} values at 13 hr (22:00, the 1st day) and 2 hr (11:00, the 8th day) and 12.75 hr (21:45, the 8th day) demonstrated an increasing trend against that at 2 hr (11:00, the 1st day). This is because plasma nateglinide concentration just before administration was not zero (about 4 μg/mL, Figure 4-1) except at the first administration. It was found that nateglinide absorption during a multiple administration was reproducible.

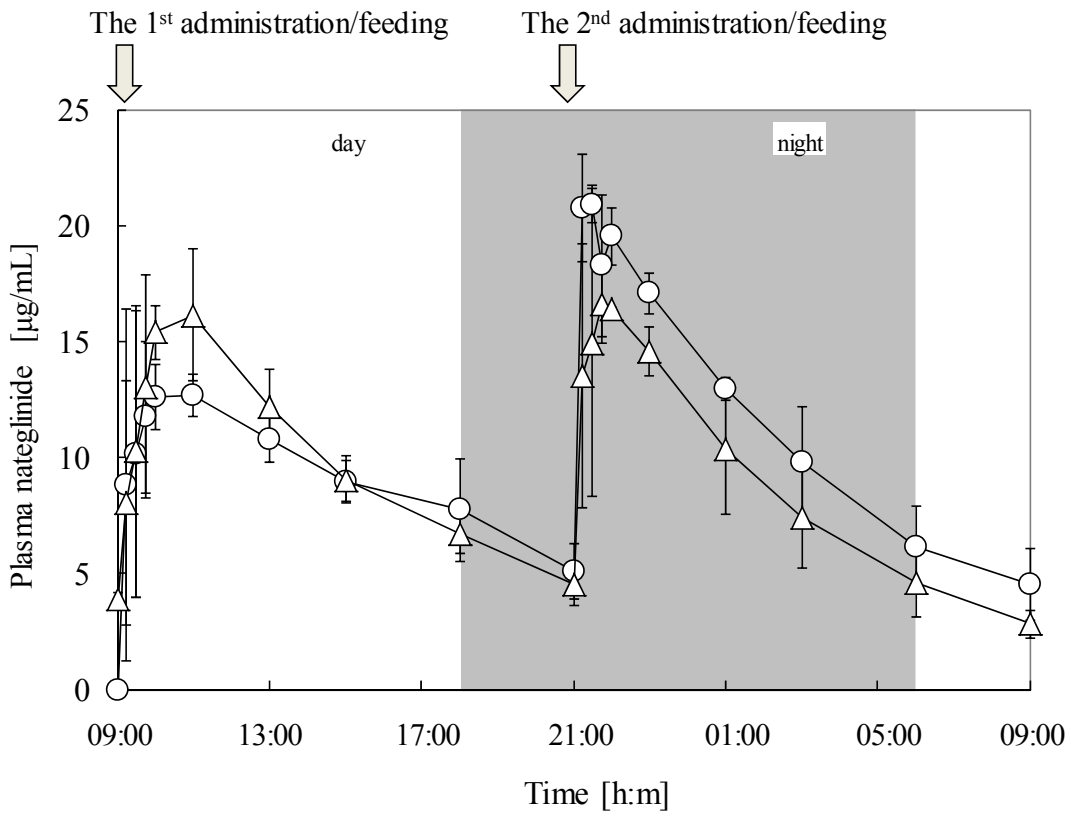


Figure 4-2: Plasma nateglinide concentration profile during a multiple oral administration of nateglinide cotrolled release tablet containing an erosion matrix tablet in fasted beagle dogs just before feeding.

Each point and vertical bar represent mean ± SEM.

○: The 1st day, n=3.

△: The 8th day, n=3.

Blood glucose level profiles are shown in Figure 4-3. Blood glucose level decreased to about 76.3% (1 hr, 10:00, the 1st day), 71.2% (6 hr, 15:00, the 1st day), 75.1% (12.25 hr, 21:15, the 1st day), 83.3% (14 hr, 23:00, the 1st day), 81.4% (1 hr, 10:00, the 8th day), 78% (6 hr, 15:00, the 8th day), 80.8% (12.25 hr, 21:15, the 8th day),

and 84.6% (14 hr, 23:00, the 8th day), as compared with blood glucose levels immediately before the 1st administration on the 1st day.

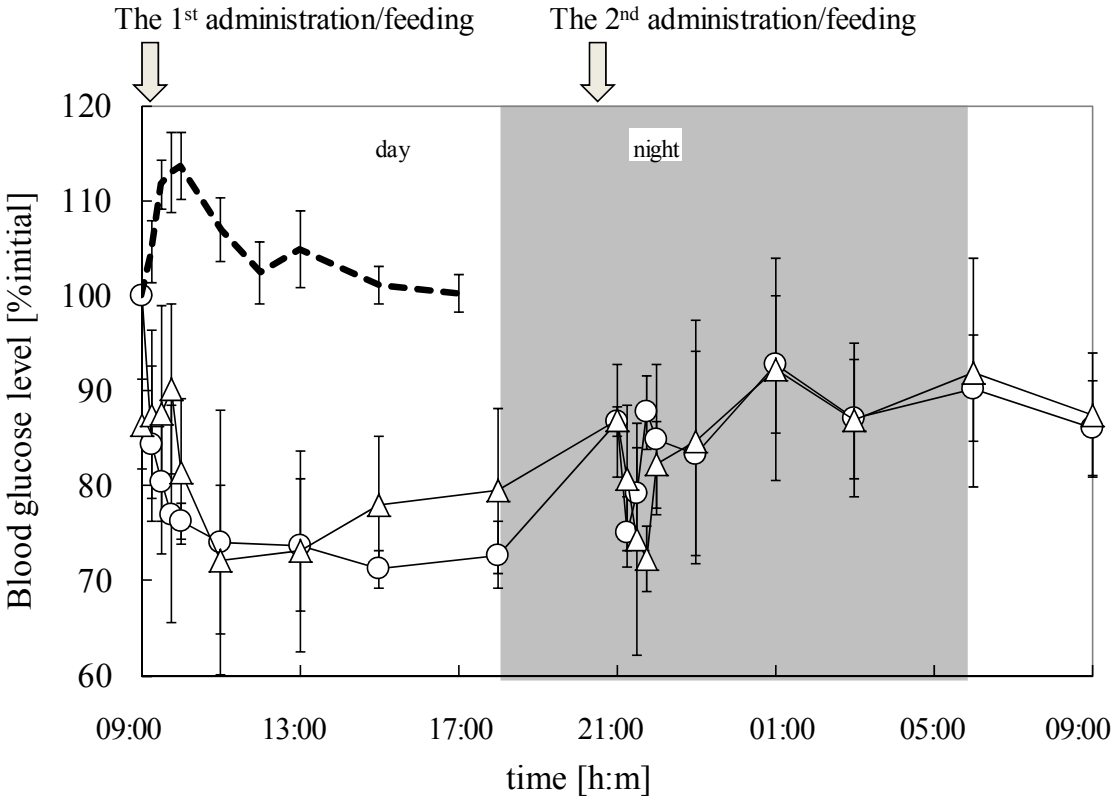


Figure 4-3: Blood glucose level during a multiple oral administration of nateglinide controlled release tablet containing an erosion matrix tablet in fasted beagle dogs just before feeding.

Each point and vertical bar represent mean ± SEM.

▬▬▬: only feeding、n=6.

○: 1st day, n=3.

△: 8th day, n=3.

Change in FBG at 9:00 A.M. during a multiple oral administration of the nateglinide controlled release tablet was evaluated. The results are shown in Figure 4-4. FBG at 9:00 decreased from the 1st day of administration to the 4th day by multiple administrations, and it rose after the 4th day.

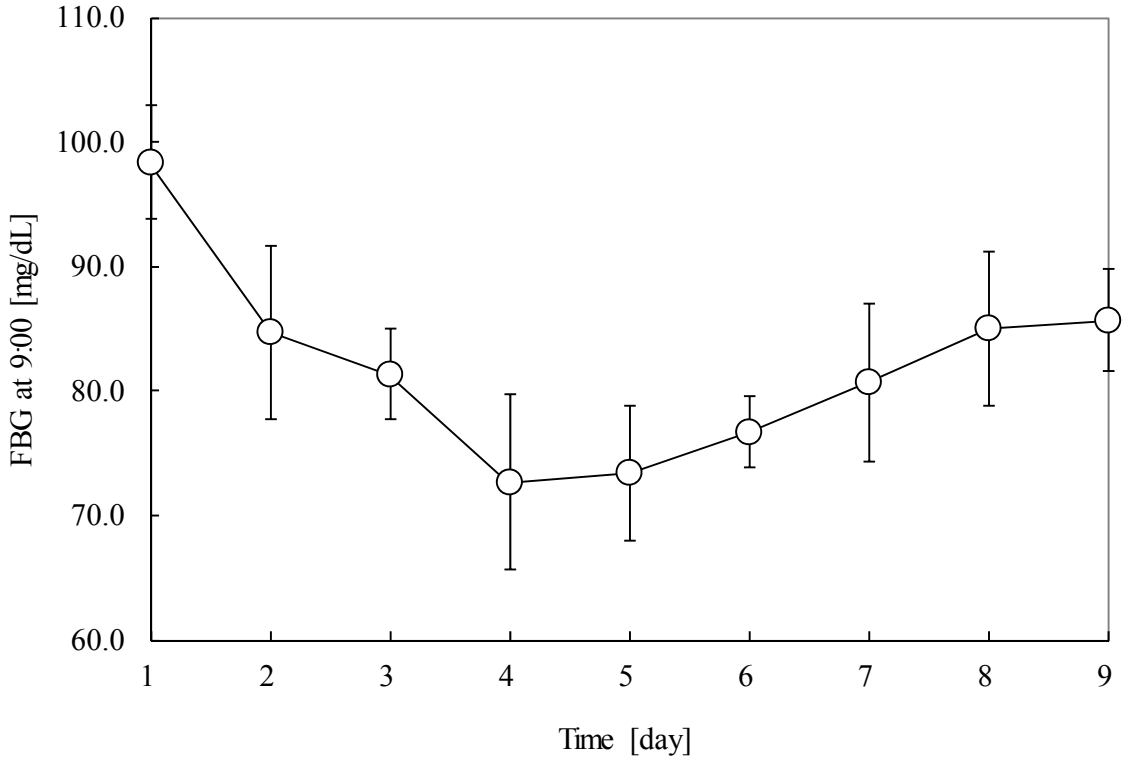


Figure 4-4: FBG at 9:00 profile in multiple administration study.

n=3, mean ± SEM.

Discussion

An *in vivo* multiple oral administration study (*b.i.d.* (interval: 12 hr), 8 days) with the nateglinide controlled release tablets using normal beagle dogs showed reproducibility of nateglinide absorption during this study. It was found that decreases in both PBG and FBG were observed. It was considered that the ability to decrease blood glucose level did not weaken during a multiple oral administration using the nateglinide controlled release tablet. Although it was initially believed that it would be difficult to continuously lower fasting blood glucose levels even if the release of an insulin secretion stimulator like nateglinide was able to be controlled, this study confirmed that the effect of decrease in both PBG and FBG did not weaken through a multiple oral administration, as shown in daytime of the 1st day with daytime of the 8th day.

The level of decrease in blood glucose level in the daytime was larger than that at night time of the 1st day and the 8th day. Beagle dogs were in a fasting state at night time, because they were sleeping and not able to eat. It was considered difficult to lower blood glucose level at night time due to glucose production by the liver at night time[39]. Further study is necessary. Such as a study would use diabetic model dogs, and so forth.

Change in FBG at 9:00 A.M. was evaluated. The reason the shape of the graph became convex below is unclear. FBG in the morning of the 4th day was about 73 mg/dL. This blood glucose level is too low for normal beagle dogs. The reason the blood glucose level rose after the 4th day is thought to be due to homeostasis. If the effect on PBG and FBG is evaluated using the nateglinide controlled release tablets with diabetic model beagle dogs of which blood glucose level is high, the graph where

FBG decreases might be obtained by a multiple oral administration.

As the above result, it is expected that a commercially available formulation will be designed, and that the effect on the blood glucose level of moderate and severe diabetes patients using nateglinide controlled release formulation will be evaluated in clinical trials. Furthermore, it is suggested that not only nateglinide but meglitinides (repaglinide, mitiglinide, etc.) can be used as an active ingredient in a controlled release tablet, because they are all classified as a short acting type insulin secretion stimulator[40].

Chapter 5

General Conclusions

With the controlled release form of insulin secretagogue, nateglinide, both PBG and FBG were able to be decreased in normal beagle dogs. A tablet that comprised of both immediate release granules and an erosion matrix tablet was considered preferable as a nateglinide controlled release formulation. In an 8 days-multiple administration study in normal beagle dogs, decrease in both PBG and FBG was observed even on 8^{th} day, furthermore, decrease in FBG in the morning was also observed. Although there was concern that the effect of decrease in blood glucose level was weakened by multiple administration of an insulin secretagogue, weakening of the effect was not observed even when multiple-administering nateglinide controlled release formulation to normal beagle dogs. Nateglinide controlled release formulation is expected to control both PBG and FBG for moderate or severe type 2 diabetes.

It is expected that a nateglinide controlled release formulation is useful for moderate or severe diabetes. However, it is only to be confirmed that both PBG and FBG are able to be decreased in an *in vivo* experiment according to the present results. For instance, only POP was confirmed. It is necessary to conduct a pre-clinical development and clinical development in order to submit a NDA in the future.

The author describe below the counterplan for issues that will occur through pre-clinical development and clinical development.

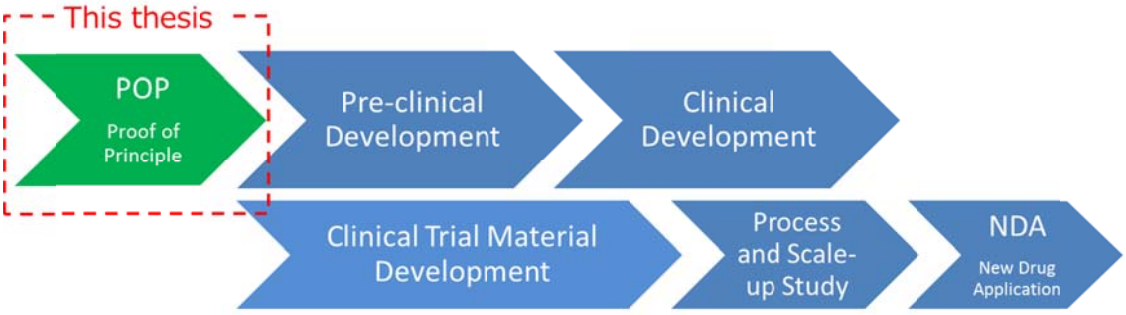


Figure 5-1: Diagram of development of nateglinide controlled release formulation. Each band shows a development stage.

Nateglinide controlled release formulation containing enteric coated granules

The issue that will be assumed when nateglinide controlled release formulation containing enteric granules is selected as a development candidate formulation, is to find the relationship between the formulation properties and the clinical trial parameters, such as dissolution pH value, dissolution rate at pH6.5, an amount of food, the kind of food. The author found that these parameters have an influence on blood glucose levels in *in vivo* study using normal beagle dogs. A clinical trial study protocol should be made in consultation with the above information.

Nateglinide controlled release formulation containing an erosion matrix tablet (A dry coated tablet)

The issue that will be assumed when nateglinide controlled release formulation containing an erosion matrix tablet is selected as a development candidate formulation, is to control dissolution rate of matrix portion. The author found the method to control dissolution rate of matrix containing nateglinide. The formulation design should be conducted in consultation with the above information.

Finally, a controlled release formulation containing a short-acting oral blood glucose regulator, not only nateglinide but meglitinides (repaglinide, mitiglinide, etc.) was suggested to enable control of both PBG and FBG for moderate and severe diabetes patients. With this controlled release formulation containing meglitinides, diabetics can have a future life without complications and with high a “Quality of Life”, which they have never experienced before.

References

1) *Treatment Guide for Diabetes*. Japan Diabetes Society (Eds.), Bunkodo, 2010, pp. 8-53.

2) Ikenoue T, Okazaki K, Fujitani S, Tsuchiya Y, Akiyoshi M, Maki T, Kondo N. Effect of a new hypoglycemic agent, A-4166 [(-)-*N*-(*trans*-4-isopropylcyclohexanecarbonyl)-D-phenylalanine], on postprandial blood glucose excursion : Comparison with voglibose and glibenclamide. *Biol. Pharm. Bull.*, **20**, 354-359 (1997).

3) Kondo N. Preclinical Studies of AY4166 (A-4166). *Jpn. J. Clin. Med.*, **55** (supple.), 159-163 (1997).

4) Package insert of Glimicron®, the 11th ed. Dainippon Sumitomo Pharm. Co., 2010.

5) Package insert and Interview form of Euglucon®, the 14th ed. Chugai Pharmaceutical Co., 2010.

6) Package insert of Amaryl®, the 21th ed. Sanofi, 2012.

7) Interview form of Fastic®, the 24th ed. Ajinomoto Pharmaceutical Co., 2012.

8) Package insert of Surepost®, the 4th ed. Dainippon Sumitomo Pharm. Co., 2012.

9) Package insert of Glufast®, the 11th ed. Kissei, 2013.

10) Package insert of Actos®, the 28th ed. Takeda Pharmaceutical, 2014.

11) Package insert of Metgluco®, the 4th ed. Dainippon Sumitomo Pharm. Co., 2010.

12) Interview form of Basen®, the 13th ed. Takeda Pharmaceutical Co., 2012.

13) Package insert and Interview form of Glucobay®, the 11th ed. Bayer, 2012.

14) Package insert and Interview form of Januvia®, the 12th ed. MSD, 2012.

15) Interview form of Deberza®, the 1st ed. Kowa, 2014.

16) Package insert of Suglat®, the 3rd ed. Astellas, 2014.

17) Interview form of Jardiance, 2nd ed. Boehringer Ingelheim, Japan , 2015.

18) Katsuno K, Fujimori Y, Takemura Y, Hiratochi M, Itoh F, Komatsu Y, Fujikura H and Isaji M. Sergliflozin, a novel selective inhibitor of low-affinity sodium glucose cotransporter (SGLT2), validates the critical role of SGLT2 in renal glucose reabsorption and modulates plasma glucose level. *J. Pharmacol. Exp. Ther.*, **320** (1), 323-330 (2007).

19) Interview form of Canaglu, the 5th ed. Tanabe-Mitsubishi, 2015.

20) Prescribing information of Avandamet®, GSK, 2011.

21) Prescribing information of Duetact®, Takeda Pharmaceutical North America, 2011.

22) Prescribing information of Actoplusmet®, Takeda Pharmaceutical North America, 2012.

23) Ohkubo T, Kurita T, Katayama S. Kanjya no byoutai byouki ni awaseta yakubutsuryouhou. *Yakkyoku*, **51** (11), 2341-2348 (2000).

24) Ogawa Y, Akiyama Y. *Formulation Design of Oral Dosage Forms*. Hashida M (Eds.), Yakugyo Jihosha, 1998, pp. 333-351.

25) Chang R K, Robinson J R. *Pharmaceutical Dosage Forms: Tablets Volume 3*. Herbert A, Lieberman, Leon Lachman and Joseph B. Schwartz (Eds.), Marcel Dekker, Inc., 1990, pp. 199-287.

26) Assmus M, Bössler H, Dreher D, Rothgang G, Weisbrod W, Peterreit H U, Beckert T, Liddiard C. *Practical Course in Film Coating of Pharmaceutical Dosage Forms with Eudragit®*. Lehmann K. (Eds), Röhm GmbH, 1999, pp. 1-38.

27) Maekawa H, Takagishi Y, Iwamoto K, Doi Y, Ogura T, Ito M, Kitamura K,

Fujimoto H. Sefarekishin no jyohouseiseizai (Prolonged formulation of cephalexin). *Jpn. J. Antibiotics*, **XXX-8**, 631-640 (1977).

Takagishi Y. Jyohouseiseizai no sekkei to baioabeirabirithi (Design of prolonged release formulation and bioavailability). *J. Jpn. Pharm. Assoc.*, **37**, 113-123 (1985).

Ogura T. Keikouyousefemukeikouseibusshitsu no seizaisekkei to seizaitokusei. *Antibiot. Chemother.,* **13** (11), 2097-2103 (1997).

28) Package insert of CONTAC®600PLUS, Glaxo Smith Kline Co., 2008.

29) Hu S, Wang S, Fanelli B, Bell PA, Dunning BE, Geisse S, Schmitz R, Boettcher BR. Pancreatic beta-cell K(ATP) channel activity and membrane-binding studies with nateglinide: A comparison with sulfonylureas and repaglinide. *J. Pharmacol. Exp. Ther.,* **293** (2), 444-452 (2000).

30) Lehmann K. Acrylic Resin Coatings for Drugs. *Drugs made in Germany*, **11**, 34-41 (1968).

Kokubo H, Obara S, Minemura K, Tanaka T. Development of cellulose derivatives as novel enteric coating agents soluble at pH 3.5-4.5 and higher. *Chem. Pharm. Bull.*, **45** (8), 1350-1353 (1997).

31) Ono I, Matsuda K, Kanno S. Determination of N-(trans-4-isopropylcyclohexanecarbonyl)-D-phenylalanine and its metabolites in human plasma and urine by column-switching high-performance liquid chromatography with ultraviolet detection. *J. Chromatography B*, **692**, 397-404 (1997).

32) Kawamori R, Watanabe K, Morishima T. Insulin, its kinetics and metabolism. *Jpn. J. Clin. Med.*, **55** (supple.), 124-132 (1997).

33) Field JB. Extraction of insulin by liver. *Ann. Rev.*, **24**, 309-314 (1973).

34) Jaspan J, Polonsky K. Glucose ingestion in dogs alters the hepatic extraction of insulin: *In vivo* evidence for a relationship between biologic action and extraction of insulin. *J. Clin. Invest.*, **69**, 516-525 (1982).

35) Cobelli C, Pacini G. Insulin secretion and hepatic extraction in humans by minimal modeling of C-peptide and insulin kinetics. *Diabetes*, **37**, 223-231 (1988).

36) Watanabe RM, Volund A, Roy S, Bergman RN. Prehepatic beta-cell secretion during the intravenous glucose tolerance test in humans: Application of a combined model of insulin and C-peptide kinetics. *J. Clin. Endocrinol. Metab.*, **69**, 790-797 (1989).

37) Shima Y, Mihara R, Suzuki M, Gonsho A. Pharmacokinetic studies of AY4166 (1), absorption, distribution and excretion in rats after single administration. *Jpn. Pharmacol. Ther.*, **25** (Suppl.), 181-193 (1997).

38) Masuda Y. *Strategy and Novel Technology on Pharmaceutical Preparations*. Takeuchi H (Supervised), CMC Press, 2007, pp.122-128.

39) Kikuchi M. Tou no nagare no kaisekihou. *Diabetes Frontier*, **6**, 642-651 (1995).

40) Kikuchi M. Non-SU, insulin secretagogue. *Nippon Rinsho*, **57**, 702-708 (1999).

Acknowledgements

The author expresses his sincere gratitude to Prof. Dr. Kouji Nakamura, University of Tsukuba for his valuable advises and encouragements as well as the kindest supports on preparation of this thesis. And the author thanks to Prof. Dr. PC Wang, Assoc. Prof. Dr. Motoo Utsumi and Assoc. Prof. Dr. Kosumi Yamada for their effort as a member of the judging committee for the doctoral thesis.

The present thesis is the collection of the studies which have been carried out under the direction of Dr. Akira Yabuki, Formulation, Pharmaceutical Research Lab., Ajinomoto Co. Ltd. during 1999-2001, for his invaluable suggestions, discussions and continuous encouragement throughout this work.

Grateful acknowledgement is made to Dr. Shigebumi Hashimoto (Ajinomoto Co. Ltd.), Mr. Kazuhiro Takanosu, Mr. Nobutaka Ninomiya, Mr. Haruo Orita (Ajinomoto Pharmaceuticals Co. Ltd.) and Mr. Hidetoshi Sakai, Dr. Akira Okano, Mr. Akinori Gonsho, Mr. Yasuhiro Tanaka, Dr. Yoshio Kawahara, Mr. Toshihisa Kato (Ajinomoto Co. Ltd.) for their constructive comments and encouragements. The author is much indebted to helpful discussions, Dr. T. Igawa and Mr. T. Sawada and coworkers at INA research (Japan) for *in vivo* studies using normal male beagle dogs, and Dr. Thomas Beckert at Röhm GmbH in Germany, and coworkers in Research Institute, Ajinomoto Pharmaceuticals Co. Ltd. and in Institute for Innovation, Ajinomoto Co. Ltd..

The author gratefully thanks to Mr. Norio Hayashi; his mother's brother, for his sincere encouragements. Finally the author expresses his deep gratitude to his father; Mr. Keisuke Makino, his mother; Mrs. Yoko Makino, his wife; Mrs. Shizu Makino, and his sons; Tsubasa and Hayato for their hearty encouragements.

List of Publication

Original papers

1) 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.

Chisato Makino, Nobutaka Ninomiya, Hidetoshi Sakai, Haruo Orita, Akira Okano, Akira Yabuki.

Chem. Pharm. Bull., **54** (4), 409-414 (2006).

2) Design of nateglinide controlled release tablet containing erosion matrix tablet and multiple administration study in normal beagle dogs.

Chisato Makino, Hidetoshi Sakai, Akira Okano, Akira Yabuki.

Chem. Pharm. Bull., **57** (9), 907-913 (2009)., Online, 2009,6,22.

3) Nateglinide controlled release tablet containing compressionable enteric coated granules.

Chisato Makino, Hidetoshi Sakai, Akira Yabuki.

Chem. Pharm. Bull., **58** (9), 1136-1141 (2010), Online, 2010,6,11.

4) A study of reducing pH dependence of dissolution profiles of nateglinide matrix granules.

Chisato Makino, Hidetoshi Sakai, Haruo Orita, Akira Yabuki.

IRYOU YAKUGAKU (*Jpn. J. Pharm. Health Care Sci.)*, **39** (10), 608-614 (2013).

5) Relationship between effect of decrease in blood glucose levels and dissolution behaviors in nateglinide enteric coated granules.

Chisato Makino, Hidetoshi Sakai, Haruo Orita, Akira Yabuki.

IRYOU YAKUGAKU (*Jpn. J. Pharm. Health Care Sci.)*, **40** (10), 602-608 (2014).

Patent applications

1) Oral preparations for diabetes. Toku-Gan-Hei 11-374959, WO 0147557.

2) Controlled Release Formulation Containing Pool-water Soluble Drug.

   Toku-Gan 2012-216647.