International Journal of Drug Delivery 3 (2011) 270-278
http://www.arjournals.org/index.php/ijdd

Original Research Article

ISSN: 0975-0215

Pharmacokinetic and pharmacodynamic evaluation of a new sustained-release capsules using starch-sponge matrix (SSM) release system for nifedipine in rats

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Abstract

We conducted a performance assessment study for a new sustained-release capsule including starch-sponge matrix (SSM). The SSM, which is a support medium for drug release, was made from 2.5% cornstarch glue by means of freezing dry method. The SSM capsule was applied for nifedipine (NFP), a calcium channel blocker, and evaluated pharmacokinetic and pharmacodynamic (PK/PD) profiles of NFP after intraduodenal administration of SSM capsules including 2.5 or 5.0 mg of NFP per capsule to rats. Plasma NFP concentrations from the SSM capsules showed dose-dependent increases with a Michaelis-Menten like behavior over 360 minutes after intraduodenal administration. The values of area under the concentration vs. time curve from time zero to 360 min (AUC_{0-360}) of NFP declined in making SSM capsules as compared to control capsules due to a simple physical mixture of NFP and cornstarch, but the values of mean residence time (MRT_{0-360}) extended and abidingness of SSM capsules were admitted with dose-dependent manner. As for a PD parameter, the mean arterial blood pressure (mABP) derived from the SSM capsules showed 15~20% decrease of baseline within 120min after intraduodenal administration, and thereafter the mABP in 2.5 mg SSM capsule was gradually recovered, while a relatively smooth and even change was found in the mABP at 5.0 mg SSM capsule. The relationships between plasma NFP concentration and sampling-time corresponding mABP after intraduodenal administration of SSM capsules showed no rapid change in the mABP, indicating that a sustained-release mechanism due to the SSM functions sufficiently to avoid a fluctuating blood pressure accompanied by going up and down of plasma levels of NFP. The SSM capsules exhibited a sustained-release pharmacokinetics of NFP, and made the fluctuation range with blood pressure small compared to the physical mixture preparations. Thus, it was evidenced that the SSM capsule is useful device to provide a sustained-release systems and optimal therapeutic efficacy of drugs.

Keywords: Controlled-release, Cornstarch, Matrix, Nifedipine, Pharmacokinetics, Pharmacodynamics.

Introduction

In the field of manufacturing medicine, there are a numerous number of reports regarding sustained-release systems of drugs, where various types of pharmaceutical additive are used for making. The sustained-release systems of drugs provide several advantages for drug therapy, for instance, increasing in patients' compliance, avoiding side effects and prolongation in efficacy periods, and so on. Cornstarch is generally used as a pharmaceutical excipient to prepare tablets or capsules. Moreover, natural starches and chemical modified starches such as substituted amylase [1], cross-linked amylase [2], starch acetate [3] or amylodextrin [4] have been extensively investigated as sustainedrelease excipients for tablets by direct compression. Thermally pregelatinaized starches also proved to be suitable for formulating hydrophilicmatrices [5–7]. In our previous report, we developed a new sustained-release system using cornstarch glue, namely, a capsule including starch-sponge matrix (SSM). The SSM capsule is composed of a gelatin capsule and SSM with drug, and the SSM has a porous and netlike structure with Krumbein's diameter of 5～22 μm, and has mucoadhesive property to increase absorption rate of a drug at intestinal tract [8]. The SSM capsule, therefore, could be applied for various types of drugs with various lipophilicity [8]. This time, we conducted a performance assessment study for this new sustained-release capsule including the SSM. This SSM capsule was applied for nifedipine (NFP), a calcium channel blocker, and evaluated pharmacokinetic and pharmacodynamic (PK/PD) profiles of NFP after intraduodenal administration of the SSM capsules including 2.5 or 5.0 mg of NFP per capsule in rats. NFP was dispersed in paste-like cornstarch (starch glue) after heating 2.5% (w/v) cornstarch suspension with electromagnetic wave at 2450 MHz (700 W) for l min. Then the drug mixture was encapsulated into a five number gratin capsule by a syringe, and the SSM capsule including NFP was prepared by means of a freezing dry method. In this study, the mean arterial blood pressure (mABP) was used as an indicator for the pharmacodynamic parameter.

Materials and methods

Chemicals and stock solutions

Cornstarch to make a sustained-released matrix in capsule, NFP (LogPow=3.23) and midazolam (MDZ, an internal standard for drug assay) were purchased from Nacalai Tesque, (Kyoto, Japan). A five number gelatin capsule (4 mmφ×10 mm, volume 150 μL), Japanese Pharmacopoeia grade, was purchased from Matsuya Co. Ltd. (Osaka Japan). All other chemicals and reagents were used of analytical grade without further purification. MDZ solution used drug assay was prepared by dissolving MDZ in methanol to a final concentration of 1 μg/mL.

Animals

Male Wistar rats of 300 to 350 g were procured from Nippon SLC Co. Ltd. (Hamamatsu, Japan). All animal experiments were performed in accordance with the guidelines for animal experimentation of Doshisha Women's College of Liberal Arts, Pharmaceutical Division and federal requirements for animal studies. Rats have free access to food and water and were housed in temperature control facility (22±2°C) with a 12-hour light/dark cycle for at least one week before use.

Preparation of SSM capsules of NFP

Preparation method for SSM capsule of NFP was previously described.[8] In brief, cornstarch was suspended in distilled water at a final concentration of 2.5% (w/v), then the mixture was microwaved at 2450 MHz (700 W) for 1 min to make starch glue. After cooling the glue in a ice bath, NFP was added and mixed well in a mortar. The final concentrations of NFP in starch glue were 16.7 or 33.3 mg/mL. After making a 300 μm i.d. hole with a micro-drill in the middle portion of a five number gelatin

capsule at the junction of cap and body, 150 µL aliquot of drug mixture with glue loaded by a syringe, then immediately the mixture loaded capsule was frozen at −80°C in a deep freezer. Then a freeze-dried method (−50°C, 9.9 Pa, for 24 hr) was applied for these preparations to make SSM capsule including 2.5 or 5 mg of NFP per capsule. As control formulations for NFP, physical mixture (PM) between NFP and cornstarch was prepared at a final concentration of 16.7%(w/w), and this diluted drug powder was encapsulated to the number five gelatin capsule at 2.5 or 5 mg/capsule, where the amount of cornstarch used to prepare capsules was the same between SSM capsules and PM capsules as control.

Animal operation and monitoring of blood pressure

After over night fasted though water allowed ad libitum for 16-18 hours prior to experiment, rats were anesthetized by intraperitoneal administration of urethane (1.0 g/kg) and placed in supine position on surgical table under an incandescent lamp to keep body temperature at 37°C. The carotid artery was cannulated with poly-ethylene tubing (i.d. 0.5 mm, o.d. 0.8 mm) containing heparinized saline (100 IU/ml) for measurement of blood pressure. A small longitudinal section was made in the skin of the rat over the left jugular vein for the collection of blood samples over time. The carotid artery was attached to blood pressure monitoring kit (Nihon Kohden, Japan) through the carotid artery cannula. The systolic and diastolic blood pressure were measured by using the Notocard HEM data acquisition and analysis system (Notocard HEM 3.5, France), and the output was continuously displayed on digital screen attached to the system.

Drug administration and sample collection

Rats received intraduodenal administration of control or SSM capsules including NFP (2.5 mg or 5 mg). After making a small incision on duodenal wall at the position of just passing over the biliary duct, a capsule was inserted. Then the wound site was made three stitches by suture thread and pasted by surgical glue. Blood samples of 120 µL each were collected into a 1.5 mL heparinized polyethylene centrifuging tube from the right jugular vein at 10, 20, 30, 60, 90, 120, 180, 240 and 360 min. The blank blood samples were taken at 5 min prior to the intraduodenal administration of capsule. The blood samples were centrifuged at 3000×g for 15 min at 4°C, and 50 µL of plasma was obtained, and then stored at −20°C until analysis.

Assay for NFP

NFP in plasma was extracted by taking 50 µL of aliquot of plasma into a polyethylene centrifuge tubes and 2.5 mL of MDZ solution (0.0025ng/mL) was added as an internal standard and mixed for 30 s, then 100 µL of 2% (w/v) $ZnSO_4$ in 50% (v/v) methanol solution was added to precipitate proteins. The mixture was mixed for 30 s and centrifuged at 3000×g for 15 min. The clear supernatant was transferred into a 1.5 mL polyethylene centrifuge tubes and 1 mL of diethyl ether was added, then the mixture was mixed for 30 s and centrifuged at 3000× g for 15 min. Decant the supernatant into a new glass test tube and evaporate in an evaporator for 30 min at 45°C. The residue was then reconstituted in 1 mL of HPLC mobile phase and 20 µL was injected into liquid chromatography-tandem mass spectrometer (LC/MS/MS) system. The LC/MS/MS (Sciex, 4000 Q TRAP with Analyst1.4.1 version) analysis was carried out by equipping with an HPLC system (Shimadzu, Kyoto, Japan). The mobile phase which consists of 90% (v/v) acetonitrile containing 0.1% formic acid was degassed before use. The sample elution was performed with a flow rate of 0.2 mL/min, and each analysis lasted for 5.0 min. The mass spectrometer was operated in the turbo-ion spray mode with positive ion detection. The flow rate of nebulizer gas, curtain gas and collision gas were set at 8, 8 and

2L/min, respectively, and the ion spray voltage and temperature were set at 5000 V and 300°C. The declustering potential, the focusing potential, the entrance potential, the collusion energy and the collision cell exit potential were set at 20, 200, -10, 30 and 6 V, respectively. Multiple reactions monitoring analysis was performed with the transition m/z 296 for NFP and m/z 326 for MDZ. All raw data were processed with Analyst Software, version 1.4.1. Taking the peak area ratio of NFP against the internal standard (MDZ), the calibration curves of NFP were made in plasma. The retention times for NFP and MDZ were 2.24 and 1.78 min, respectively. Calibration curve of NFP were linear and passed through the origin with correlation coefficients of 0.999 or over. The limits of detection were NFP was 0.005 μg/mL.

Pharmacokinetic (PK) and pharmacodynamic (PD) analysis

PK parameters from each of the individual rats plasma concentration–time profiles of NFP were analyzed by non-compartmental method. The area under the plasma concentration–time curve from time 0 to the last time ($AUC_{0\text{-}360}$) was calculated by using a linier trapezoidal rule. The area under the first moment curve to the last measurable concentration ($AUMC_{0\text{-}360}$) was also calculated by a linier trapezoidal rule, and the mean residence time to the last measurable concentration ($MRT_{0\text{-}360}$) was calculated by an equation of $AUMC_{0\text{-}360}/AUC_{0\text{-}360}$. Then, the variance of $MRT_{0\text{-}360}$ ($VRT_{0\text{-}360}$) was calculated. As for PD parameters, the mean arterial blood pressure (mABP) was calculated every 5 minutes using continues record of systolic and diastolic blood pressure before and after intraduodenal administration of the SSM or control capsules using full excerpts of diastolic or systolic arterial blood pressure from continuous Notocord HEM 3.5 records and noted mean values corresponding at 0, 10, 20, 30, 60, 90, 120, 180, 240 and 360 min after intraduodenal administration of NFP capsules.

Statistical analysis

Values are expressed as their mean or mean±SD. Statistical significance for the mean values was accepted when the p-value was less than 0.05, using a one-way analysis of variance and a multiple comparison test.

Results

Absorption efficacy of NFP from the SSM capsule after intraduodenal administration was studied in rats. Figure 1 shows the plasma NFP concentration vs. time curves after administration of 2.5 mg/capsule or 5.0 mg/capsule with or without the SSM. After administration of PM capsules, plasma NFP concentrations showed dose-dependent increases with maximum concentrations around 180 min, and thereafter decreased gradually.

Table 1 Pharmacokinetic parameters of NFP after intraduodenal administration of SSM capsules by non-compartmental method.

	NFP 2.5 mg/capsule		NFP 5.0 mg/capsule	
	Control[a)]	SSM	Control[a)]	SSM
$AUC_{0\text{-}360}$ (μg・min/mL)	3180±890	1115±221	5790±1765	2387±239
$MRT_{0\text{-}360}$ (min)	205±10	233±21	208±8	219±11
$VRT_{0\text{-}360}$ (min^2)	222267±36674	555612±29173**	119779±45097	273036±72764**

Values are expressed as mean ± SD; n= 6 in each group. **): $p<0.01$ against respective control. a) Control capsules were prepared by mixing NFP with cornstarch (physical mixture).

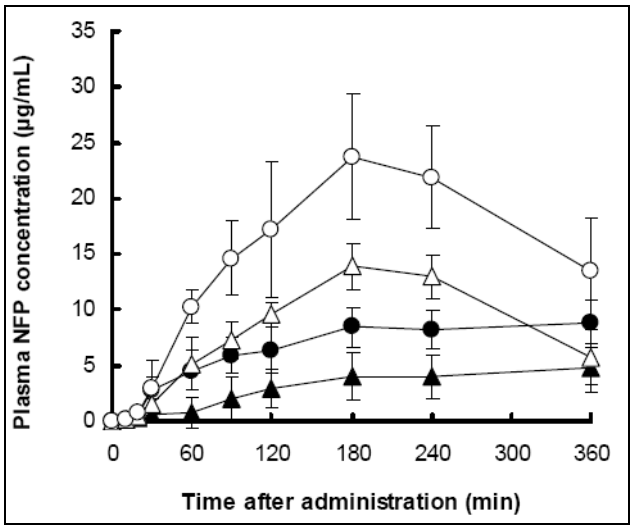

Fig. 1: Plasma NFP concentration-time curve after intraduodenal administration of SSM capsules of NFP to rats.

Each value represents the mean±SD of 6 rats in each group.

Key: Δ, PM capsule (2.5 mg/rat); ▲, SSM capsule (2.5 mg/rat); ○, PM capsule (5.0 mg/rat); ●, SSM capsule (5.0 mg/rat)

On the other hand, after administration of SSM capsules, its plasma NFP concentrations showed dose-dependent and Michaelis-Menten like increases without peak concentrations over 360 minutes. PK parameter values, which were calculated using the non-compartmental analysis method, are listed in Table 1. The $AUC_{0\text{-}360}$ values for NFP with PM capsules including 2.5 mg or 5.0 mg NFP were 3180±890 or 5790±1765 μg・min/mL, respectively, whereas those for NFP capsules with SSM were 1115±221 or 2387±239 μg・min/mL, respectively. On the other hand, the $MRT_{0\text{-}360}$ vales for NFP capsules with PM including 2.5 mg or 5.0 mg NFP were 205±10 or 208±8 min, respectively, whereas those for NFP capsules with SSM were 233±21 or 219±11 min, respectively. The $VRT_{0\text{-}360}$, which values are related to the effectiveness of capsules, for NFP capsules with PM including 2.5 mg or 5.0 mg NFP were 222267±36674 or 119779±45097 min^2, respectively, while those for NFP capsules with SSM were 555612±29173 or 273036±72764 min^2, respectively.

As for PD parameter, we used the mean arterial blood pressure (mABP). Figure 2 shows changes in mABP after intraduodenal administration of NFP capsules. The data show percent changes of mABP in rats. After intraduodenal administration of NFP capsules without SSM in rats, consecutive decrease in the relationship between mABP and time, and 5 mg capsule without SSM showed a profound effect to reach at 40% decrease of baseline during 180 to 360 min. On the other hand, after intraduodenal administration of NFP capsules with SSM, the mABP of both capsules, including 2.5 mg or 5.0 mg NFP, showed 15~20% decrease of baseline within 120min. Thereafter, the mABP in 2.5 mg SSM capsule was recovered gradually, while a relatively smooth and even change was found in 5.0 mg SSM capsule.

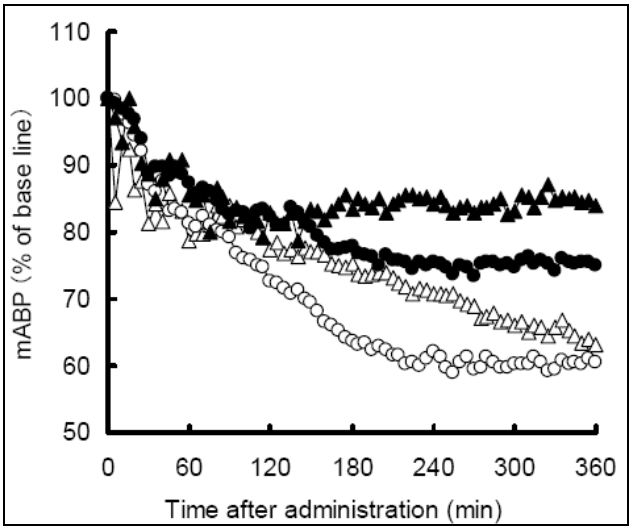

Fig. 2: Mean arterial blood pressure (mABP)-time curve after intraduodenal administration of SSM capsules of NFP to rats.

Each value represents the mean of 6 rats in each group.

Key: Δ, PM capsule (2.5 mg/rat); ▲, SSM capsule (2.5 mg/rat); ○, PM capsule (5.0 mg/rat); ●, SSM capsule (5.0 mg/rat)

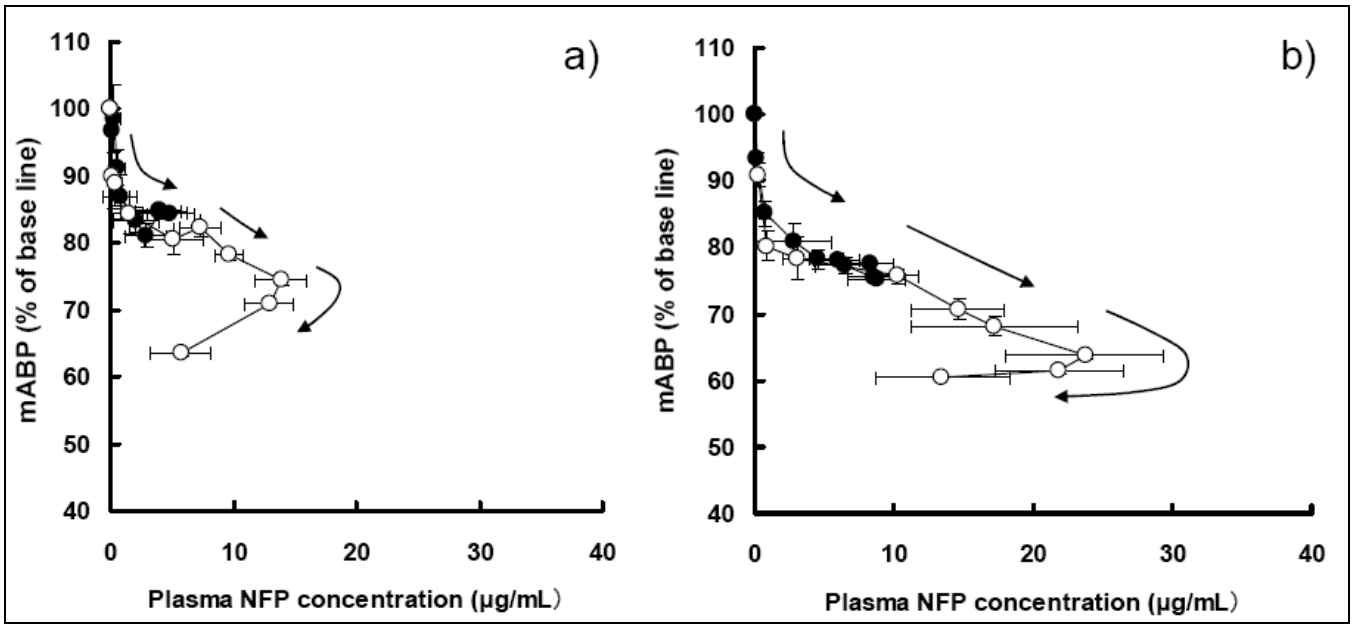

Fig.3: Relationship between plasma NFP concentration and mean blood pressure after intraduodenal administration of SSM capsules of NFP to rats. Rats received 2.5 mg NFP (a) or 5.0 mg NFP (b) by PM capsule or SSM capsule. Arrowed lines represent proceeding with time from zero to 360 min. Each value represents the mean±SD of 6 rats in each group. Key: ○, PM capsule; ●, SSM capsule.

To evaluate the relationship between plasma NFP concentration and mABP, the values of mABP corresponding to sampling times were calculated using full excerpts from continuous Notocord HEM 3.5 records. Figure 3 represents the relationships between plasma NFP concentration and the sampling-time corresponding mABP. In the PM capsules, even if NFP concentration in plasma passed the peak level, the mABP continued to fall. However, in case of the SSM capsules, a rapid change was not found in the values of mABP, indicating that a sustained-release of NFP from the SSM functions sufficiently.

Discussion

Oral delivery of drugs is the most attractive route of administration, where sustained release system is one of major field in pharmaceutics to provide reasonable therapeutic effects of drug as well as to increase the patient's conveniences [9]. There are a large number of reports about sustained-release drug delivery system to increase oral bioavailability using excipients beside of starches, therapeutic effect and duration of action of drugs. Solid dispersion systems using polyvinilpirolidone[10], polyethylenglycol [11] or fine porous silica particles [12,13] are useful method to improve drug release and solubility. Moreover, the hydrophilic nanoparticles or microspheres using polymers such as chitosan or poly(D,L-lactic-co-glyceric acid) have received much attention to deliver therapeutic peptide, protein, antigen, oligonucleotide and gene by intravenous, oral or mucosal administration [14,15]. In addition, nanoparticles or microspheres using such biodegradable pharmaceutical products have a mucoadhesive property to increase drug bioavailability [17-18]. Recently, we developed a new sustained-release system for therapeutic drugs using starch glue, namely, a starch-sponge matrix (SSM) sustained capsule [8]. The SSM capsule we developed was made from one of natural starches, corn starch, and the SSM had a matrix structure to regulate the drug release in vitro and in vivo [8]. The SSM capsule based on starch glue provided sustained release following Higuchi's diffusion controlled model [19], because the SSM had a ghost matrix after drug release in vitro. Moreover, it was found by X-

ray contrast examination that the SSM has local-residential and mucoadhesive properties. This mucoadhesive property is an important characteristic for the sustained-release system to maintain a constant plasma drug levels [20-22]. In this study, as a next step, we evaluated the PK/PD aspects of the SSM capsule for NFP.

NFP, a hypertensive calcium channel blocker, is commonly administered to subjects with coronary heart disease who often exhibited hyperlipidemia or diabetics mellitus [23]. In the current study, doses of 2.5 mg/rat and 5.0 mg/rat were used to examine the usefulness of the SSM capsule. However, therapeutic dose of oral NFP is <1 mg/kg in human patients [24]. If assuming that rat's body weight was 300 grams, the applied-doses of NFP in this study were 8.3 mg/kg and 16.7 mg/kg, respectively, and those were apparently over-dose.

As NFP is more stronger in the affinity to the peripheral-vessel than in the heart, an overdose of NFP causes a reflectivity pulsus frequens due to blood pressure fall as a result of restraining Ca^{2+} entry to the vascular smooth muscle [25]. In deed, the heart rate in rats after intraduodenum administration of SSM capsule of NFP increased (data not shown). In this study, however, we focused to investigate about pharmaceutical property of NFP capsule with SSM. As shown in Figs 2 and 3, a sustained-release aspects were brought and the fluctuation range with blood pressure became small in the SSM capsules as compared to the capsules of physical mixture between NFP and cornstarch. The values of $AUC_{0\text{-}360}$ for NFP declined in making SSM capsules, but the values of $MRT_{0\text{-}360}$ extended and abidingness of SSM capsules was admitted, and moreover they were dose-dependent. Thus, it was evidenced that the SSM capsule is useful device to provide a sustained release systems and optimal therapeutic efficacy of drugs.

Essentially, drug-free SSM has a porous and netlike structure, and the distribution aspect of model drugs in the SSM depends on physicochemical properties between cornstarch glue and drugs. In our previous report, three representative drugs (uranine, indomethacin and nifedipine) with different physicochemical properties were selected as model drugs and in vitro release and in vivo pharmacokinetics were examined [8]. Uranine with much lower lipophilicity exists in continued phase of SSM, and indomethacin or NFP with a moderate or a higher lipophilicity exist in continues phase or porous space of the SSM. In the in vitro dissolution study, the release rate of drug from the SSM was mainly dependent on the lipophilicities of drugs, showing a rank order of the release rate as uranine > indomethacin > NFP. Moreover, the sustained-release effect of SSM capsule was enhanced with an increase in the lipophilicity of a drug, and local-residential and mucoadhesive properties of SSM in the intestine provided stable supply of drugs from the SSM. Moreover, the in vitro release rate for each drug was well regulated by changing the initial concentration of cornstarch suspension. In addition, in vivo absorption studies in rats, after intraduodenal administration of SSM capsules including those model drugs revealed that the sustained-release effects also could be regulated by the initial concentration of starch suspension. As evaluated here, the SSM capsules for NFP showed a sustained-release profiles of NFP and the small fluctuation in blood pressure as compared to the control capsules, suggesting that the SSM capsules can contribute to avoid abrupt increase or drastic decline in plasma NFP levels and side effects which are related to fluctuation of cardiovascular parameters. The SSM capsule we developed, therefore, shows promising results as an oral drug delivery system for sustained-release regulation or target specificity in the lumen.

Conclusion

A new sustained-release capsule using the SSM derived from cornstarch glue was applied for NFP, and its PK/PD aspects in rats were evaluated. The SSM capsule exhibited a

sustained-release pharmacokinetics of NFP, and made the fluctuation range with blood pressure small as compared to the capsule of physical mixture. The SSM capsule is useful device to provide a sustained-release systems and optimal therapeutic efficacy of drugs.

Acknowledgement

This study was supported by Project Research Grants in Doshisha Women's College of Liberal Arts (2007).

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