

Pharmacokinetic and Pharmacodynamic Evaluations of Novel Oral Morphine Sustained Release Granules

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Pharmacokinetics and pharmacodynamics of novel oral sustained-release granules based on swelling polymer incorporation layer system (SPILA granules) containing morphine hydrochloride was evaluated. SPILA granules were designed to release morphine faster in neutral environment than in acidic one to keep higher plasma levels over a protracted period, especially after 12 h post dose. SPILA granules were orally administered to beagle dogs to compare the pharmacokinetics with commercially available twice-a-day dosage form, MS Contin[®]. T_{max} and AUC_{0-24h} values of SPILA granules were 6 h and 191 $\mu\text{g}\cdot\text{h}/\text{ml}$, respectively. T_{max} and AUC_{0-24h} values of MS Contin[®] were 2 h and 146 $\mu\text{g}\cdot\text{h}/\text{ml}$, respectively. Relative bioavailability following SPILA granules administration to twice-a-day MS Contin[®] (30 mg) administration was 131%. In rats, analgesic effect was evaluated over 24 h. SPILA granules and aqueous solutions were administered to rats to compare the analgesic effect. AUC_{0-24h} value for SPILA granules was 8.88 $\mu\text{g}\cdot\text{h}/\text{ml}$, which was a little lower than that for the aqueous solution (10.1 $\mu\text{g}\cdot\text{h}/\text{ml}$), whereas the analgesic effect after SPILA granules once-a-day administration expressed as $AURC$ (1701% Analgesia $\cdot\text{h}$) was similar to that after the aqueous solution 4 times-a-day administration (1603% Analgesia $\cdot\text{h}$). These results indicate that SPILA granules based on the pH-dependent release regulating polymer system can be a good candidate for an oral once-a-day sustained-release dosage form.

Key words swelling polymer incorporation layer; sustained-release granules; pH-dependent release; morphine; analgesic effect; once-a-day administration

Pain control is crucial for a good quality of life for patients with severe cancer pain, namely, the complete management of cancer pain can help patients lead the same life style as healthy people. For cancer pain control, we have no analgesic which is superior to morphine.^{1,2)} Especially for the care at home or long lasting care, pain self-control using oral sustained-release dosage forms containing morphine is inevitable.

We designed oral once-daily sustained-release granules based on a swelling polymer incorporation layer system (SPILA granules) containing morphine hydrochloride for the treatment of chronic cancer pain.³⁾ Prerequisites were; a) once-daily administration, b) duration of constant pharmacological (analgesic) effect due to constant plasma morphine levels. Generally, oral SR formulations tend to provide lower bioavailability compared to IR formulations, because release from the SR formulations, especially the formulations containing basic drugs, are not complete during the transit through the GI tract. The lower bioavailability after administration of oral SR formulations would result from a reduction in release in the lower small intestine and colon due to lack of fluid and lower solubility of basic drugs.⁴⁻⁶⁾ SPILA granules were designed to release morphine hydrochloride faster in neutral environment than in acidic one due to a coating layer with pH-dependent swelling polymer, carboxyvinyl polymer (CP) (Fig. 1). SPILA granules which consists of core granules coated with a mixture of CP, water insoluble polymer, and water soluble polymer can be prepared by only simple coating of core granules. SPILA granules were designed as a multiple unit system because the system could spread out over a large area of intestine and provide more stable levels of drug in the plasma after oral administration.⁷⁾

In SPILA granules, ethylcellulose (EC) was used as a water insoluble polymer, and one or two polymers were selected from polyethyleneglycol (PEG), hydroxypropyl methylcellulose (HPMC) and hydroxypropylcellulose (HPC) as water soluble polymer.

The purpose of this study was to evaluate SPILA granules containing morphine hydrochloride pharmacokinetically and pharmacodynamically. In pharmacokinetic evaluation of oral solid dosage forms, beagle dogs have usually been used. On the other hand, in the pharmacodynamic evaluation of morphine, beagle dogs have not been used but rats and mice. For analgesic evaluation of morphine, tail-flick test or hot-plate test using rats or mice has been adopted,⁸⁾ and morphine is usually administered in the form of aqueous solutions orally or injectably in the tests. In addition, long-lasting analgesic tests have hardly reported. In this paper, the efficiency of this pH-dependent SPILA granules containing morphine hydrochloride was evaluated by comparing the pharmacokinetic profile with commercially available morphine sulfate sustained-release tablet, MS Contin[®], in beagle dogs. In addition,

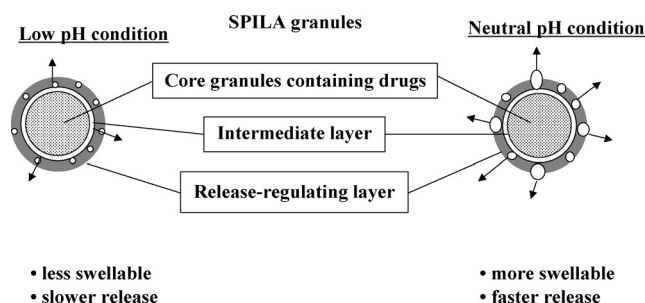


Fig. 1. Drug Release Mechanism from SPILA System

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tion, both pharmacokinetic profile and analgesic profile after SPILA granules administration were compared with those after aqueous solution administration in rats for 24 h.

MATERIALS AND METHODS

Materials Morphine hydrochloride was provided by Takeda Pharmaceutical Company Limited (Osaka, Japan). Microcrystalline cellulose (MCC, Asahi Kasei Chemicals, Japan), corn starch (Nihon Corn Starch, Japan), low-substituted hydroxypropyl cellulose (L-HPC, Shinetsu Chemicals, Japan), polyvinylpyrrolidone (PVP, BASF Takeda Vitamin, Japan), PEG (PEG6000, Sankyo Chemical Industries, Japan), HPMC (TC-5, Shinetsu Chemicals, Japan), HPC (HPC-L, Nisso Co., Japan), talc (Matsumura Sangyo, Japan) and cethyl alcohol (Kao Co., Japan) were used in Japanese Pharmacopoeia grade (JP). EC (Ethocel, Dow Chemical, MI, U.S.A.) and CP (Hiviswako, Wako Pure Chemical, Japan) were used in Japanese Pharmaceutical Excipients grade (JPE). All other reagents used were of analytical grade.

Preparation of SPILA Granules SPILA granules were prepared by coating of release-regulating membrane on the core granules made by the extrusion-spheronization method as previously described.³⁾ In brief, powder mixtures comprising morphine hydrochloride, MCC, corn starch, L-HPC, PVP, PEG, HPC and tartaric acid were blended and then water was added to the blended mixture to yield a wet mass with the proper consistency for wet granulation. The wet mass was passed through a Dome gran DG-L1 (Fuji Powdal Co., Japan) fitted with 0.6-mm screen and operated at 50 rpm. The cylindrical extrudate was immediately spheronized in a Marumerizer OJ-230G (Fuji Powdal Co., Japan). Granules were dried under vacuum for 16 h at 40 °C and collected 14/30-mesh (500—1180 μm) fraction. The obtained core granules were placed in the prewarmed chamber of coating apparatus FD-S-2 (Fuji Powdal Co., Japan). After coating an ethanolic-water suspension of HPMC, tartaric acid and talc as an intermediate layer to a 5% weight increase, an ethanolic-water suspension of EC, CP, PEG, HPC, tartaric acid, cethyl alcohol and talc was also sprayed onto the granules as a release-regulating membrane resulting in a 26% weight increase. The granules after being dried at 70 °C for 1 h and collected in 12/30-mesh (500—1400 μm) fraction were used as sustained-release SPILA granules.

In Vitro Release Test *In-vitro* release of morphine hydrochloride from SPILA granules was evaluated using the dissolution apparatus No. 2 (paddle) of the JPXII at a rotation speed of 100 rpm at 37 °C, in 900 ml of pH 1.2 (JPXII, first fluid) and pH 6.8 (JPXII, second fluid).

The release of morphine hydrochloride was determined by HPLC with fluorescence detection (ex. 280 nm, em. 344 nm). Separation on the octadecylsilane column (YMC-Pack ODS-AM, 150 mm length \times 4.6 mm diameter) was achieved at 40 °C and a 1 ml/min flow rate. The mobile phase was a mixture of 10 mM phosphate buffer (containing 1 mM sodium dodecyl sulfate), adjusted to pH 2.1 : acetonitrile (74 : 26).

The mean *in vitro* dissolution time (MDT) was calculated according to the Eq. 1:

$$MDT = ABC_{in\ vitro} / M_{\infty} \quad (1)$$

where $ABC_{in\ vitro}$ is the area between the release curve and its

asymptote, calculated by the trapezoidal rule from time zero to the last measured time point, and M_{∞} is the total amount of released drug at this time point.⁹⁾

In Vivo Experimental Procedure Using Beagle Dogs

Five male beagle dogs weighing 9—13 kg were fasted 20 h before administration until the last blood sample was taken, with free access to water. SPILA granules at a dose of 60 mg as morphine hydrochloride and MS Contin[®] at a dose of 30 mg as morphine sulfate were administered orally with 50 ml water. Blood samples were collected at predetermined times. Plasma samples were separated and frozen at -20 °C until assay. Necessary approvals for the experimental protocol of animals were obtained from the Ethical Committee of Takeda Pharmaceutical Company Limited.

Analysis of Plasma Samples The concentration of morphine in the plasma was determined by the method of Svensson *et al.*^{10,11)} with some modifications. In brief, 800 μl of plasma was added to a test-tube containing 3 ml of 0.5 M ammonium buffer (pH 9.3) and 200 μl of 0.1 M sodium pentan-sulfonate. This mixture was applied to Bond Elute C₁₈ cartridge (Varian, U.S.A.). The cartridge was washed with 10 ml of 5 mM ammonium buffer (pH 9.3), and 0.5 ml of distilled water. Morphine was eluted with 3 ml of methanol. The elute was evaporated to dryness at 60 °C under nitrogen gas. The residue was redissolved in the HPLC mobile phase (300 μl) and determined by HPLC with electrochemical detection (coulochem II, Eg: 500 mV, E₁: 250 mV, E₂: 450 mV, ESA, MA, U.S.A.). Separation on the octadecylsilane column (YMC-Pack ODS-AM, 150 mm length \times 4.6 mm diameter) was achieved at 40 °C and a 1 ml/min flow rate. The mobile phase was a mixture of 10 mM phosphate buffer (containing 10 mM dodecyl sulfate and 30 nM EDTA \cdot 2Na), adjusted to pH 2.5 : methanol : acetonitrile (68 : 5 : 27).

In Vivo Experimental Procedure of Rats Seven-week-old male Sprague-Dawley (SD) rats (Japan Clea Inc., Japan) were randomized to three treatment groups. Rats were fasted overnight and until 3 h after administration, 6—15 h and 18—24 h with free access to water. Water (control) was administered to rats in Group 1. SPILA granules were administered to rats in Group 2, once-a-day at a dose of 160 mg/kg. Aqueous solutions of morphine hydrochloride were administered to rats in Group 3, 4 times-a-day at a dose of 40 mg/kg every 6 h (Group 3). At predetermined intervals, the analgesic effect was evaluated. Immediately after determination of the analgesic response, blood was taken from the abdominal aorta under anesthesia. Plasma samples were separated and frozen at -20 °C until assay. Necessary approvals for the experimental protocol of animals were obtained from the Ethical Committee of Takeda Pharmaceutical Company Limited.

Analgesic Effect Evaluation Analgesic effect of SPILA granules of morphine was evaluated using tail-flick test in rats.¹²⁾ An automated tail-flick analgesimeter MK-330A (Muromachi Kikai, Japan) was used. Tail-flick latencies were measured in three different areas of the rat tail at intervals of 1 min.¹³⁾ The mean of three consecutive measures was retained as the latency. Percentage analgesia was calculated according to Eq. 2:

$$\% \text{ analgesia} = (R_{\text{post}} - R_{\text{cont}}) / (\text{cut-off time} - R_{\text{cont}}) \times 100 \quad (2)$$

where R_{cont} is control response and R_{post} is post-dose re-

sponse. The intensity of the thermal stimulus when an aliquot of water as vehicle control was administered to rats was adjusted to obtain latency as 7 s. A cut-off time of 15 s was used to prevent any injury to the tail.

Pharmacokinetic and Statistical Analysis The maximum plasma concentration (C_{max}), and the time to reach the maximum concentration (T_{max}) were directly obtained from the observed values. The area under the curve until 24 h after administration (AUC_{0-24h}) was calculated by the trapezoidal rule from the observed values, the area under the response curve (AURC) was calculated by the trapezoidal rule from the observed % analgesia values.

Statistical analysis was performed by the Student's *t* test, and $p < 0.01$ was used to indicate statistical significance.

RESULTS

Release patterns of morphine hydrochloride from SPILA granules are shown in Fig. 2. Mean dissolution time at pH 1.2 and pH 6.8 were 7.2 h and 3.7 h respectively. Morphine hydrochloride was released almost 2 times slower in the medium of pH 1.2 than in the medium of pH 6.8.

Plasma profiles of morphine after once-a-day oral administration of SPILA granules or twice-a-day oral administration of MS Contin[®] to beagle dogs are shown in Fig. 3. The whole plasma profile following MS Contin[®] administration was calculated assuming that in case of the second dosing after 12 h, the same plasma profiles as the first dosing could be obtained. Pharmacokinetic parameters are shown in Table 1. For SPILA granules and MS Contin[®], T_{max} values were 6 h and 2 h, and AUC_{0-24h} values were 191 $\mu\text{g}\cdot\text{h}/\text{ml}$ and

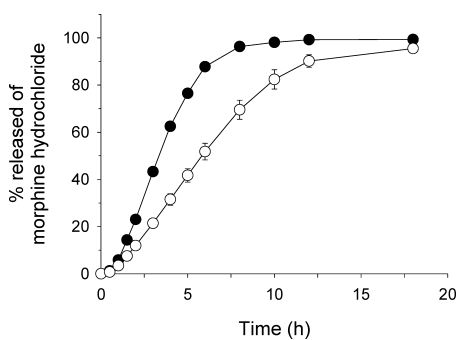


Fig. 2. Release Profiles of Morphine Hydrochloride from SPILA Granules in the Medium of pH 1.2 (○) and pH 6.8 (●) Solutions ($n=6$, Mean \pm S.D.)

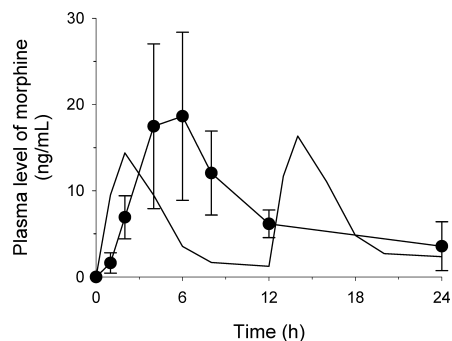


Fig. 3. Plasma Levels of Morphine after Once-a-Day Administration of SPILA Granules (60 mg Morphine Hydrochloride, ●) and Twice-a-Day Administration of MS Contin[®] (60 mg Morphine Sulfate, ○) to Fasted Beagle Dogs ($n=5$, Mean \pm S.D.)

146 $\mu\text{g}\cdot\text{h}/\text{ml}$, respectively. Relative bioavailability of morphine after SPILA granules administration, based on simulated morphine plasma profiles following MS Contin[®] administration, were 131%.

Plasma profiles of morphine after once-a-day oral administration of SPILA granules at a dose of 160 mg/kg or 4-times-a-day oral administration of aqueous solutions at doses of 40 mg/kg to rats are shown in Fig. 4. After oral administration of aqueous solutions, plasma levels of morphine rose rapidly (T_{max} : 1 h) and decreased to lower level within 6 h. For SPILA granules, plasma levels of morphine were maintained higher than 250 ng/ml for 24 h after administration. Pharmacokinetic and pharmacodynamic parameters are shown in Table 2. AUC_{0-24h} values for SPILA granules was 8.88 $\mu\text{g}\cdot\text{h}/\text{ml}$, and a little lower than that for solution (10.1 $\mu\text{g}\cdot\text{h}/\text{ml}$).

The analgesic profile obtained after SPILA granules administration reflected the plasma profile as shown in Fig. 5. Percentage analgesia after SPILA granules administration was higher than 50% from 4 h to 24 h post dose, while % analgesia after aqueous solutions administration was less than 50% even at 6 h after each dosing. The analgesic effect after SPILA granules administration expressed as the area under the response curve (AURC, 1701% Analgesia \cdot h) was similar to that after aqueous solutions administration (1603% Analgesia \cdot h).

Table 1. Pharmacokinetic Parameters for Morphine after Oral Administration of SPILA Granules and MS Contin[®] to Fasted Beagle Dogs

Formulation	T_{max} (h)	C_{max} (ng/ml)	AUC_{0-24h} (ng \cdot h/ml)	rBA (%)
MS Contin [®]	1.8 \pm 0.5	14.8 \pm 5.3	145.9	100
SPILA granules	5.6 \pm 1.7**	20.7 \pm 8.4	190.9 \pm 63.3	130.9

Data are shown as mean \pm S.D. ($n=5$). rBA: relative bioavailability. ** $p < 0.01$.

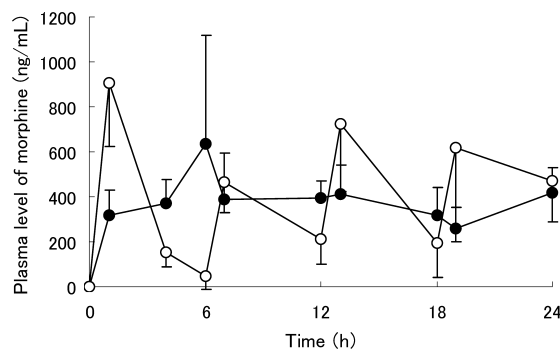


Fig. 4. Plasma Levels of Morphine after Once-a-Day Administration of SPILA Granules (●) and 4-Times-a-Day Administration of Aqueous Solutions (○) to Fasted Rats ($n=5$, Mean \pm S.D.)

Table 2. Pharmacokinetic and Pharmacodynamic Parameters for Morphine after Oral Administration of SPILA Granules and Solution to Fasted Rats

Formulation	AUC_{0-24h} (ng \cdot h/ml)	AURC _{0-24h} (% analgesia \cdot h)
Solutions	10.1	1603
SPILA granules	8.88	1701

Data are shown as mean ($n=5$).

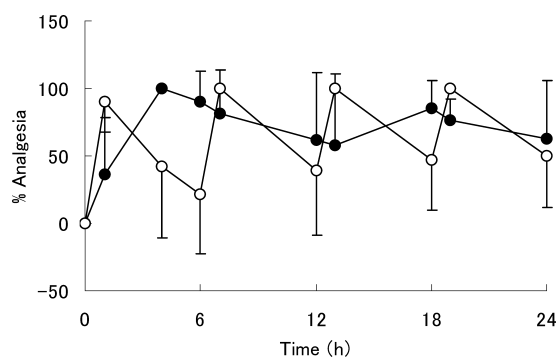


Fig. 5. Analgesic Effect of Morphine after Once-a-Day Administration of SPILA Granules (●) and 4-Times-a-Day Administration of Aqueous Solutions (○) to Fasted Rats ($n=5$, Mean \pm S.D.)

DISCUSSION

Generally, bioavailability, after administration of oral sustained-release dosage forms is known to be lower than that after administration of fast release dosage forms, due to lack of fluid in the lower small intestine and the colon, even though a drug is absorbed from all parts of the GI tract.^{4,14} Lack of fluid would make the contents in lower GI viscous, especially in the colon, and would restrict fluid movement around the dosage forms and reduce in thereby drug release. As a result, it would make the maintenance of drug levels difficult after administration of oral sustained-release dosage forms. In order to prevent the sustained-release dosage forms from reducing in drug release, SPILA granules system was designed to release morphine hydrochloride faster in pH 6.8 medium than in pH 1.2 medium (Fig. 2), *i.e.*, in order to maintain plasma levels of morphine over 24 h, SPILA granules were formulated to provide faster release rate in the lower small intestine or colon than that in the stomach by coating the core granules with EC film containing CP, which showed higher swelling property in the neutral medium than in the acidic medium.

Morphine has been reported to be absorbed in every part of the GI except for the stomach.¹⁵ The plasma profile after oral administration of SPILA granules to beagle dogs was compared with that after administration of MS Contin[®], which contained 30 mg of morphine sulfate and which released morphine sulfate pH-independently. It has been widely available as a twice-a-day dosage form for cancer pain. T_{max} after oral administration of SPILA granules, 6 h, was significantly delayed, compared with that after administration of MS Contin[®], 2 h (Fig. 3). Relative bioavailability of SPILA granules was 131% based on AUC of MS Contin[®] (Table 1). The result indicates that the pH-dependent swellable polymer in the film composition of SPILA granules could play an important role for keeping the higher morphine plasma levels over a protracted period, especially after 12 h post dose, because the water-absorbing and swollen polymer in the film composition could make it possible to continue to release morphine even in the distal small intestine and the colon and enhance absorption of morphine. Enteric polymers are usually used to make an oral sustained-release formulation like Kadian[®], orally once a day morphine sulfate sustained-release formulation. SPILA granules have characteristic to use pH dependent water-absorbing and swollen poly-

mer, which remains in the release-regulating layer with water instead of enteric polymers, which are dissolved at high pH condition. It is expected that the higher the amount of water in the release-regulating layer, the more the release amount of morphine rises even in the distal small intestine and the colon that are lack of fluid.

Analgesics after SPILA granules administration were evaluated by comparing with multi-dosing of aqueous solutions. Studies using analgesic tests such as tail-flick test and hot plate test, after administration of oral dosage forms have been hardly reported in rats or mice, because of the difficulty in administration of dosage forms to small animal models. SPILA granules were successfully administered to rats because the granule size was 500–1400 μ m. In this study, effect of orally administered SPILA granules at a dose of 160 mg/kg as a morphine hydrochloride once a day to rats was compared with that of orally administered aqueous solutions at a dose of 40 mg/kg as a morphine hydrochloride every 6 h to rats.

A dose of 160 mg/kg/d as morphine hydrochloride seemed to be high, considering the effective analgesic dose in humans. The reason why a dose of 160 mg/kg/d was chosen as follows; In our preliminary test using rats, about 500 ng/ml of morphine in plasma was found to be required for 100% analgesic effect. And a dose of 40 mg/kg as morphine hydrochloride aqueous solution administration was found to be required for 500 ng/ml plasma concentration. Clinically, oral aqueous solutions or fast release formulations of morphine such as tables or powders, are administered 6-times a day (every 4 h) or 5-times a day. For aqueous solution of morphine hydrochloride, $t_{1/2}$ after oral administration to rats was 4.76 h (data are not shown), which was 1.64 times longer compared with that after oral administration to human (2.90 h).¹⁶ In this study, based on the information, aqueous solutions were administered to rats 4-times a day. Therefore a dose of 160 mg/kg once-a-day to rats, when SPILA granules being administered, was selected as the same dose as the dose of solutions administered.

The analgesic effect of SPILA granules expressed as $AURC$ was similar to that of the aqueous solutions (Table 2). In addition, analgesic profiles after administration of both SPILA granules and aqueous solutions containing morphine hydrochloride to rats reflect the morphine plasma profile (Figs. 3, 4). The result indicates that once-a-day administration of SPILA granules to man could provide the similar analgesic effects to multiple-dosing of fast release formulations, when the same dose was administered in either dosage form, *i.e.*, SPILA system is expected to provide once a day sustained-analgesic effect all day long.

CONCLUSIONS

Morphine hydrochloride was released faster from SPILA granules in the medium of pH 6.8 than in the medium of pH 1.2. In beagle dogs, SPILA granules provided more delayed T_{max} , and greater AUC_{0-24h} than MS Contin[®]. In the test using rats, the analgesic effect of SPILA granules was similar or almost the same to that of the aqueous solutions, when the same dose was administered in either dosage form. SPILA granules would be pharmacokinetically and pharmacodynamically accepted as a once-a-day oral dosage form.

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