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New clopidogrel napadisilate salt and its solid dispersion with improved stability and bioequivalence to the commercial clopidogrel bisulphate salt in beagle dogs

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ABSTRACT

The purpose of this study was to develop a novel clopidogrel napadisilate-loaded solid dispersion with improved stability and bioequivalence to the clopidogrel bisulphate-loaded commercial product. Clopidogrel napadisilate prepared in this study appeared as a white crystalline powder unlike clopidogrel base. However, this salt did not improve the solubility of clopidogrel, even with improved stability compared to clopidogrel bisulphate. To improve the solubility of clopidogrel napadisilate, a novel clopidogrel napadisilate-loaded solid dispersion was prepared by the spray-drying technique using HPMC and colloidal silica, and the physicochemical properties, dissolution and bioavailability in beagle dogs were evaluated compared to the clopidogrel bisulphate-loaded commercial product. The solid dispersion composed of clopidogrel napadisilate, HPMC and colloidal silica at a weight ratio of 11.069/3/3.5 improved solubility by 6.5-fold compared to clopidogrel napadisilate, even if it did not improve drug solubility compared to clopidogrel bisulphate. However, unlike clopidogrel bisulphate, this formulation improved the stability of clopidogrel. Furthermore, the clopidogrel napadisilate solid dispersion-loaded tablet showed similar dissolution to the clopidogrel bisulphate-loaded commercial product and was bioequivalent to the commercial product in beagle dogs. Thus, this clopidogrel napadisilate-loaded solid dispersion could be a promising candidate for improving the stability and bioavailability of clopidogrel.

1. Introduction

Clopidogrel [methyl(+)-(S)-alpha-(o-chlorophenyl)-6,7-dihydrothieno[3,2-a]pyridine-5(4*H*)-acetate] (Fig. 1) is a routine component of the clinical management of patients after acute coronary syndrome, in whom it has been reported to reduce rates of major cardiovascular adverse events (Diener et al., 2005; Fox and Chelliah, 2007). It is approved for the reduction of atherosclerotic events in patients with stroke, myocardial infarction, cardiovascular disease and acute coronary syndrome. Its action may be related to an adenosine diphosphate (ADP) receptor on platelet cell membranes (Antić et al., 2007; Savi et al., 2006). It specifically and irreversibly inhibits the platelet $P2Y_{12}$ subtype of the ADP receptor, which is important in the aggregation of platelets and cross-linking by the protein fibrin. As a result, activation of the glycoprotein IIb/IIIa complex, which is involved in platelet activation and stabilisation of the platelet aggregate, is also inhibited (Park et al., 2010a,b; Shim et al., 2010).

Clopidogrel, a prodrug, has no activity in itself. To exert its anti-aggregatory effect, it requires biotransformation into its active metabolite, 2-oxo-clopidogrel by the hepatic cytochrome P450. Furthermore, the other active metabolite, a thiol derivative, is formed by subsequent hydrolysis of 2-oxo-clopidogrel. Mostly, carboxylesterase converts clopidogrel to an inactive carboxylic acid metabolite, clopidogrel carboxylic acid derivative [(S)-(2-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4*H*)-acetic acid; SR26334] (Antić et al., 2007; Savi et al., 2006; Silvestro et al., 2010). Following oral administration in humans, the plasma levels of clopidogrel are very low due to extensive metabolism and are difficult to quantify. Furthermore, the active metabolites such as 2-oxo-clopidogrel and the thiol derivative are highly labile and remain undetected in plasma. Thus, neither the parent drug nor the active metabolite is detected in plasma. However, the main circulating metabolite (the carboxylic acid derivative; SR26334) represents 85% of the circulating metabolites in plasma, even if

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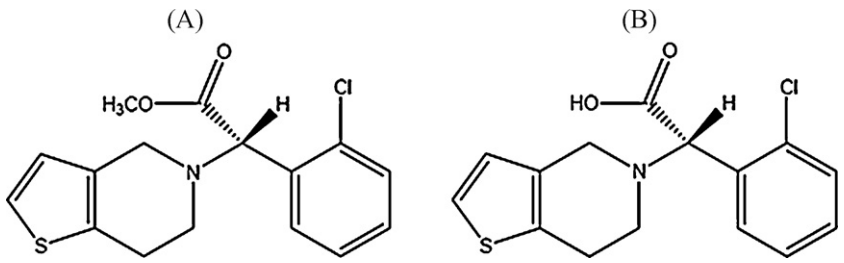

Fig. 1. Chemical structure: (A) clopidogrel; (B) SR26334.

it is pharmacologically inactive. Thus, the quantification of the inactive carboxylic acid metabolite of clopidogrel, which is the most abundant species circulating in the blood, has emerged as an indirect approach for studying the pharmacokinetics of clopidogrel (Mitakos and Panderi, 2002; Silvestro et al., 2010; Singh et al., 2005).

Clopidogrel base has not been used as a commercial drug, since it is an oil phase which is difficult to purify and handle. Thus, clopidogrel bisulphate, a salt form, has been mainly used in a commercial product (Plavix®; Sanofi-Aventis Korea Co.) because of its crystalline form and improved solubility of about 90 mg/ml (Di Girolamo et al., 2010; El Ahmady et al., 2009). However, this commercial drug has been reported to be unstable under accelerated moisture and heat condition, resulting in the production of significant amounts of degradants (Gomez et al., 2004; Raijada et al., 2010; Skillman et al., 2010). At accelerated conditions of 40 °C/75%RH, the compound is hydrolyzed to the hydrolyzed degradant, which has no biological activity. Moreover, it can be transformed into the isomer, racemized degradant with much less pharmacological activity (Mitakos and Panderi, 2002, 2004). Thus, another salt must be developed in order to improve the stability and bioavailability of clopidogrel.

2. Materials and methods

2.1. Materials

Clopidogrel base was obtained from Hanmi Pharm. Co. (Hwasung, South Korea). Hydroxypropylmethylcellulose (HPMC 2910) was purchased from Shin-Etsu Co. (Tokyo, Japan). D-Mannitol, crospovidone, colloidal silica and sucrose ester of fatty acid supplied by Hanmi Pharm. Co. (Hwasung, South Korea) were of USP grade. The commercial product (Plavix®; in tablet form) was purchased from Sanofi-Aventis Korea Co. (Seoul, South Korea). All other chemicals were of reagent grade and were used without further purification.

2.2. Animals

All animal care and procedures were conducted according to the Guiding Principles in the Use of Animals in Toxicology, as adopted in 1989, revised in 1999 and amended in 2008 by the Society of Toxicology (SOT, 2008). The protocols for the animal studies were also approved by the Institute of Laboratory Animal Resources of Yeungnam University. Twenty-four male beagle dogs weighing 9–11 kg were fasted for 16 h prior to the experiments but were allowed free access to water.

2.3. Preparation of clopidogrel napadisilate

First, 34.8 g of 2-naphthalenesulfonic acid monohydrate was dissolved in a mixture of 150 ml of ethyl acetate and 5 ml of water. Over a period of 30 min, this solution was added dropwise to 100 ml of ethyl acetate in which 50 g of clopidogrel base was dissolved. This mixture was stirred at 25 °C for 12 h and continuously at 4 °C for 4 h. The precipitates were obtained by filtering through a membrane filter (0.45 μm), washed with 30 ml of ethyl acetate and dried in an oven at 50 °C for 3 h.

2.4. Solubility of clopidogrel napadisilate and clopidogrel bisulphate

Excessive amounts of clopidogrel napadisilate and clopidogrel bisulphate (about 10 g) were added to 10 ml of water, 0.1 N HCl (pH 1.2), phosphate buffered solution (pH 4.0) and phosphate buffered solution (pH 6.8), respectively. They were shaken in a water bath at 25 °C for 3 days, centrifuged at 3000 × *g* for 10 min using a 5415C centrifuge (Eppendorf; Hauppauge, NY, USA) and filtered through a membrane filter (0.45 μm) to obtain a clear solution. The concentration of clopidogrel in the resulting solution was analysed by HPLC as described below.

2.5. Stability of clopidogrel napadisilate and clopidogrel bisulphate

To investigate the comparative stability of clopidogrel napadisilate and clopidogrel bisulphate, they were kept at the accelerated condition of 40 °C/75% RH for 7 weeks (Radhakrishna et al., 2000; Raijada et al., 2010). Their water content, drug content and degradants were analysed at pre-determined time intervals. Water contents were analysed using the Karl–Fisher method (Zupančič et al., 2005). To assay the drug contents and the impurities of clopidogrel, the compounds were dissolved in the mobile phase and filtered through a membrane filter (0.45 μm). The resulting solution 10 μl was analysed by HPLC (Hitachi, Tokyo, Japan) equipped with an Ultron ES-OVM column (Rockland Technologies, 0.5 μm, 15 cm × 0.46 cm i.d.) and a UV detector (Model L-7400). The mobile phase consisted of phosphate buffered solution (pH 4.8) and acetonitrile (80:20, volume ratio). The eluent was monitored at 220 nm with a flow rate of 1.0 ml/min (Dantu et al., 2010; Mitakos and Panderi, 2002, 2004). The retention times were as follows: hydrolyzed degradant, 2.7 min; clopidogrel, 9.3 min and racemized degradant, 22.8 min.

2.6. Preparation of clopidogrel napadisilate-loaded solid dispersion

A Büchi 190 nozzle type mini spray dryer (Flawil, Switzerland) was used for the preparation of the clopidogrel napadisilate-loaded solid dispersion (Lee et al., 1999; Li et al., 2008). Various amounts of HPMC and colloidal silica were dissolved or dispersed in 110 ml ethanol–methylene chloride mixture (10:100, volume ratio), and clopidogrel napadisilate was dissolved in this solution. The detailed compositions of spray-drying solutions for the preparation of the clopidogrel napadisilate-loaded solid dispersion are given in Table 1. The resulting suspension was delivered to the

Table 1
Composition of clopidogrel napadisilate-loaded solid dispersions.

Ingredients	I	II	III	IV	V
Clopidogrel napadisilate (g)	11.069	11.069	11.069	11.069	11.069
HPMC (g)	0.5	1.0	2.0	3.0	4.0
Colloidal silica (g)	3.5	3.5	3.5	3.5	3.5

nozzle (0.7 mm diameter) at a flow rate of 5 ml/min using a peristaltic pump and spray-dried at 90–100 °C inlet temperature. The pressure of spray air was 4 kg/cm^2 and the flow rate of drying air was maintained at the aspirator setting of 10 which indicated the pressure of aspirator filter vessel to be −30 mbar. The direction of air flow was the same as that of the sprayed products.

2.7. Solubility of clopidogrel napadisilate in solid dispersions

Excessive amounts of solid dispersions (about 10 g) were added to 10 ml of water, 0.1 N HCl (pH 1.2), phosphate buffered solution (pH 4.0), and phosphate buffered solution (pH 6.8), respectively. The solubility of clopidogrel napadisilate in solid dispersions was determined by the same method as described above.

2.8. Shape and size of clopidogrel napadisilate-loaded solid dispersion

The shape and surface morphology of clopidogrel bisulphate, clopidogrel napadisilate and the clopidogrel napadisilate-loaded solid dispersion were examined using a scanning electron microscope (TM3000, Hitachi, Japan). The powders were fixed on a brass specimen club using double-sided adhesive tape and made electrically conductive by coating in a vacuum (6 Pa) with platinum (6 nm/min) using a Hitachi Ion Sputter (E-1030) for 300 s at 15 mA. Moreover, their sizes were also measured using a high-resolution laser diffraction spectroscopy (HELOS, Sympatec, Clausthal-Zellerfeld, Germany).

2.9. Crystallinity of the clopidogrel napadisilate-loaded solid dispersion

The thermal characteristics of clopidogrel napadisilate, the physical mixture and clopidogrel napadisilate-loaded solid dispersion were investigated using a differential scanning calorimeter (DLUS, Rheometric Scientific, USA). The physical mixture was prepared by physically mixing clopidogrel napadisilate, HPMC and colloidal silica at a weight ratio of 11.1:3:3.5. About 2 mg of samples were placed in sealed aluminium pans before heating under nitrogen flow (10 ml/min) at a heating rate of 10 °C/min from 50 to 200 °C. Furthermore, their powder crystallinity were assessed by powder X-ray diffraction (M18XHF-SRA, Mac Science, Japan) conducted at room temperature using monochromatic Cu Kα-radiation (λ = 1.5406 Å) at 40 mA and 40 kV in the region of $3.5^\circ \leq 2\theta \leq 40^\circ$ with an angular increment of 0.02° per second.

2.10. Preparation of clopidogrel napadisilate-loaded tablets

As shown in Table 2, clopidogrel napadisilate (or the clopidogrel napadisilate-loaded solid dispersion), crospovidone, D-mannitol and sucrose ester of fatty acid were well mixed. A clopidogrel napadisilate-loaded tablet (CNT) and a clopidogrel napadisilate solid dispersion-loaded tablet (CNSDT), with a diameter of 6 mm and a hardness of 8–10 KP were prepared by compressing the resulting mixtures using the ERWEKA tablet machine (GmbH, Frankfurt, Germany).

Table 2
Composition of two kinds of clopidogrel napadisilate-loaded tablets.

Ingredients (mg/tablet)	CNT[a]	CNSDT[b]
Clopidogrel napadisilate	11.069	–
Clopidogrel napadisilate-loaded solid dispersion	–	17.569[c]
Crospovidone	4.0	4.0
D-Mannitol	3.0	3.0
Sucrose ester of fatty acid	1.0	1.0

[a] Clopidogrel napadisilate-loaded tablet.
[b] Clopidogrel napadisilate solid dispersion-loaded tablet.
[c] Equivalent dose of 11.069 mg clopidogrel napadisilate.

2.11. Dissolution of clopidogrel napadisilate-loaded tablets

The dissolution test was performed using USP XXXII dissolution apparatus II with 900 ml water, 0.1 N HCl (pH 1.2), phosphate buffered solution (pH 4.0) and phosphate buffered solution (pH 6.8) as the dissolution media at 37 ± 0.5 °C. The speed of the paddle was adjusted to 50 rpm. The CNT, CNSDT and the clopidogrel bisulphate-loaded commercial tablet at an equivalent dose of 75 mg clopidogrel base were placed into a dissolution tester (Shinseang Instrument Co., South Korea), respectively (Newa et al., 2007; Piao et al., 2008; Yong et al., 2005). At pre-determined intervals, 1 ml of the medium was sampled and filtered through a membrane filter (0.45 μm). The concentration of clopidogrel in the filtrate analysed by the HPLC method as mentioned above.

2.12. Oral administration

Beagle dogs, divided into three groups, were fasted overnight and restrained by means of a dog sling (Alice King Chatham Medical Arts, Los Angeles, CA, USA) during the 48 h experimental period. The beagle dogs in each group were orally administered with two CNT, CNSDT or clopidogrel bisulphate-loaded commercial products equivalent to 150 mg clopidogrel base (Di Girolamo et al., 2010; El Ahmady et al., 2009). About 1.5 ml of blood was collected from the cephalic vein at pre-determined time intervals. These samples were immediately centrifuged at 3,000 × *g* at 4 °C for 15 min using a centrifuge (5415C; Eppendorf, Hamburg, Germany) and stored at −80 °C prior to analysis.

2.13. Blood sample treatment

Plasma (400 μl) was mixed with 500 μl of methanol solution containing tichlopidine (60 ng/ml) as an internal standard. Then, 4 μl of formic acid was added, followed by liquid–liquid extraction for 10 min with 1 ml of methyl tert-butyl ether. The organic layer was separated and removed at 30 °C in a heated centrifugal evaporator (EYELA CVE-200D; Tokyo Rikakikai Co., Tokyo, Japan). The residue was reconstituted in 100 μl of the mobile phase by vortex-mixing for 15 s, and 10 μl of this solution was injected onto the column.

2.14. HPLC–MS-MS conditions

The plasma concentrations of clopidogrel base and its metabolite SR26334 were quantified using an Waters 2795 HT LC/MS/MS system (Waters, Milford, MA, USA) equipped with an electrospray ionisation interface that was used in the positive ion mode ($[M+H]^+$). The compounds were separated on a X-terra MS C18 column (2.1 mm × 50 mm, 3.5 μm, Waters, Milford, MA, USA) with a mobile phase that consisted of 0.1% TFA adjusted to pH 6 with ammonia/methanol/distilled water (10:850:5, volume ratio). The column was heated to 30 °C and the mobile phase was eluted at

0.15 ml/min (Mitakos and Panderi, 2002, 2004). The ESI-MS data were acquired in the positive mode and the conditions of MS analysis were as follows: drying gas (argon) flow rate, 10 l/min; drying gas temperature, 280 °C; capillary voltage, 3.39 kV; fragmentor, 0.3 V. Clopidogrel, SR26334 and tichlopidine (internal standard) gave mainly protonated molecules at m/z 321.8, 308.1 and 264.1, respectively. Furthermore, the product ions were scanned in Q3 after collision with argon in Q2 at m/z 212.1, 198.0 and 154.0 for clopidogrel base SR26334 and tichlopidine, respectively. Quantification was performed by multiple reaction-monitoring (MRM) of the protonated precursor ions and the related product ions, using the ratio of the area under the peak for each solution and a weighting factor of $1/y^2$. The analytical data were processed with Analyst software (MassLynx, version 4.0) (Cho et al., 2010; Lim et al., 2010). The calibration curve was constructed over a range of 1–500 (R^2 = 0.9993) and 10–2500 ng/ml (R^2 = 0.9992) in plasma and with a lower limit of quantification (LLOQ) of 1 and 10 ng/ml for clopidogrel and SR26334, respectively. For the validation, inter- and intra-day differences were conducted and the differences were found to be within an acceptable range.

2.15. Pharmacokinetic data analysis and statistical analysis

The area under the drug concentration time curve from zero to infinity (AUC), the elimination constant (K_{el}) and the half life ($t_{1/2}$) were calculated using non-compartmental analysis (WinNonlin; professional edition, version 2.1; Pharsight Co., Mountain View, CA, USA). The maximum plasma concentration of the drug (C_{max}) and the time taken to reach the maximum plasma concentration (T_{max}) were directly obtained from the plasma data (Gibaldi and Perrier, 1982). Levels of statistical significance ($P < 0.05$) were assessed using the Student's t-test between the two means for unpaired data. All data are expressed as the mean ± standard deviation (S.D.) or as the median (ranges) for T_{max}.

3. Results and discussion

3.1. Clopidogrel napadisilate

Clopidogrel base is very unstable, and appears as an oil form which is difficult to purify and handle. Thus, its ester salt, clopidogrel bisulphate, has been used as a commercial drug. The aqueous solubility of clopidogrel bisulphate is about 90 mg/ml, which indicated that this salt was water-soluble (Di Girolamo et al., 2010; El Ahmady et al., 2009). As shown in Fig. 2, the solubility of clopidogrel bisulphate in the medium was in the order of pH 6.8 < pH 4.0 < water < pH 1.2. In particular, the solubility of clopidogrel bisulphate in pH 1.2 is higher than in water due to its basic property.

In this study, clopidogrel napadisilate monohydrate (Fig. 3; NMR and IR data not shown), another salt form, was easily prepared with clopidogrel base and 2-naphthalenesulfonic acid monohydrate (2:1, molar ratio). It gave the yield of about 85% and appeared as a white crystalline powder unlike clopidogrel base. Similarly, among the media tested, clopidogrel napadisilate gave the highest solubility in pH 1.2. However, the clopidogrel napadisilate prepared in this study gave significantly lower solubility than did clopidogrel bisulphate in all aqueous solutions. In particular, the aqueous solubility of clopidogrel napadisilate was about 70-fold lower compared to clopidogrel bisulphate (1.3 ± 1.1 mg/ml vs. 88.7 ± 5.4 mg/ml). Thus, the poorly water-soluble napadisilate salt could not improve the solubility of clopidogrel, even if it was difficult to purify and handle due to its solid form.

To compare the stability of clopidogrel napadisilate and clopidogrel bisulphate, both compounds were kept at the accelerated condition of 40 °C/75% RH for 7 weeks, and then their water content,

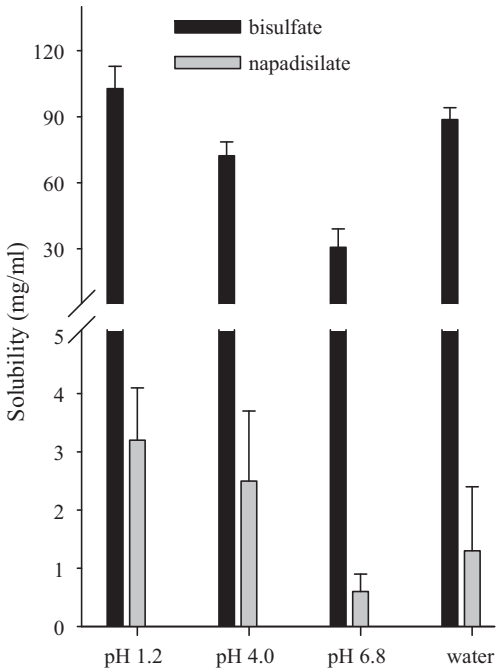

Fig. 2. Solubility of clopidogrel napadisilate and clopidogrel bisulphate. Each value represents the mean ± S.D. (n = 3).

drug content and degradants were analysed. At accelerated conditions of 40 °C/75%RH, clopidogrel was hydrolyzed to the hydrolyzed degradant [(*S*)-(+)-α-(o-2-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4*H*)-acetic acid] and transformed to the isomer, racemized degradant [methyl (*R*)-(−)-(o-2-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4*H*)-acetate] (Gomez et al., 2004; Raijada et al., 2010; Skillman et al., 2010). In this study, the drug, hydrolyzed degradant and racemized degradant were simultaneously determined using HPLC (Fig. 4). Furthermore, another degradant, methyl(±)-(o-2-chlorophenyl)-6,7-di hydrothieno[3,2-c]pyridine-5(4*H*)-acetate was negligible due to its very small concentrations.

At 0 week, clopidogrel bisulphate gave a negligible water content of 0% due to its anhydrous property (Fig. 5A). However, it showed no changes in water content until 2 weeks followed by increased water content. In particular, this salt gave a water content of about 5% at 5 weeks, suggesting that clopidogrel bisulphate is hygroscopic. On the other hand, clopidogrel napadisilate gave a water content of about 2% at 0 week because of its monohydrous property. However, this salt gave no changes in water content up to 5 weeks, indicating that clopidogrel bisulphate is not hygroscopic.

As shown Fig. 5B, the drug contents in the clopidogrel bisulphate gradually decreased up to 3 weeks followed by a rapid decrease. In particular, this salt had a reduced drug content of about 87% at 5 weeks, indicating that clopidogrel bisulphate was an unstable salt form under the accelerated condition (Agrawal et al., 2003; Raijada et al., 2010; Skillman et al., 2010). However, in the case of clopidogrel napadisilate, there were no significant changes in drug

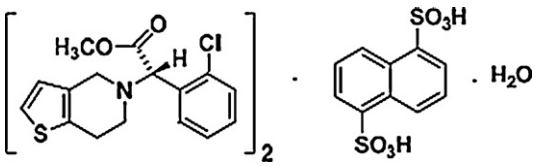

Fig. 3. Chemical structure of clopidogrel napadisilate.

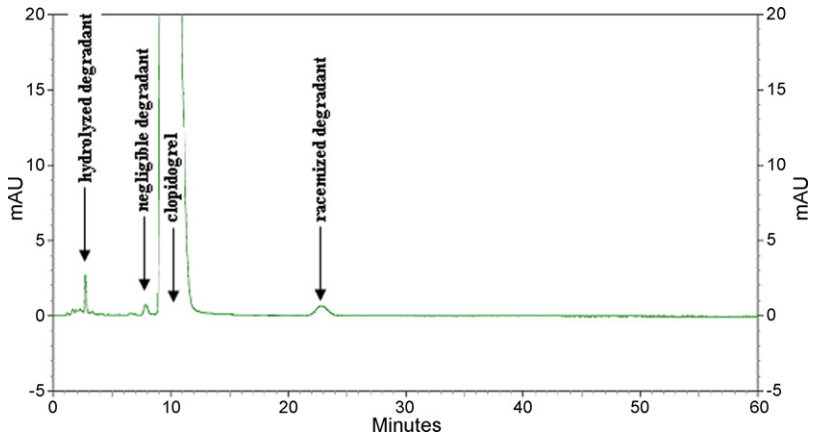

Fig. 4. Chromatographic selectivity of impurities at 60 °C/75% RH for 7 weeks.

content, suggesting that clopidogrel napadisilate was stable under the accelerated condition.

As shown in Fig. 5C and D, clopidogrel bisulphate gave no significant changes in hydrolyzed and racemized degradants until 3 weeks followed by rapidly increased levels of the hydrolyzed and racemized degradants. In particular, the hydrolyzed and racemized degradants increased to about 5% and 1% at 5 weeks, respectively, indicating that clopidogrel bisulphate was an unstable salt form

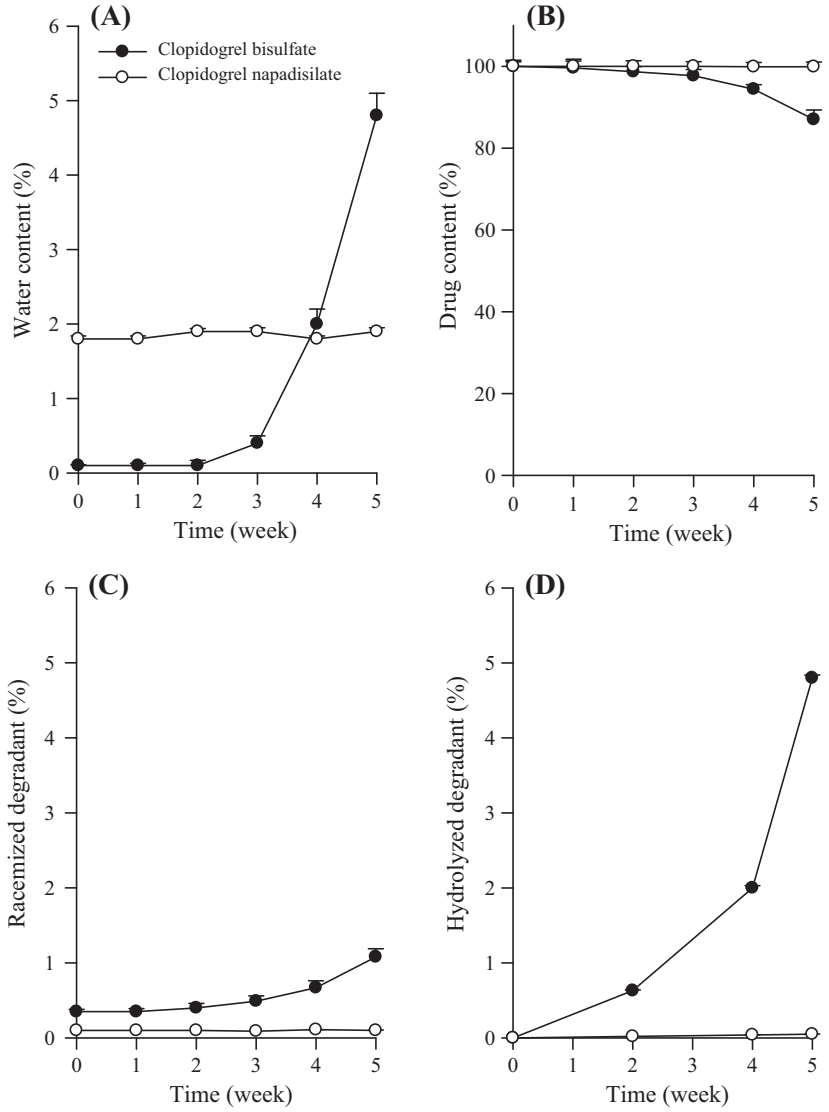

Fig. 5. Stability of clopidogrel salts at the accelerated condition of 40 °C/75%RH: (A) water content; (B) drug content; (C) racemized degradant; (D) hydrolyzed degradant. Each value represents the mean ± S.D. ($n = 3$).

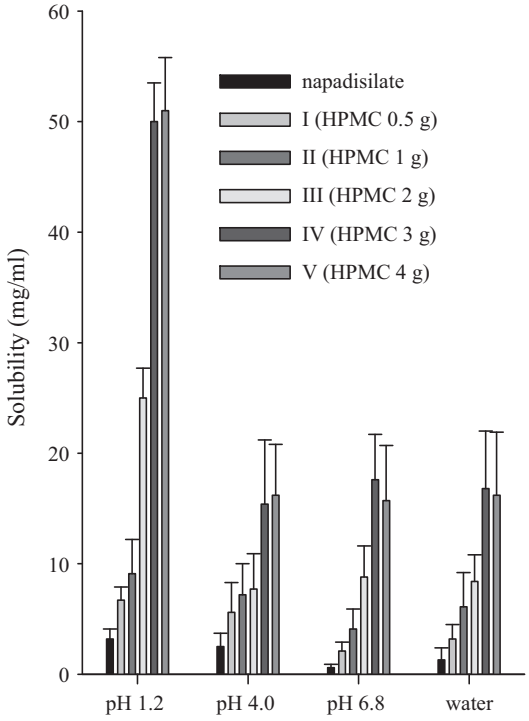

Fig. 6. Effect of HPMC on the solubility of clopidogrel napadisilate in the solid dispersions. Each value represents the mean ± S.D. ($n = 3$).

(Agrawal et al., 2003; Raijada et al., 2010; Skillman et al., 2010). However, in the case of clopidogrel napadisilate, there were no significant changes in the hydrolyzed and racemized degradants. Thus, unlike clopidogrel bisulphate, clopidogrel napadisilate improved the stability of clopidogrel. On the other hand, the hydrolyzed and racemized degradants showed the similar profiles to water content. Our results suggested that the stability of clopidogrel salts was dependent upon their water content. Clopidogrel bisulphate with a hygroscopic character was degraded, but clopidogrel napadisilate with a non-hygroscopic character was not degraded under the accelerated condition.

From these findings, clopidogrel napadisilate was determined to be a salt form without improved solubility but with enhanced stability of clopidogrel compared to clopidogrel bisulphate.

3.2. Clopidogrel napadisilate-loaded solid dispersion

In this study, the clopidogrel napadisilate-loaded solid dispersion was prepared using a spray-drying technique with HPMC in order to improve the solubility of poorly water-soluble clopidogrel napadisilate. HPMC and colloidal silica were dissolved or dispersed in an ethanol and methylene chloride solution, and the poorly water-soluble drug was dissolved in this solution. The resulting solutions were spray-dried so that the clopidogrel napadisilate formed a solid dispersion together with HPMC, a hydrophilic polymer (Joe et al., 2010). In the absence of colloidal silica, we observed strong electrostatic interactions among solid dispersions which arose from friction in the spray dryer, making the powder fly in all directions, which caused difficulty in handling. Colloidal silica was used to avoid attaching the solid dispersion to the inner wall of spray-drying chamber and to produce a free-flowing powder (Lee et al., 1999).

To investigate the effect of HPMC on the solubility of clopidogrel napadisilate in the solid dispersions, various solid dispersions were prepared with different amounts of HPMC (Table 1) and their solubilities were evaluated. All clopidogrel napadisilate-loaded solid

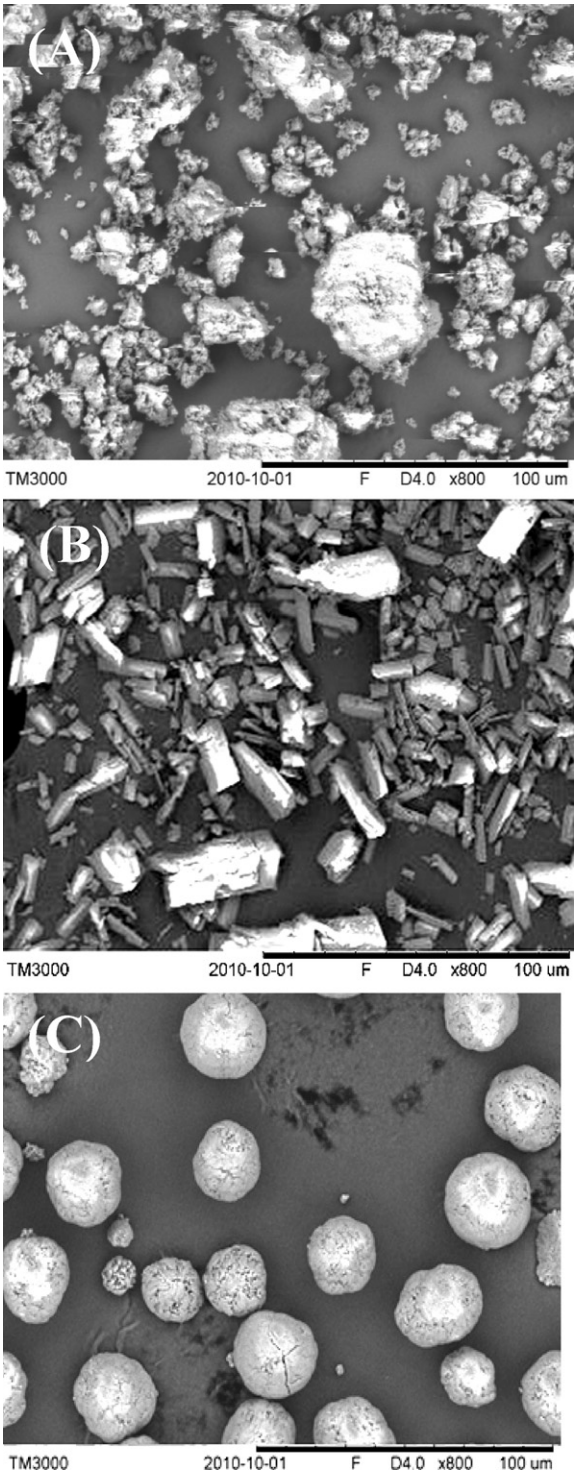

Fig. 7. Scanning electron micrographs (X800): (A) clopidogrel bisulphate; (B) clopidogrel napadisilate; (C) solid dispersion.

dispersions improved the solubility of the clopidogrel napadisilate at pH 1.2, 4.0, 6.8 and water (Fig. 6). The more HPMC were added to 3 g (against 11.069 g clopidogrel napadisilate), the more increased the drug solubility in the solid dispersions (Lim et al., 2010; Okimoto et al., 1997). However, the solid dispersion prepared with HPMC 4 g (formulation V) hardly improved drug solubility in all solutions compared to those with HPMC 3 g (formulation IV). Thus, with respect to increased drug solubility in the preparation of clopidogrel napadisilate-loaded solid dispersion, the HPMC

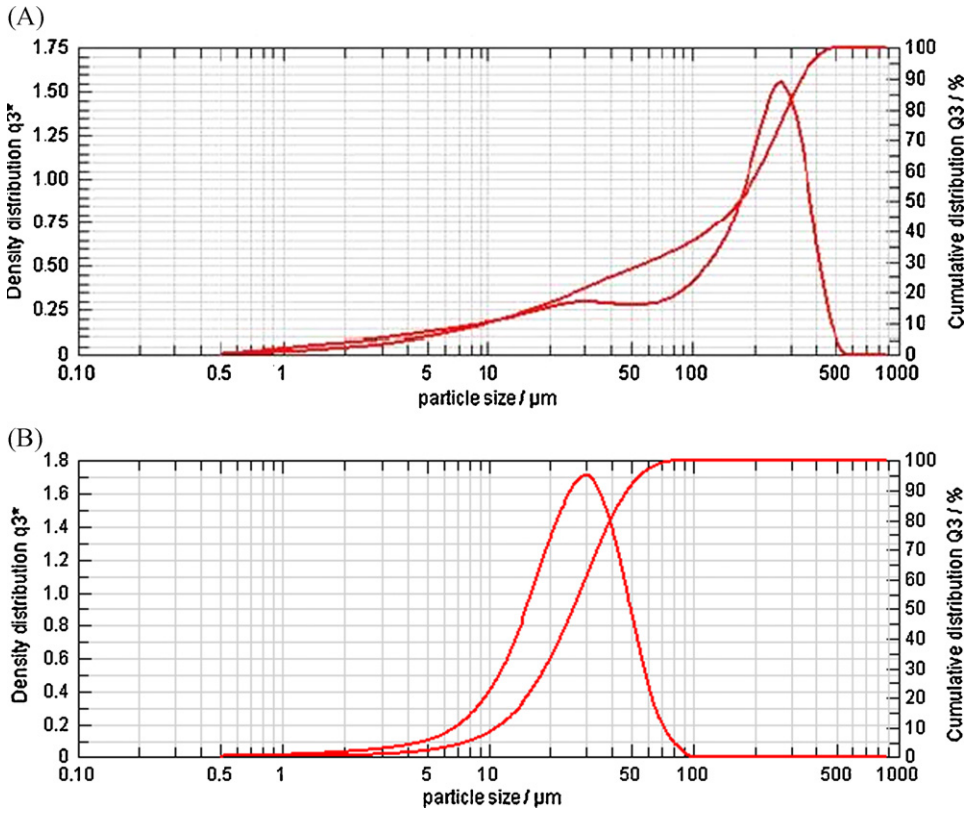

Fig. 8. Particle size: (A) clopidogrel napadisilate; (B) solid dispersion. Each value represents the mean ± S.D ($n = 3$).

amount was fixed to 3 g. In particular, formulation IV improved the solubility of clopidogrel napadisilate in water by 6.5-fold.

To evaluate the stability of the clopidogrel napadisilate-loaded solid dispersion, formulation IV was kept at the accelerated condition of 40 °C/75% RH for 7 weeks, and then its water content, drug content and degradants were analysed. Like clopidogrel napadisilate, this solid dispersion showed no significant changes in water content, drug contents and degradants after 7 weeks (data not shown). Thus, like clopidogrel napadisilate, the clopidogrel napadisilate-loaded solid dispersion improved the stability of clopidogrel.

From Fig. 6, the solid dispersion could not improve the drug solubility as high as clopidogrel bisulphate, even if it improved the drug solubility compared to clopidogrel napadisilate. In particular, formulation IV gave one-tenth of aqueous solubility of the clopidogrel bisulphate (8.4 ± 2.4 mg/ml vs. 88.7 ± 5.4 mg/ml). However, formulation IV improved the stability of clopidogrel unlike clopidogrel bisulphate. Thus, the clopidogrel napadisilate-loaded solid dispersion composed of clopidogrel napadisilate, HPMC and colloidal silica at the weight ratio of 11.069/3/3.5 was selected for further study, as this solid dispersion was useful for improving the solubility of poorly water-soluble clopidogrel napadisilate and the stability of clopidogrel.

The scanning electron micrographs of clopidogrel bisulphate, clopidogrel napadisilate and the clopidogrel napadisilate-loaded solid dispersion are shown in Fig. 7. Clopidogrel bisulphate (Fig. 7A) showed irregular crystals with a rough surface. Clopidogrel napadisilate (Fig. 7B) appeared as smooth-surfaced rectangular crystals in shape. However, the solid dispersion gave a spherical shape with a smooth surface (Fig. 7C). Moreover, clopidogrel napadisilate powder (Fig. 8A) and the clopidogrel napadisilate-loaded solid dispersion (Fig. 8B) gave a mean particle size of about 165 μm and 25 μm, respectively, indicating that the solid dispersion greatly reduced the particle size of the drug.

The thermal behaviour of clopidogrel napadisilate, the physical mixture and the clopidogrel napadisilate-loaded solid dispersion are given in Fig. 9. The thermograms of clopidogrel napadisilate (Fig. 9A) exhibited an endothermic peak at about 170 °C corresponding to its melting point, indicating its crystalline nature. HPMC (Fig. 9B) and colloidal silica (Fig. 9C) showed no intrinsic peak. The melting peak which appeared in the drug was shown in the physical mixture (Fig. 9D), indicating that the drug did not interact with other ingredients. However, the endothermic peaks of the drug were absent in the solid dispersion (Fig. 9E). Our results suggested that the drug might be in an amorphous state in the solid dispersion (Newa et al., 2007).

The powder X-ray diffractometry patterns are presented in Fig. 10. Clopidogrel napadisilate had sharp peaks at diffraction angles showing a typical crystalline pattern (Fig. 10A). HPMC (Fig. 10B) and colloidal silica (Fig. 10C) showed no intrinsic peak. Furthermore, all major characteristic crystalline peaks which appeared in the drug were observed in the physical mixture

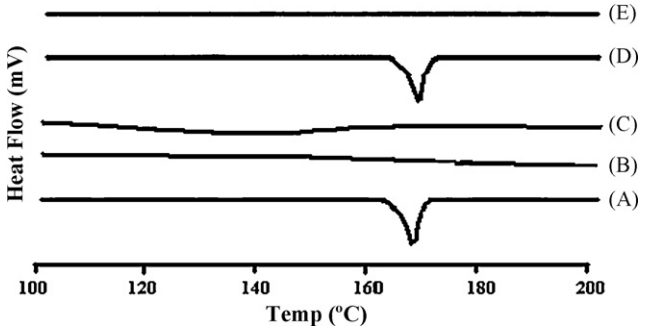

Fig. 9. Differential scanning calorimetric thermograms: (A) clopidogrel napadisilate; (B) HPMC; (C) colloidal silica; (D) physical mixture; (E) solid dispersion.

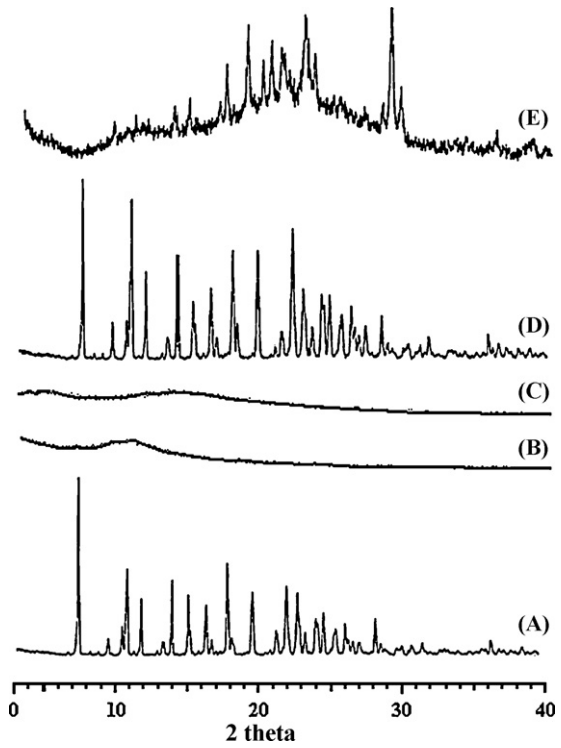

Fig. 10. Powder X-ray diffraction: (A) clopidogrel napadisilate; (B) HPMC; (C) colloidal silica; (D) physical mixture; (E) solid dispersion.

(Fig. 10D). However, unlike the drug and the physical mixture, the solid dispersion gave another peak at a diffraction angle showing an amorphous pattern (Fig. 10E). Thus, together with DSC results, clopidogrel napadisilate was present in a changed amorphous state in the solid dispersion.

It is generally well known that a drug in a solid dispersion system often exists in an amorphous form. The amorphous form of a drug has a higher thermodynamic activity than its crystalline form, leading to improved solubility (Joe et al., 2010). Similarly, such improved solubility was due to the change of drug crystallinity to the amorphous form in the clopidogrel napadisilate-loaded solid dispersion. Furthermore, clopidogrel napadisilate dispersed in polymeric carriers may achieve the highest levels of particle size reduction and surface area enhancement (Oh et al., 2011; Park et al., 2010a,b).

3.3. Clopidogrel napadisilate-loaded tablets

The clopidogrel napadisilate-loaded tablet (CNT) and a clopidogrel napadisilate solid dispersion-loaded tablet (CNSDT) were prepared by compressing the mixtures composed of clopidogrel napadisilate (or clopidogrel napadisilate-loaded solid dispersion), crospovidone, D-mannitol, and sucrose ester of fatty acid.

To evaluate whether the solid dispersion affected the dissolution rates of the drug, dissolution studies on the CNT, CNSDT and the clopidogrel bisulphate-loaded commercial product were performed in pH 1.2, 4.0, 6.8 and water (Fig. 11). At pH 1.2 and pH 4.0, two kinds of clopidogrel salts showed almost complete dissolution above 70% within 30 min. However, they showed a small amount of dissolution after 1 h at pH 6.8 due to their basic molecules. The CNSDT gave a lower initial dissolution rate of the drug compared to CNT at pH 1.2 (Fig. 11A). However, from 30 min onwards, the amounts of clopidogrel dissolved from the CNT did not significantly differ from those from CNSDT. At pH 4.0, the CNSDT gave a lower initial dissolution rate of the drug compared to CNT to 10 min, followed by higher dissolution rate of the drug to 60 min (Fig. 11B). As shown in Fig. 11C, there were no significant differences between the amounts dissolved from the CNT and CNSDT at pH 6.8. However, in water, the dissolution rate of the drug from the CNSDT was very high compared to the CNT (Fig. 11D). After 60 min, there was about a 2-fold increase in the dissolution rate of the drug from the CNSDT compared to the CNT ($90.2 \pm 6.0\%$ vs. $49.0 \pm 4.6\%$), because clopidogrel napadisilate might not overcome relatively low drug solubility in pH 6.8 unlike in pH 1.2 and 4.0.

The CNSDT showed similar dissolution of clopidogrel to the commercial product at pH 1.2 and pH 6.8, and higher dissolution of clopidogrel than the commercial product at pH 4.0 and water, although there were no significant differences.

The *in vitro* release profiles of the solid dispersion and commercial product were compared using the difference factor (f_1) and similarity factor (f_2), as defined by the following equation (Lim et al., 2010):

$$f_1 = \left[\frac{\sum(R_t - T_t)}{\sum(R_t + T_t)/2} \right] \times 100 \quad (1)$$

$$f_2 = 50 \times \log \left\{ \left[1 + \frac{1}{n} \sum (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \quad (2)$$

where n is the number of time points, and T_t and R_t are percentage releases at time point (t) for the commercial product and tablet, respectively. In these equations, $0 < f_1 < 15$ and $50 < f_2 < 100$ mean a similar correlation between the dissolution patterns of the two products.

The f_1 and f_2 values between CNT and commercial product were 20.25 and 31.82, 17.54 and 42.95, 10.24 and 75.55, and 39.58 and 28.55 at pH 1.2, 4.0, 6.8 and water, respectively. Except at pH 6.8, the CNT gave no similar correlation of dissolution profiles to the commercial product. On the other hand, the difference factor (f_1) values between CNSDT and commercial product were 3.22, 9.33, 3.80 and 10.65 at pH 1.2, 4.0, 6.8 and water, respectively. Furthermore, they had similarity factor (f_2) values of 71.98, 61.28, 94.52 and 59.14 at pH 1.2, 4.0, 6.8 and water, respectively. Thus, the CNSDT and commercial product showed a similar correlation of dissolution profiles over the entire pH range.

Fig. 12 shows the change in mean plasma concentration of clopidogrel (A) and its major metabolite, SR26334 (B) after oral administration of the clopidogrel bisulphate-loaded commercial product (Plavix®), CNT and CNSDT and at a dose of 15 mg/kg clopidogrel base in beagle dogs. Clopidogrel, a prodrug, has no activity in itself. The *in vivo* effects of clopidogrel are predominantly the result of the actions of its metabolites such as 2-oxo-clopidogrel and the thiol derivative (Mitakos and Panderi, 2002, 2004). However, these active metabolites are highly labile and remain undetected in plasma. In animals, clopidogrel is rapidly metabolised to its major metabolite, clopidogrel carboxylic acid derivative [(S)-(2-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4*H*)-acetic acid; SR26334] (Antić et al., 2007; Lagorce et al., 1998; Savi et al., 2006; Silvestro et al., 2010), even if it is pharmacologically inactive. Thus, SR26334 was assayed as an indirect approach for studying the pharmacokinetics of clopidogrel (Mitakos and Panderi, 2002; Singh et al., 2005). The CNT gave lower total plasma concentrations of the parent drug and SR26334 compared to the commercial product and CNSDT. In particular, the plasma concentrations of the parent drug and SR26334 in CNT, from 0.75 h to 8 h, were significantly lower compared with those in the commercial product and CNSDT ($P < 0.05$). The lower plasma concentrations in the CNT were due to lower dissolution of the drug resulting from relatively lower drug solubility. However, the total plasma concentrations of the parent drug and SR26334 in the CNSDT did not significantly differ from

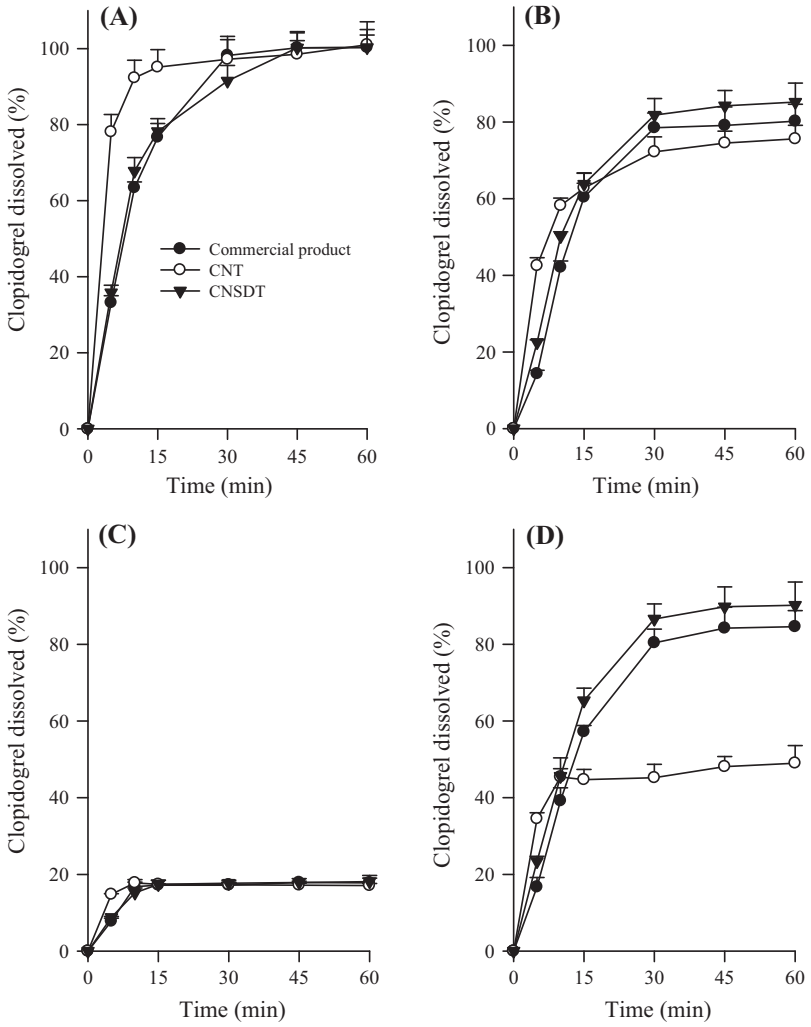

Fig. 11. Dissolution profile of drug from the clopidogrel bisulphate-loaded commercial product, clopidogrel napadisilate-loaded tablet (CNT) and clopidogrel napadisilate solid dispersion-loaded tablet (CNSDT): (A) pH 1.2; (B) pH 4.0; (C) pH 6.8; (D) water. Each value represents the mean ± S.D. ($n = 6$).

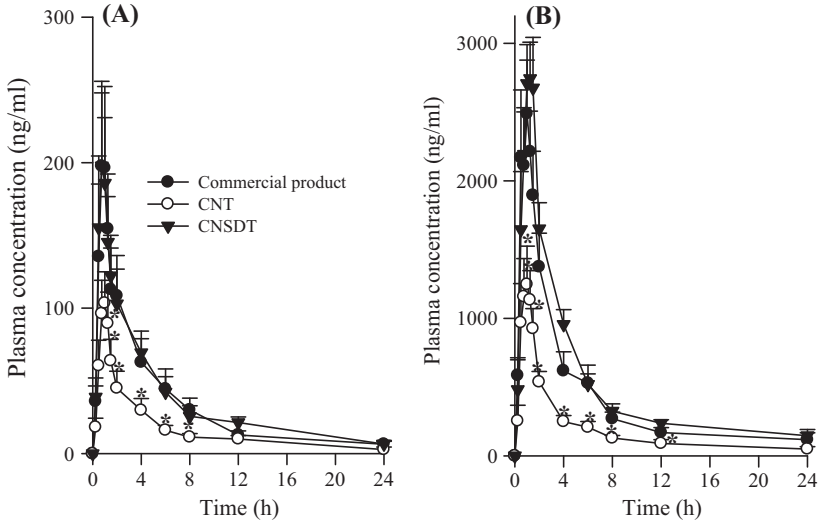

Fig. 12. Plasma concentration-time profiles of drug after oral administration of the clopidogrel bisulphate-loaded commercial product, clopidogrel napadisilate-loaded tablet (CNT) and clopidogrel napadisilate solid dispersion-loaded tablet (CNSDT) at a dose of 15 mg/kg clopidogrel base to dogs: (A) clopidogrel; (B) SR26334. Each value represents the mean ± S.D. ($n = 8$). $^{*}P < 0.05$ compared with the commercial product and solid dispersion-loaded tablet.

Table 3
Pharmacokinetic parameters of clopidogrel and SR26334 after oral administration of the clopidogrel bisulphate-loaded commercial product, clopidogrel napadisilate-loaded tablet and solid dispersion-loaded tablet at a dose of 15 mg/kg clopidogrel base to beagle dogs.

Parameters	AUC (ng h/ml)	C_{max} (ng/ml)	T_{max} (h)	$t_{1/2}$ (h)	K_{el} (h^{-1})
Clopidogrel					
Commercial product	804.63 ± 175.38	239.87 ± 54.19	0.88 ± 0.50	6.30 ± 2.29	0.11 ± 0.04
CNT[a]	401.29 ± 113.89*	126.92 ± 34.85*	0.97 ± 0.31	5.33 ± 1.69	0.13 ± 0.05
CNSDT[b]	865.92 ± 157.86	234.75 ± 45.12	1.28 ± 1.13	5.58 ± 1.41	0.12 ± 0.05
SR26334					
Commercial product	11011.82 ± 1377.82	3099.94 ± 306.53	0.91 ± 0.38	7.71 ± 3.42	0.09 ± 0.04
CNT[a]	4536.85 ± 753.44*	1403.57 ± 383.19*	1.00 ± 0.33	6.34 ± 2.29	0.11 ± 0.04
CNSDT[b]	12210.25 ± 879.66	3193.66 ± 305.72	1.25 ± 0.23	6.93 ± 2.08	0.10 ± 0.03

Each value represents the mean ± S.D. ($n = 8$).
[a] Clopidogrel napadisilate-loaded tablet.
[b] Clopidogrel napadisilate solid dispersion-loaded tablet.
* $P < 0.05$ compared with commercial product and CNSDT.

those in the commercial product in beagle dogs. Our results suggested that the higher plasma concentrations of the parent drug and SR26334 in CNSDT were due to the increase in solubility of clopidogrel from CNSDT in beagle dogs (Balakrishnan et al., 2009).

The pharmacokinetic parameters are shown in Table 3. The CNSDT gave a significantly higher AUC and C_{max} of the parent drug and SR26334 than the CNT. In particular, the AUC of the parent drug and SR26334 from CNSDT was about 2.2- and 2.7-fold higher than that from CNT, respectively. Our results suggest that the enhanced relative oral bioavailability of clopidogrel in the CNSDT was contributed to by the marked increase in the absorption of the drug due to its improved solubility in beagle dogs (Balakrishnan et al., 2009; Yan et al., 2010). However, the K_{el} and $t_{1/2}$ values of the parent drug and SR26334 from the CNSDT were not significantly different from those from the CNT. On the other hand, there were no significant differences in the AUC, C_{max} and T_{max} values between the CNSDT and the commercial product, suggesting that the CNSDT might be bioequivalent to the commercial product in beagle dogs (Di Girolamo et al., 2010; El Ahmady et al., 2009). Furthermore, the K_{el} and $t_{1/2}$ values of the parent drug and SR26334 from the CNSDT were not significantly different from those of the commercial product. Thus, from the pharmacokinetic view, the CNSDT showed similar drug efficacy compared to the commercial product in beagle dogs.

4. Conclusion

In conclusion, the clopidogrel napadisilate-loaded solid dispersion composed of clopidogrel napadisilate, HPMC and colloidal silica at a weight ratio of 11.069/3/3.5 improved the solubility of clopidogrel napadisilate, even if it did not improve drug solubility compared to clopidogrel bisulphate. However, unlike clopidogrel bisulphate, this formulation improved the stability of clopidogrel. Furthermore, the clopidogrel napadisilate solid dispersion-loaded tablet showed similar dissolution to the clopidogrel bisulphate-loaded commercial product and was bioequivalent to the commercial product in beagle dogs. Thus, the clopidogrel napadisilate solid dispersion-loaded tablet prepared with HPMC and colloidal silica could be a promising candidate for improving the stability and bioavailability of clopidogrel. For the development of a novel the clopidogrel napadisilate solid dispersion-loaded tablet, further study on its bioequivalence in human subjects will be performed.

Acknowledgements

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