Factors Affecting the Absorption of Nilvadipine from Disintegration-Controlled Matrix Tablet in Dogs

Toshiro SAKAI,*a Kazuhiro SAKO,a and Masahiro HAYASHIB

*a Pharmaceutical Research and Technology Laboratories, Astellas Pharma Inc.; 180 Ozumi, Yaizu, Shizuoka 425–0072, Japan: and b Department of Absorption and Pharmacokinetics, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences; 1482–1 Horinouchi, Hachioji, Tokyo 192–0392, Japan.

Received June 12, 2011; accepted August 31, 2011; published online September 2, 2011

The purpose of this study was to investigate the pharmacokinetics of nilvadipine (NiD) from disintegration-controlled matrix tablets (DCMT). A further purpose was to clarify biological factors that affect the absorption of NiD from DCMT. Two DCMT formulations, which released approximately 80% of NiD in 6 h (DCMT-M) and 10 h (DCMT-S) in vitro, were prepared and compared with immediate-release (IR) tablets. The T\textsubscript{max} and mean residence time from DCMT-M and DCMT-S were significantly longer than those from IR tablets in fasted dogs. The area under the plasma concentration–time curve (AUC) (0–infinity) from DCMT-M in both fed and fasted dogs and IR tablets were comparable in both fed and fasted dogs, indicating complete drug release and absorption without food effect. In contrast, the AUC from DCMT-S was significantly lower than the AUC from IR tablets in fasted dogs. The AUC from DCMT-S increased in fed dogs, but it was still lower than the AUC from IR tablets. In vivo absorption profiles calculated by deconvolution method suggested that the duration of drug absorption from DCMT-S was prolonged from 6 h in fasted condition to 8 h in fed condition, suggesting longer gastrointestinal (GI) transit time in fed condition allowed longer drug release duration from DCMT-S. Regional drug absorption was also evaluated using NiD solution. The results indicated NiD was almost completely absorbed from canine jejunum, ileum and colon, indicating drug permeation is not a rate-limiting factor of NiD absorption. Therefore, limited GI transit time is the primary factor that affects the drug release from DCMT and subsequent NiD absorption.

Key words nilvadipine; disintegration; sustained-release; solid dispersion; absorption

Nilvadipine (NiD), a dihydropyridine calcium antagonist, was developed by Astellas Pharma Inc. (Tokyo, formerly Fujisawa Pharmaceutical Co., Ltd.) as an antihypertensive agent. The immediate release (IR) tablet is marketed at a required twice-daily dose.\textsuperscript{1)} Since crystalline NiD is poorly soluble in water (approximately 1 \(\mu\)g/ml), a solid dispersion (SD) technique was used for the IR tablet to improve NiD oral bioavailability by increasing dissolution rate and apparent solubility.\textsuperscript{2)} Although SD is suitable for use as an immediate release formulation of NiD, the development of a sustained-release formulation is hampered by the re-crystallization of NiD after supersaturation-dissolution.\textsuperscript{3)} To provide a once-daily dosing with precise control of release rate while preventing the re-crystallization of NiD in gastro-intestinal (GI) tract, a sustained-release formulation, named the disintegration-controlled matrix tablet (DCMT), was developed.\textsuperscript{4)} The authors reported that fast- and moderate-release types of DCMT successfully sustained the absorption of NiD longer than IR tablets in beagle dogs.\textsuperscript{5)} However, the in vivo absorption of NiD from the slow-release type of DCMT has not yet been investigated. Furthermore, regional absorption of NiD, which is one of the most effective factors on the absorption of the drug form a sustained-release formulation, have not been investigated.

Consequently, pharmacokinetics of slow-release DCMT (DCMT-S) were investigated in fasted and fed dogs. Also, biological factors which affect the release or absorption of NiD from DCMT were investigated.

MATERIALS AND METHODS

Materials NiD and the IR tablet containing 4 mg of NiD were provided by Astellas Pharma Inc., formerly Fujisawa Pharmaceutical Co., Ltd. (Tokyo, Japan). Hydroxypropylmethylcellulose (HPMC) 2910 (labeled viscosity, 6 mm\(^2\)/s) and L-HPC (grade, LH21) were purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). Lactose, hydrogenated soybean oil (HSO) and magnesium stearate (MS) were purchased from DMV Japan (Tokyo, Japan), Miyoshi Oil and Fat Co., Ltd. (Tokyo, Japan) and Taihei Chemical Industrial Co., Ltd. (Osaka, Japan), respectively. All other materials were analytical reagent grade.

Preparation of DCMT DCMT-M and DCMT-S were prepared according to the method of Tanaka et al.\textsuperscript{4)} In brief, the SD granules were prepared using solvent-evaporation method with NiD, HPMC, L-HPC, lactose, ethanol, and dichloromethane. Subsequently, HSO was melted and mixed with SD granules at 85°C. The mixture was cooled to room temperature, and sized with an 850 \(\mu\)m sieve. Finally, DCMT were prepared by compressing the HSO-treated SD granules with MS. Table 1 shows the composition of DCMT-M and DCMT-S.

Table 1. Formulation of DCMT-M and DCMT-S

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>DCMT-M</th>
<th>DCMT-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>NiD</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>HPMC</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Lactose</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>L-HPC</td>
<td>36</td>
<td>24</td>
</tr>
<tr>
<td>HSO</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>MS</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Total (mg/tablet)</td>
<td>160.3</td>
<td>160.3</td>
</tr>
</tbody>
</table>

The amount of NiD and excipients is given in mg per tablet.
**In Vitro Release Study** In vitro release was tested by Japanese Pharmacopoeia (JP) dissolution test (paddle method) in 900 ml of JP first medium (pH 1.2) and JP second medium (pH 6.8) at 37 °C. The paddle rotation speed was 100 rpm. Samples were taken at intervals, and NiD concentrations were determined with an UV spectrometer (HP-8451A, Hewlett Packard, California, U.S.A.) at 246 nm.

**In Vivo Oral Absorption Study for DCMT** In vivo absorption was studied in six male beagle dogs (Japan Laboratory Animals, Inc., Tokyo, body weight of 10.5 to 14.0 kg) under fasted and fed conditions. The animals were maintained at 23 °C and 55% relative humidity. For the studies under the fasted condition, dogs were fasted overnight but allowed free access to water before drug administration. For the studies under the fed condition, dogs were given 300 g of solid food (Oriental Yeast Co., Ltd., Tokyo, Japan) 30 min before drug administration. A dose of 8 mg of NiD was orally administered by DCMT or IR tablets with 40 ml of water. Approximately 5 ml of blood was withdrawn into a heparinized syringe at 0.5, 1, 2, 4, 6, 8, 12, and 24 h after drug administration. The samples were immediately centrifuged and the plasma was stored at −20 °C until analysis. All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Astellas Pharma Inc., in accordance with “Principles of Laboratory Animal Care” (NIH publication #85-23).

**Determination of Plasma Concentration** A mixture of 1.5 ml of 0.1 M borate–HCl buffer (pH 9.0) and 4 ml of benzonene/hexane (1:1 (v/v)) was added to a 10 ml centrifuge tube containing 0.5 ml of plasma. The mixture was shaken for 5 min and centrifuged at 19000 m/s² for 10 min; 3 ml of organic layer was transferred to a 10 ml centrifuge tube and evaporated under a stream of nitrogen. The residue was reconstituted with 100 μl of ethyl acetate containing 100 ng of 4-acetoxy-1-[5-chloro-2-oxo-3-benzothiazolyl]acetyl]piperidine as an internal standard and 5 μl aliquot was injected onto Hewlett Packard (California, U.S.A.) 5890 gas chromatograph equipped with a 63Ni electron capture detector and an integrator (model 3392A; Hewlett Packard). A 3 mm×950 mm glass column packed with 3% Silicon OV-101 on Gas Chrom Q (100—200 mesh, GL Sciences Inc., Tokyo) was used at the following operating conditions; column temperature 250 °C, injector temperature 300 °C, detector temperature 300 °C, and flow rate of argon/methane (95:5 (v/v)) carrier gas 60 ml/min. The concentration was calculated from the peak area ratio of NiD to the internal standard; the coefficient of variation for the calibration curves (0.5 to 140 ng/ml NiD) was 1—3% and the correlation coefficient was >0.99.

**Pharmacokinetic (PK) Analysis** Area under the plasma concentration–time curve from 0 to infinity (AUC₀→∞) was calculated by trapezoidal method. Maximum plasma concentration (Cmax) and time to reach Cmax (Tmax) were obtained from individual plasma concentration–time curve. The mean residence time (MRT) from 0 to infinity was also calculated by trapezoidal rule based on the statistical moment theory.

**In Vivo Absorption Profiles** In vivo absorption profiles of NiD after oral administration of DCMT was analyzed by deconvolution method. Previous reports found NiD elimination from plasma in dogs follows a two-exponential equation. The following equation was determined as a weight function based on the elimination of NiD after administration of IR tablets at the dose of 8 mg/body in dogs; $C_p (ng/ml) = 23.3±3.8e^{-0.179t}+136.5e^{-1.002t}$, where $C_p$ = blood plasma concentration at time (t) in hours.

**In Situ Closed Loop Study for NiD Elimination** Three beagle dogs, 10.0 to 14.0 kg, were anesthetized with 20 mg/kg thiopental sodium (Tanabe Seiyaku Co., Ltd., Osaka, Japan). Animals were fasted overnight before the experiment but allowed free access to water. The intestine was exposed through a midline abdominal incision and four loops (20 cm each, at duodenum, jejunum, ileum, and colon) were prepared in each of the animals. The luminal surface of the loop was washed with 25 ml of saline. SD granules containing 180 μg of NiD was dissolved in 30 ml of saline. The solution was warmed to 37 °C and then injected into each of the loop, followed by suturing the incised abdominal muscle and skin. At 60 min after the injection, the abdomen was incised again in the median line and the loops were excised. The luminal surface of the loop was washed with 25 ml of saline, and 25 ml of ethanol. The washings were collected separately and volume of each washing was measured to estimate the residual NiD in the loop and adsorbed NiD on the luminal surface. NiD concentration in the washings was determined using the method described in following section.

**Determination of NiD Concentration in Washings** NiD concentrations were analyzed by HPLC. After centrifuging the sample at 19000 m/s² for 5 min, 1 ml of the supernatant was collected into 10 ml centrifuge tube with 1 ml of water. The solution was mixed with a Vortex mixer and 40 μl aliquot was injected into HPLC (Waters Co., New Jersey, U.S.A.) equipped with a 6000A pump, a WISP-710A injector, a 440 UV detector and a Data Module and a 4 mm×150 mm column (Develosil® ODS-5, 5 μm, Nomura Chemical Co., Ltd., Aichi, Japan). The operating conditions were: column temperature 25 °C, UV wavelength 254 nm, flow rate of acetonitrile/water/sodium perchlorate/dichloromethane (600 ml/400 ml/3 g/10 ml) mobile phase at 1.0 ml/min. The concentration was calculated from the ratio of peak area of NiD to standard solution (2.0 μg/ml); the coefficient of variation for calibration curves of standard solution at 0.1 to 10 μg/ml was <2% and the correlation coefficient was >0.99.

**Statistical Analysis** Results are expressed as the mean ± S.E. of six measurements for in vivo absorption studies and three measurements for the other studies. Differences among groups were tested by analysis of variance (ANOVA) and significant difference of the means was tested by Dunnett’s test.

**RESULTS AND DISCUSSION**

**In Vitro Characteristics of DCMT** Release profiles of NiD from DCMT-M and DCMT-S were evaluated to confirm the in vitro performance. IR tablets were also evaluated as the comparative formulation. Since Tanaka et al. reported that the release rate of NiD from DCMT could be regulated by changing the amounts of HSO and L-HPC, both tablets were prepared containing the equal amount of HSO and different amounts of L-HPC (Table 1).

As the results shown in Fig. 1, drug release from both DCMT-M and DCMT-S was prolonged compared to that
from IR tablets. IR tablets released 95% of NiD within 1 h. In contrast, DCMT-M and DCMT-S released 81 and 80% of NiD at 6 and 10 h, respectively. Also, it was visually confirmed that the tablets were completely disintegrated after the dissolution test. To confirm the release kinetics of DCMT-M and DCMT-S, the release profiles were analyzed using the Hixon–Crowell’s model, which is known as one of the most suitable models for describing the drug release from DCMT.\(^4\) The drug release mechanism of DCMT was reported as follows: (i) the penetration rate of water into the DCMT is controlled by a wax layer; (ii) L-HPC in SD granules swells under the influence of penetrating water; (iii) the swollen SD granules separate from the surface of the tablet; and (iv) drug is released from the separated SD granules.\(^4\) Among these mechanisms composed of four processes, the rate-limiting step in drug release from DCMT is (i), the penetration rate of water.\(^4\) The penetration rate was not affected by agitation intensity nor pH of dissolution media.\(^4\)

As shown in Table 2, coefficients of determination were more than 0.99, indicating that the release profiles of DCMT-M and DCMT-S were well-fitted to the model. The calculation results support that the drug release rates from DCMT-M and DCMT-S were controlled by the disintegration from the tablet surface, indicating the difference of L-HPC and lactose amounts affected the drug release rate, but not altered the release mechanism of DCMT. Tanaka et al. reported HSO was used to limit the penetration rate of water to the surface layer of the DCMT.\(^4\) L-HPC was included to separate the SD granules swollen by penetrating water from the rest of the tablet part.\(^4\) The tablets which contain less than 80 mg of HSO did not disintegrate completely and formed swollen gel tablet in the dissolution process, suggesting the water penetration was faster than the separation of SD granules from the tablet surface.\(^4\) Since the SD which contacts with water for longer period could lead the re-crystallization of NiD, the phenomenon must be avoided to achieve the complete release and maintain the bioavailability of NiD.

Based on these results, it was indicated that DCMT-M and DCMT-S, which have different drug release rates, were appropriately prepared for in vivo absorption experiments.

---

**Table 2. Release Rate Constant \(k_r\) of NiD from DCMTs and Coefficient of Determination \(r^2\) of Their Release Profiles Based on Hixon-Crowell’s Model**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DCMT-M</th>
<th>DCMT-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>(r^2)</td>
<td>0.994</td>
<td>0.996</td>
</tr>
<tr>
<td>(k_r) (mg(^{1/3})/h)</td>
<td>0.146</td>
<td>0.086</td>
</tr>
</tbody>
</table>

---

**Table 3. Pharmacokinetic Parameters of NiD after Oral Administration of DCMTs to Beagle Dogs under Fasted Conditions**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>(AUC_{\text{cmax}}) (ng·h/ml)</th>
<th>(C_{\text{max}}) (ng/ml)</th>
<th>(T_{\text{max}}) (h)</th>
<th>MRT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR tablets</td>
<td>281±75</td>
<td>102±20.0</td>
<td>0.583±0.083</td>
<td>3.66±0.07</td>
</tr>
<tr>
<td>DCMT-M</td>
<td>231±35</td>
<td>36.0±5.5**</td>
<td>3.67±0.33**</td>
<td>5.45±0.34**</td>
</tr>
<tr>
<td>DCMT-S</td>
<td>109±22**</td>
<td>23.8±4.2**</td>
<td>3.33±0.42**</td>
<td>5.17±0.35**</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E. \((n=6)\). * \(p<0.05\), ** \(p<0.01\), compared with the corresponding parameter of IR tablets.

---

**In Vivo Performance of DCMT in Fasted Dogs**  Pharmacokinetics of NiD after administration of IR tablet, DCMT-M, and DCMT-S was evaluated in fasted dogs. Figure 2 shows the mean plasma concentration profiles of NiD after oral dosing of these tablets. The pharmacokinetics parameters of these formulations are shown in Table 3. The pharmacokinetics profiles of NiD differed significantly depending on whether the drug is administered as a DCMT or as an IR tablet. NiD was rapidly absorbed from IR tablets \((T_{\text{max}}<1\ h)\) but absorption was significantly prolonged when NiD was administered via DCMT-M or DCMT-S \((T_{\text{max}}>3\ h)\). Moreover, MRT was also significantly prolonged and \(C_{\text{max}}\) was significantly reduced by DCMT-M or DCMT-S compared to IR tablets, indicating slower absorption of NiD from DCMTs were achieved. The relative \(AUC\) of DCMT-M and DCMT-S to IR tablet were 82 and 39%, respectively.

These results indicated that sustained-release of NiD from DCMT-M resulted in the sustained absorption of NiD in vivo; DCMT-M significantly delayed \(T_{\text{max}}\) and prolonged MRT, and maintained \(AUC\). In contrast, the \(AUC\) following administration of DCMT-S was significantly lower than that.
following IR tablets despite the complete in vitro drug release (86% at 12 h).

This limited drug absorption from DCMT-S suggests the possibility of incomplete drug release or limited permeation in the GI tract in vivo.

**Effect of Foods on in Vivo Absorption** It has been reported that DCMT-M showed similar PK profile of NiD in both of the fasted and fed dogs, and the absorption continued at least for 6 h. However, the effects of foods on the absorption of NiD from a DCMT-S have not been investigated. The absorption of NiD from DCMT-M and DCMT-S was compared in fasted and fed dogs.

As the results, DCMT-M showed similar plasma concentration profiles of NiD in fasted and fed conditions (Fig. 3, Table 4). In contrast, the PK parameters of NiD after administration of DCMT-S in fed dogs tended to be higher than those in fasted dogs (Fig. 4, Table 5). Especially, DCMT-S showed significantly longer MRT (6.29 h) than DCMT-M (5.45 h) in fed state.

Subsequently, absorption profiles of drug from both formulations were calculated by deconvolution method using the weighing function which calculated from the results of IR tablets (Figs. 5, 6). Drug absorption from DCMT-M both in fasted and fed conditions at 6 h were 83%. The results indicate that absorption of NiD from DCMT-M was not affected by the food. In addition to that, since both of the in vitro drug release and in vivo absorption from DCMT-M were similarly completed in 6 h, NiD in DCMT-M was confirmed to be released and absorbed in vivo, as expected from the in vitro release results.

In contrast, the drug absorption profiles from DCMT-S in fasted and fed conditions were different. Drug absorption from DCMT-S in fasted and fed conditions at 10 h were 42.8 and 60.2%, respectively, although the in vitro drug release at

![Fig. 3: Plasma Concentration Profiles of NiD after Oral Administration of DCMT-M to Beagle Dogs in Fasted and Fed Conditions](image1)

![Fig. 4: Plasma Concentration Profiles of NiD after Oral Administration of DCMT-S to Beagle Dogs in Fasted and Fed Conditions](image2)

![Fig. 5: In Vitro Release and in Vivo Absorption Profiles of NiD from DCMT-M in Beagle Dogs under Fasted and Fed Conditions](image3)
highly lipophilic drugs, were considered. Potential effective effects of GI physiology on absorption of drugs, especially intestinal epithelial membrane should be considered. via drug release in GI tract and limited drug permeation absorption of NiD from DCMT-S, possibility of limited in vivo absorption in fasted condition is 3 to 4 h, NiD can be released from DCMT/S in fasted dogs, both of the DCMT-M and DCMT-S showed continuous absorption of NiD up to 6 h. Considering the reports that the mouth-to-cecum transit time of non-disintegrating tablets in fasted dogs is 3 to 4 h, NiD can be released from DCMT even after reaching colon for 2 to 3 h. The mean SITT in dogs was not affected by the food, and almost completely absorbed after oral administration of IR tablets with solid dispersion granules. Although these reports suggested that NiD was highly permeable drug in upper bowel, the permeability in distal bowel has not been clarified. Thus, regional difference in intestinal absorption of NiD was evaluated in dogs using in situ closed loop, by obtaining the decrease of NiD concentration in solution from duodenal, jejunal, ileal, and colonic loops. To correct residual of NiD in the closed loops, it was eluted using saline and ethanol as the washing solvent because it was considered that NiD is adsorbed on the intestinal cell membrane because of its high-lipophilicity.

As the results shown in Table 6, less than 2.52 and 1.97% of injected NiD was recovered from the residual solution and the washing solvent, respectively. The results suggest that more than 95% of NiD is absorbed from duodenum, jejunum, ileum, and colon in 60 min when the drug was administered in solution, indicating the permeation of NiD is not the rate limiting step of absorption when the drug is released to GI fluid.

Considering the release mechanism of the DCMT, the penetration of water into the system is absolutely necessary to disintegration of tablet surface followed by the drug release. Meanwhile, the existence of the water is extremely low in the distal bowel, especially in distal colon. Therefore, GI transit time is suggested as primary factor which affect the drug release and absorption from DCMT.

Based on the above findings, it is considered that the application of DCMT for long-acting type system (i.e. DCMT-S) is not appropriate in the viewpoint from complete drug absorption. In particular, the amount of L-HPC must be carefully adjust to complete the disintegration of tablet surface before reaching it to the colon. For further development of the sustained-release system of NiD based on the DCMT, it is considered that the system which releases the NiD within 6 h would be more secure to avoid the food effect and the lower bioavailability.

CONCLUSION

DCMT-S significantly reduced the AUC compared to IR tablet in fasted dogs. The GI transit time was considered as a primary factor which affects the absorption of NiD from DCMT. The data suggest that the drug release from DCMT terminated at 2 to 3 h after the formulation reached the colon. Since the key process controlling drug release from DCMT is water penetration into tablet surface followed by the disintegration, drug release can be terminated in the distal colon where there is less water. NiD administered as solution was absorbed from jejunum to colon, and this result indicates that

**Table 6. Residual and Adsorbed amount of NiD 60 min after Administration of NiD Solution to in Situ Closed Loop of Beagle Dogs**

<table>
<thead>
<tr>
<th>Absorption site</th>
<th>Residual of NiD (%)</th>
<th>Adsorption of NiD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>2.40±0.78</td>
<td>1.15±0.19</td>
</tr>
<tr>
<td>Jejunum</td>
<td>1.91±1.41</td>
<td>2.16±0.40</td>
</tr>
<tr>
<td>Ileum</td>
<td>2.52±2.23</td>
<td>1.97±0.37</td>
</tr>
<tr>
<td>Colon</td>
<td>0.933±0.218</td>
<td>1.80±0.44</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E. (n=6) and is also shown as the percent of the input amount into the lumen.
permeability of NiD did not limit rate and extent of drug absorption. Therefore, it is concluded that drug release from DCMT should be completed within about 6 h considering the transit time from mouth to the colon and the absorption time in the colon to achieve the complete release and absorption in GI tract.

REFERENCES