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## SHORT COMMUNICATION

# Pharmacokinetics of Oral Amlodipine Orotate in Vagotomized Dogs

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**ABSTRACT:** It was reported that gastric motility was delayed and gastric acid secretion was reduced in vagotomized dogs which mimics a low gastric acidity in humans. A delay in gastric motility causes long residence of amlodipine in the stomach. More unionized fractions of amlodipine could exist in less acidic conditions of gastrointestinal fluids, since amlodipine is a weak basic drug with pKa of 8.7. Hence, gastrointestinal absorption of amlodipine is expected to be enhanced and the time to reach a peak plasma concentration of amlodipine ($T_{max}$) is faster in vagotomized dogs. This was proven after oral administration of an amlodipine orotate tablet at a dose of 5 mg as amlodipine in vagotomized dogs. For example, in vagotomized dogs, the total area under the plasma concentration–time curve from time zero to the last measured time, 48 h, in plasma ($AUC_{0-48\,h}$) was significantly greater (725 versus 348 ng h/ml) and $T_{max}$ was significantly shorter (1.50 versus 5.00 h) than those in dogs without vagotomy. Copyright © 2006 John Wiley & Sons, Ltd.

**Key words:** amlodipine orotate tablet; vagotomized dogs; dissolution rates; gastric motility; gastric acid secretion; pharmacokinetics

## Introduction

Amlodipine (2-[(2-aminoethoxy)methyl]-4(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine) is a third generation dihydropyridine calcium channel antagonist. Amlodipine is a basic drug with a pKa value of 8.7, hence it mainly exists in its ionized form at physiological pH [1]. The amlodipine orotate salt was synthesized (Research Laboratory, Dong-A Pharmaceutical Company, Yongin, Republic of Korea) to increase the dissolution rate in wide pH ranges. Hence, amlodipine orotate salt may enhance the gastrointestinal absorption of amlodipine in patients with low gastric acidity, because more unionized forms of amlodipine are available in low gastric acidity since amlodipine is a weak basic drug, if the gastrointestinal absorption of amlodipine follows the pH-partition hypothesis [2]. In humans, absorption of amlodipine from the gastrointestinal tract is high with extensive metabolism in the liver (but there is no significant presystemic or first-pass metabolism) [1,3]. The extent of absolute oral bioavailability is 60%–65% in humans [1].

The aim of this study is to report the enhanced absorption of amlodipine orotate tablet in vagotomized dogs. Vagotomy, in general, delays gastric motility and reduces gastric acid secretion

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in dogs [4]. In vagotomized dogs, the pH of the gastric juice is increased considerably [5]. For example, the pH ranges of most regions of the stomach in vagotomized dogs were 4–7; the high acid values in normal dogs dropped in the entire area of the stomach in vagotomized dogs [6]. Hence, vagotomy would mimic patients with low gastric acidity.

## Materials and Methods

### *Chemicals*

Amlodipine orotate tablets (Research Laboratory of Dong-A Pharmaceutical Company; lot number, AOT 3001) and amlodipine powder were supplied from the Research Laboratory of Dong-A Pharmaceutical Company. Zoletil (1:1 mixture of zolazepam and tiletamin) and tiropramide (an internal standard of HPLC/MS/MS analysis of amlodipine) were purchased from Virbac Corporation (Fort Worth, TX) and Sigma–Aldrich Corporation (St Louis, MO), respectively. Other chemicals were of reagent grade or HPLC grade.

### *Dissolution test*

The dissolution rates of amlodipine orotate tablets were evaluated in three dissolution media, with pHs of 2.0 (0.01 N HCl buffer), 4.0 (acetate buffer) and 6.8 (Sørensen phosphate buffer). The dissolution test was performed with six tablets using USP XXVIII apparatus 2 (paddle method) (VanKel VK 7000; Varian Inc., Palo Alto, CA) in 500 ml of each media kept at $37^\circ \pm 0.5^\circ$C and at a rate of 50 revolutions per min (rpm). At 0, 10, 20, 30 and 60 min, a 1 ml aliquot of sample was collected and the same volume of dissolution medium was replaced as soon as each sample was collected. The collected sample was centrifuged and the supernatant was stored in a $-80^\circ$C freezer (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC) before HPLC analysis of amlodipine [6].

### *Animals*

Twelve male beagle dogs (weighing 8.3–10.5 kg) were purchased from the Kwang-San Laboratory Animal Company (Suwon, Republic of Korea). They were randomly divided into two groups; without ($n = 4$) and with ($n = 8$) vagotomy. The vagotomized group was further divided into two groups; sham-operated and selective gastric complete vagotomy ($n = 4$; each). Two weeks after the surgery, gastric motility was measured. One week after measurement of gastric motility, the pharmacokinetic studies were performed for the selective gastric complete vagotomized dogs ($n = 4$). Pharmacokinetic studies were also performed in dogs without vagotomy ($n = 4$).

### *Surgery for sham-operation and selective gastric complete vagotomy, and measurement of gastric motility*

The procedures for vagotomy [7] and for the measurement of gastric motility [8] in dogs were similar to the reported methods. Food was withheld for 20 h with free access to water before surgery. Each dog was anesthetized with intramuscular zoletil (20 mg/kg; total injection volume of approximately 2 ml). The abdomen was then opened with a midline laparotomy. The stomach was gently mobilized, and the five major branches and two trunks of the abdominal vagus were identified. In the vagotomized groups ($n = 4$), a 2 cm portion of each anterior and posterior gastric vagi was gently transected with preservation of the anterior and posterior hepatic and celiac branches; all vessels to the minor curvature from the cardia to the pylorus were ligated. Sham operation was also performed ($n = 4$). Animals were then sewn up and treated with intramuscular cefradine (20 mg/kg). The validity of vagotomy was confirmed by ultrasound examination. Briefly, all dogs were supine-positioned and hair was clipped so that the probe could make contact direct with the skin. Ultrasound examinations were performed with a high-resolution real-time scanner (SonoAce 9900; Medison, Seoul, Republic of Korea). With the dogs positioned in dorsal recumbency, transverse images were obtained by placing the transducer in a transverse plane of the cranial abdomen, parallel to the right costal arch. By rotating the transducer at a $90^\circ$ angle in a clockwise direction, longitudinal images were made and the frequency of antral contractions was measured. The frequency of antral

contractions was defined as the numbers of contractions per min after feeding commercial canned food.

### *Oral administration*

One tablet of amlodipine orotate, 5 mg as amlodipine, was administered orally with 20 ml of water to dogs with and without vagotomy ($n = 4$; each). Approximately $\sim$0.5 ml aliquot of blood was collected via the jugular vein into heparin-vacutainer tubes at 0 (to serve as a control), 1, 2, 4, 6, 8, 10, 12, 24 and 48 h after oral administration. After centrifugation of the blood sample, a 0.2 ml aliquot of plasma sample was stored in a $-80^{\circ}$C freezer until HPLC/MS/MS analysis of amlodipine [9].

### *Analysis of amlodipine*

The concentrations of amlodipine in the dissolution sample were analysed by the HPLC method [6]. A 0.02 ml aliquot of sample was injected directly onto the reversed-phase ($C_{18}$) column. The mobile phase, methanol:0.03 M $KH_2PO_4$ (60:40; v/v) was run at a flow rate of 1.5 ml/min, and the column effluent was monitored by an UV detector set at 237 nm.

The concentrations of amlodipine in dog plasma were analysed by the reported HPLC/MS/MS method [9]. To a 200 µl aliquot of the plasma sample, a 20 µl aliquot of acetonitrile containing an internal standard (40 ng/ml) and a 1.3 ml aliquot of tert-butylmethylether were added. The organic layer was collected and then separated on an Atlantis reversed-phase ($C_{18}$) column (2.1 mm. i.d. $\times$ 100 mm. *l.*; particle size; 5 µm; Waters, Milford, MA) using isocratic elution of acetonitrile:methanol:0.2% formic acid (5:55:40; v/v/v) at a flow rate of 0.2 ml/min. The column and autosampler tray temperatures were 40° and 15°C, respectively. The eluent was introduced directly into the positive ionization electrospray source of a tandem quadrupole mass spectrometer (Quattro Micro; Micromass, Amnchester, UK). The ion source and desolvation temperatures were held at 120° and 250°C, respectively. The optimum cone voltages and collision energy were 29 V and 20 eV, and 11 V and 10 eV for tiropramide (an internal standard) and amlodipine, respectively. Selected reaction monitoring (SRM) using the precursor product ion transitions of $m/z$ 409.1 > 238.1 and $m/z$ 468 .1 > 367.3 were used to quantify amlodipine and tiropramide, respectively. The linear calibration curves were obtained in the concentration ranges 0.5–100 ng/ml. The quantification limit of amlodipine was 0.5 ng/ml. The within- and between- day coefficients of variation were below 10%.

### *Pharmacokinetic analysis*

The total area under the plasma concentration–time curve from time zero to the last measured time, 48 h, in plasma ($AUC_{0-48\,h}$) were calculated using WinNonlin 3.1™ (Pharsight Corporation, Mountain View, CA). The maximum plasma concentration ($C_{max}$) and the time to reach a $C_{max}$ ($T_{max}$) were read directly from the experimental data.

### *Statistical analysis*

A value of $p < 0.05$ was considered to be statistically significant using an unpaired *t*-test, or a Duncan's multiple range test of Statistical Package of Social Sciences (SPSS) *posteriori* analysis of variance (ANOVA) program among the three means for the unpaired data. All data are expressed as mean $\pm$ standard deviation.

## Results

### *Dissolution study*

The dissolution rates of amlodipine from six amlodipine orotate tablets in a medium of pH 2.0 were approximately $70.5 \pm 3.9\%$, $85.3 \pm 4.6\%$, $91.9 \pm 4.2\%$ and $98.5 \pm 3.5\%$ at 10, 20, 30 and 60 min, respectively. However, the rates were slower in media of pHs 4.0 and 6.8; the corresponding values were $51.7 \pm 5.3\%$, $66.6 \pm 3.6\%$, $74.7 \pm 2.9\%$ and $90.1 \pm 2.3\%$, and $35.0 \pm 3.3\%$, $39.5 \pm 4.7\%$, $41.8 \pm 2.4\%$ and $44.9 \pm 3.2\%$ in media of pHs 4.0 and 6.8, respectively. The values at 20, 30 and 60 min in media of pHs 4.0 and 6.8 were significantly slower than those in the pH 2.0 medium.

### *Measurements of gastric motility*

The mean numbers of contractions of the pyloric antrum in sham-operated dogs were $27.4 \pm 4.13$,

25.6 ± 3.24 and 28.6 ± 2.83 contractions/min for 10, 30 and 70 min, respectively; each value was not significantly different. The corresponding values in vagotomized dogs were 22.6 ± 4.62, 18.5 ± 1.76, and 13.3 ± 3.37 contractions/min; the values at 30 and 70 min were significantly smaller than that at 10 min. The values in vagotomized dogs were significantly smaller than those in sham-operated dogs at all times.

### *Pharmacokinetics of amlodipine in dogs with or without vagotomy*

The mean venous plasma concentration–time profiles of amlodipine following oral administration of an amlodipine orotate tablet in dogs with and without vagotomy are shown in Figure 1, and some relevant pharmacokinetic parameters are listed in Table 1. In vagotomized dogs, the plasma concentrations of amlodipine were higher, $C_{max}$ was significantly higher (89.4% increase), $T_{max}$ was significantly shorter and the $AUC_{0-48\,h}$ was significantly greater (108% increase) than those in unvagotomized dogs. Note that there were multiple peaks in the plasma concentrations of amlodipine after oral administration (Figure 1). The possible reasons for the multiple peaks phenomena could be due to irregular blood partition of drugs between plasma and blood cells, enterohepatic recycling of drugs, rapid uptake of the drugs in the tissues and then late release from the tissues to blood, and/or the gastric emptying pattern of drugs [10,11].

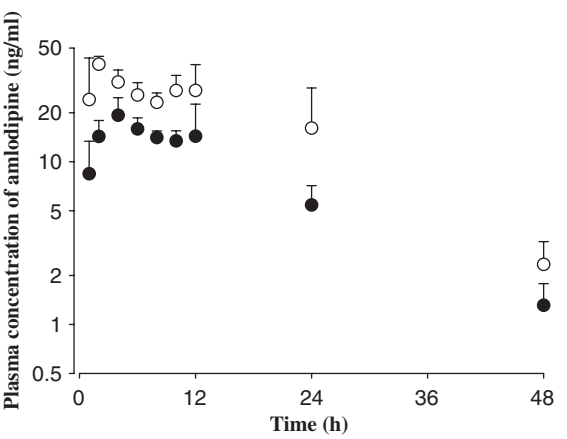


Figure 1. Mean venous plasma concentration–time profiles of amlodipine after oral administration of amlodipine orotate tablet at a dose of 5 mg as amlodipine in dogs with (○) and without (●) vagotomy ($n = 4$; each). Bars represent standard deviation

Table 1. Mean (± standard deviation) pharmacokinetic parameters of amlodipine after oral administration of amlodipine orotate tablet at a dose of 5 mg as amlodipine in dogs without or with vagotomy ($n = 4$; each)

| Parameter | Without vagotomy | With vagotomy |
|---|---|---|
| $AUC_{0-48\,h}$ (ng h/ml) | 348 ± 84.0 | 725 ± 109[a] |
| $C_{max}$ (ng/ml) | 21.8 ± 5.83 | 41.3 ± 4.78[a] |
| $T_{max}$ (h) | 5.00 ± 4.76 | 1.50 ± 0.71[a] |

[a] Significantly different ($p < 0.05$) from without vagotomy.

## Discussion

The present study was performed to determine whether the absorption of amlodipine is enhanced after oral administration of amlodipine orotate tablet in vagotomized dogs, which mimics a low gastric acidity (hypo- or anacidity) condition in patients. The $AUC_{0-48\,h}$ of amlodipine increased approximately 2-fold in vagotomized dogs compared with that in unvagotomized dogs (Table 1). This could be due to a delay in gastric motility as mentioned earlier and a reduction in gastric acid secretion in vagotomized dogs [5]. Gastrointestinal motility is one of the most important factors that can influence drug absorption from the gastrointestinal tract [12]. For example, the absorption of compound UK 81252 (a dual inhibitor of angiotensin-converting enzyme and neutral peptidase with potential application as an antihypertensive agent as well as a treatment of congestive heart failure) in dogs was markedly enhanced by caprylocaproyl macrogolglycerides, a lipid-based surface-active agent, due to inhibition of the gastric emptying time [13]. The slower gastric emptying time causes longer residence of amlodipine, hence, absorption of amlodipine could be enhanced. The reduction of gastric acid secretion (low gastric acidity) in vagotomized dogs could also contribute to the significantly greater $AUC_{0-48\,h}$ of amlodipine in vagotomized dogs, since more unionized forms of amlodipine are present

in the less acidic condition. Although the dissolution rates of amlodipine orotate tablet are significantly slower in dissolution media of pHs 4.0 and 6.8 than in 2.0, the reduction of gastric motility and low gastric acidity contribute considerably to the significantly faster $T_{max}$ and greater $AUC_{0-48\,h}$ of amlodipine orotate tablets in vagotomized dogs. The above data could suggest that amlodipine orotate tablets could be used in patients with low gastric acidity. Human studies are required to prove the above hypothesis.

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