Evaluation of Acid Tolerance of Drugs Using Rats and Dogs Controlled for Gastric Acid Secretion

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ABSTRACT: We attempted to establish animal models to evaluate the effects of drug degradation in the stomach on oral bioavailability. In addition, we assessed the utilization of animal studies in determining the need for enteric-coated formulations. In order to control the gastric pH in rats and dogs, appropriate dosing conditions were investigated using pentagastrin and rabeprazole, which stimulate and inhibit gastric acid secretion. Using animals controlled for gastric acid secretion, the area under curve (AUC) ratios (AUC with rabeprazole/AUC with pentagastrin) of all compounds unstable under acidic conditions were evaluated. The AUC ratios of omeprazole and erythromycin, which are administered orally to humans, as enteric-coated tablets, were greater than 1.9 in the rats and dogs controlled for gastric acid secretion. On the contrary, the AUC ratios of clarithromycin, azithromycin, and etoposide (commercially available as a standard immediate-release form) were less than 1.3 each. In conclusion, *in vivo* models using rats and dogs were optimized to evaluate the effects of gastric acid on the oral bioavailability of drugs, and demonstrated that *in vivo* models can lead to a better understanding of the oral bioavailability, with respect to the formulation development. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:2887–2893, 2015

Keywords: gastric pH; acid tolerance; pentagastrin; gastric acid secretion; stability; oral bioavailability; pharmacokinetics; formulation; enteric coating

INTRODUCTION

The rapid degradation of drugs under acidic conditions reduces their oral bioavailability. In addition, it may result in inter- and intraindividual variability in the pharmacokinetic profile of the drug. It is essential to know the magnitude of any degradation occurring prior to the absorption of oral doses for unstable compounds under acid conditions. It is also important to determine the need for enteric coating of such compounds during the optimization process, as an enteric-coated formulation might delay the absorption of a drug and increase the cost and time required for development.[1,2]

Rat is the most extensively used animal model for bioavailability studies, because of its ease of handling. The use of rats is preferred in the drug discovery process, as it reduces the quantity of the drug required at such an early stage. In addition, the gastric pH of rats under fasting conditions is closer to that of humans.[3,4] However, rats have not been widely used in the optimization of formulations because of technical problems, such as the size of the tablets and gavage needles. Beagle dogs are commonly used in the study of oral bioavailability, as they share many similarities with humans in terms of the gastrointestinal anatomy and physiology.[5,6] Therefore, these dogs are utilized to optimize the design of formulations for clinical analysis.[7–12] However, dogs are known to express a high-gastric pH during fasting conditions.[13]

Gastric pH influences the degradation of unstable drugs and differs between dogs and humans during fasting conditions.[6] In addition, some studies have observed some remarkable interindividual variations in the gastric pH in untreated dogs.[13,14] Hence, the gastric pH in dogs must be controlled in order to estimate its effect on oral bioavailability in humans. Pentagastrin stimulates gastric acid secretion,[15,16] whereas proton pump inhibitors and H_2 blockers have been reported to increase gastric pH content and assist in maintaining a neutral pH in the stomach.[13,17–20] These drugs are widely used to control gastric pH in animal pharmacokinetic studies.

Proton pump inhibitors and macrolide antibiotics have been discovered to undergo rapid decomposition under acidic conditions.[2,21–24] Several compounds such as omeprazole, lansoprazole, and erythromycin are commercially available as enteric-coated tablets. Although the formulation of these compounds has been optimized in humans and dogs,[7,11,25,26] the process with which the need for enteric-coated formulations can be determined remains to be elucidated. This is because very few studies have investigated the effect of degradation in the stomach on the oral bioavailability of a standard immediate-release form or suspension.[1]

The major focus of this study is to determine the effect of acidic stability on oral bioavailability, using animal models. Animal models with controlled gastric pH were established, and the effect of degradation in the stomach on oral bioavailability was evaluated. Through this study, we propose the practical use of *in vivo* models to determine the need for enteric-coated formulations.

MATERIAL AND METHODS

Materials

Acetaminophen, caffeine, carbamazepine, erythromycin, etoposide, omeprazole, theophylline, and tolbutamide were

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purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Alprenolol was obtained from Sigma Chemicals Company (St. Louis, Missouri). Azithromycin, pentagastrin, rabeprazole, clarithromycin, and methyl cellulose were purchased from Astatech, Inc. (Bristol, Pennsylvania), Bachem AG (Bubendorf, Switzerland), MolBridge LLC (Princeton, New Jersey), Tokyo Chemical Industry Company, Ltd. (Tokyo, Japan), and Shin-Etsu Chemical Company, Ltd. (Tokyo, Japan), respectively. All other reagents and solvents of analytical or HPLC grade were obtained commercially.

Animal Models and Handling

Male beagle dogs (1–2 years, 7–11 kg) were purchased from KITAYAMA LABES Company, Ltd. (Chiba, Japan), and housed under controlled conditions of temperature (22°C–27°C) and humidity (40%–70%), and a 12 h light/dark cycle. The dogs were fed laboratory chow (DS-A; Oriental Yeast Company, Ltd., Tokyo, Japan) at 9 am every day. Free access to water was provided. Male Crl–CD (SD) rats (8–11-weeks-old, 246–428 g) were purchased from Charles River Japan Inc. (Shiga, Japan) and housed under controlled temperature and humidity conditions and a 12-h light/dark cycle. The animals were provided with free access to laboratory chow (CE-2; Clea Japan, Inc., Tokyo, Japan) and water. All animal protocols were approved by the Institutional Animal Care and Use Committee of the Shonan Research Center, Takeda Pharmaceutical Company Ltd. (Kanagawa, Japan; Approval No. 00005679-100-000, 00005973-101-000, 00005391-101-000).

Determination of Gastric pH

The dogs were made to fast overnight prior to and for 8 h after drug administration, whereas access to water was provided *ad libitum*. The same dogs were used in all studies including the pentagastrin and rabeprazole treatment. The drugs were administered at a minimum of 1-week intervals. Pentagastrin was dissolved in 10% (v/v) ammonia solution. This was further diluted in saline, and the final pH was adjusted to 8.0 using 0.1 N HCl. Rabeprazole was dissolved in saline. Pentagastrin (0.01 mg/kg) was administered via intramuscular injections 30 min before and 15 min after oral administration of 0.5% (w/v) methylcellulose (0.5% MC) solution. Rabeprazole (1 mg/kg) was administered intravenously 60 min before the oral administration of 0.5% MC solution. Each dog was provided with 20 mL of water immediately after the oral administration of 0.5% MC solution (2 mL/kg). A catheter was inserted into the stomach of each dog immediately or 1 h after oral administration, and a small volume of the gastric fluid was aspirated through the catheter. The pH of the stomach fluids was determined using a glass electrode (9618–10D; Horiba, Ltd., Kyoto, Japan).

The rats were made to fast overnight prior to and for 4 h after drug administration. Access to water was provided *ad libitum*. Pentagastrin (0.01 mg/kg) was administered via intramuscular injections 30 min before oral administration of 0.5% MC solution. Rabeprazole (10 mg/kg) was administered intraperitoneally 30 min before the oral administration of 0.5% MC solution. The rats were sacrificed immediately or 1 h after the oral administration of 0.5% MC solution (5 mL/kg), and the stomachs were removed. The pH of the stomach fluids was measured using a glass electrode (9618-10D; Horiba, Ltd.).

Dosing and Blood Sampling

Fasting dogs were pretreated with pentagastrin and rabeprazole as described above. All tested compounds were suspended in 0.5% MC solution. Each dog was provided with 20 mL of water immediately after the oral administration of the test compounds suspended in 0.5% MC solution (2 mL/kg). Acetaminophen (3 mg/kg) was administered orally to these dogs in order to determine gastric emptying. Carbamazepine (1 mg/kg), theophylline (0.5 mg/kg), and tolbutamide (0.2 mg/kg) were coadministered orally in order to evaluate the drug–drug interactions with pentagastrin and rabeprazole. Omeprazole, erythromycin, etoposide, azithromycin, and clarithromycin were administered orally at a dose of 1 mg/kg each, in order to investigate the stability of the drugs in the stomach. Blood samples were collected from the forearm cephalic vein of all test dogs.

Fasting rats were pretreated with pentagastrin and rabeprazole as described above. All tested compounds were suspended in 0.5% MC solution. Acetaminophen (3 mg/kg), omeprazole (5 mg/kg), erythromycin (5 mg/kg), etoposide (3 mg/kg), azithromycin (5 mg/kg), and clarithromycin (5 mg/kg) were administered orally. Carbamazepine (1 mg/kg), theophylline (0.5 mg/kg), and tolbutamide (0.2 mg/kg) were also coadministered orally. Blood samples were collected from the tail vein of all rats.

Sample Preparation

Plasma was separated from the blood samples by centrifugation at approximately 8000*g* for 5 min, and stored at −80°C until further analysis. The plasma samples (30 μL) were transferred to a 96-well plate, and mixed with 90 μL of acetonitrile, containing the internal standards alprenolol (20 ng/mL), caffeine (100 ng/mL), and acetaminophen-$^{13}C_2$, ^{15}N (20 ng/mL). All samples were centrifuged at approximately 4000*g* for 5 min at 4°C. The supernatants (10 μL) were mixed with 90 μL of 0.01 mol/L ammonium formate (pH 3.0) for acetaminophen, carbamazepine, theophylline, and tolbutamide or 0.01 mol/L ammonium acetate (pH 7.0) for the other drugs. An aliquot of each solution was injected into a liquid chromatography–tandem mass spectrometry (LC–MS/MS) column.

Quantification

The LC–MS/MS was performed using a Shimadzu series 20AD-VP LC system (Shimadzu, Kyoto, Japan) equipped with binary pumps, a degasser, and a SIL-HTc autosampler (Shimadzu).

For acetaminophen, samples were subjected to LC–MS/MS using the Capcell Pak C18 AQ analytical column [3.0 mm internal diameter (i.d.) × 50 mm, 3.0 μm; Shiseido, Tokyo, Japan]. The mobile phase, consisting of 0.2% (v/v) formic acid in 0.01 mol/L ammonium formate (pH 3.0; solvent A) and acetonitrile (solvent B), was delivered at a flow rate of 0.7 mL/min. The initial mobile phase composition (60% A and 40% B (v/v)) was maintained for the first 0.8 min. Following this, the percentage of B was increased to 95%. This concentration was held for 1.2 min. The amount of solvent B was again lowered to 10% (within 0.01 min), followed by re-equilibration for 1.5 min. The total cycle time for each injection was 3.5 min.

The separation of the analytes in all samples excluding acetaminophen was accomplished on a Shim-pack XR-ODS column (2.0 mm i.d. × 30 mm, 2.2 μm; Shimadzu). For the analysis of the carbamazepine and tolbutamide, solvent A and B were delivered at a flow rate of 0.7 mL/min.

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