Quantification of in vivo gastric fluid volume in Bama miniature pigs in fasted state

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Abstract:	Although the study of bioequivalence waivers in humans is already well-established, their application and translation into animals, which are complicated by differences in physiology, have only recently become subjects of interest. The main purpose of this paper is to quantify the liquid volume affecting drug dissolution in pig stomachs. We used magnetic resonance imaging (MRI) to scan 18 Bama miniature pigs weighing 15, 30 or 50 kg. Amira 6.0.1 software was used for 3D image processing. We found that the gastric fluid volume had a linear relationship with the weight of pig (R2 = 0.9935) over this weight range. The pig weight therefore could be used as a surrogate for the fasted gastric fluid volume. After combining data of gastric fluid secretion and drinking water volumes, our results could be used as a reference for the evaluation of oral drug absorption in pigs.

Quantification of *in vivo* gastric fluid volume in Bama miniature pigs in fasted state

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ABSTRACT

Although the study of bioequivalence waivers in humans is already well-established, their application and translation into animals, which are complicated by differences in physiology, have only recently become subjects of interest. The main purpose of this paper is to quantify the liquid volume affecting drug dissolution in pig stomachs. We used magnetic resonance imaging (MRI) to scan 18 Bama miniature pigs weighing 15, 30 or 50 kg. Amira 6.0.1 software was used for 3D image processing. We found that the gastric fluid volume had a linear relationship with the weight of pig (R^2 = 0.9935) over this weight range. The pig weight therefore could be used as a surrogate for the fasted gastric fluid volume. After combining data of gastric fluid secretion and drinking water volumes, our results could be used as a reference for the evaluation of oral drug absorption in pigs.

KEYWORDS:

Pig; Drug absorption; Magnetic resonance imaging; Stomach fluid.

INTRODUCTION

In vivo bioequivalence waivers are currently available for many human drugs. The World Health Organization (WHO), the US Food and Drug Administration (FDA), the European Medicines Agency (EMA) and the China Food and Drug Administration (CFDA) have all published detailed biowaiver guidelines (FDA, 2015; EMEA, 2010; WHO, 2006; CFDA, 2016). Bioequivalent waivers, which rely on the biopharmaceutical classification system (BCS) framework to classify drugs, are selective waivers in which *in vitro* studies are accepted in lieu of *in vivo* studies. The BCS is based on the water solubility and intestinal permeability of the active pharmaceutical ingredient (API) in oral solid dosage forms. Further BCS subclasses for *in vivo* predictive dissolution have been proposed in particular for BCS II and IV drugs (Tsume, Mudie, Langguth, Amidon, & Amidon, 2014).

However, differences in physiology mean that existing bioequivalence studies in humans cannot be directly extrapolated to animals (Martinez et al., 2004). As drug solubility is strongly influenced by pH, solvent composition, volume, temperature and effective dosage (Martinez & Fahmy, 2012), differences in first-pass metabolism and pH of the gastrointestinal tract may lead to differences in oral bioavailability between humans and animals (Dressman, 1986). Current classifications of human oral drug solubility are based on a volume of 250mL, which is the volume of a standard glass of water ingested upon oral drug administration (FDA, 2015). However, pigs have a larger stomach volume than humans (Karali, 1995), therefore it is necessary to factor in the *in vivo* gastric fluid volume when calculating oral drug absorption in pigs.

Magnetic resonance imaging (MRI) has previously been used to study gastrointestinal absorption of drugs in humans (Mudie *et al*, 2014). In that study, twelve healthy volunteers underwent an upper abdomen scan before and after drinking 240 mL of water. The study showed that a fasted stomach contained 35 ± 7 mL of resting water. Immediately after water drinking, the gastric fluid volume rose to

242 ± 9 mL (mean ± SEM). These data help to reveal the physiological relevance of *in vitro* testing methods and computer-based drug transport analyses. However, this method has not been applied to animals yet, to our knowledge.

The aim of this study is to use MRI to image the abdomens of miniature pigs with different weights, and to establish the relationship between the volume of gastric fluid and the body weight of pigs, so as to aid the prediction of the pharmaceutical performance of oral solid drugs in our future studies.

MATERIALS AND METHODS

Materials and equipment

Image scans were performed using a 1.5 T Siemens Symphony MRI scanner (Siemens Healthcare, Munich, Germany). Sodium pentobarbital was purchased from the Sigma Chemical Co. (St. Louis, MO). An anesthesia agent Sumianxin II was purchased from the Shengda Animal Pharmaceutical Co. (Jilin, China).

Animals

This study was performed using three groups of Bama miniature pigs with six pigs in each group, weighing 15, 30, or 50 kg, respectively. Pigs were purchased from the Shichuang Experimental Animal Center (Beijing, China) and housed using a 12 h light–dark cycle. Pigs were fasted for 18 h and deprived of water for 6 h prior to experiments. Sumianxin II (0.2 mL/kg) was injected intramuscularly at first, then anesthesia was administered by intravenous injection of sodium pentobarbital (0.1 mL/kg) ten minutes later. This procedure has a minimal effect on gastrointestinal secretion. This study was approved by the Qingdao Agricultural University Animal Experiment Committee [license Number: SYXK (SD) 20170005] and the animals were maintained in accordance with Qingdao Agricultural University guidelines for the care and use of laboratory animals.

Experiments

Pigs were placed at a prone position with a two-channel circular polarized abdominal coil wrapped around the abdomen (Figure 1). During the scan, at least one person was present to observe the state of the pigs in case of an emergency. T2-weighted MRI sequences were used to image the abdominal organs. The parameters used to scan the transverse and sagittal planes were a TR of 1100 ms, TE of 122 ms, slice thickness of 4 mm, and FOV of 308×380 mm. The characteristic sequence used to scan the coronal plane was a TR of 4.5 ms, and TE of 2.25 ms. The average scanning time per pig was about 15 min. After a scan

was completed, the pig was gently removed from the scanning room and allowed to wake up. All pigs had stable anesthesia during the scan and reached a sober state within the prescribed time after completion of the trial. No severe complications were observed, and good quality images were obtained from all pigs. After fasting for 18 h, the stomachs were shrunken but liquid could still be identified as bright regions in the images (Figure 2). The Amira 6.0.1 graphics software was used for 3D image processing. The MRI images were manually segmented by two independent investigators skillful in using the software to determine the amount of stomach fluid in each pig. Both investigators were blind to the protocol to prevent biasing of the study results. After interactive segmentation the gastric fluid was volume rendered and measured. The maximum and minimum differences between the two investigators were 12.5, and 3.2 mL. The final volume was an average of the two calculations.

Data analysis

Statistical analysis was carried out using GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA). The data were tested for normality using paired Student's *t*-test.

RESULTS

Continuous cross-sectional scanning images were used for 3D image processing. Measured gastric fluid volumes are given in Table 1. The stomach fluid volumes of pigs in the 15, 30, and 50 kg groups in the fasting state were 32.51 ± 4.19 mL, 78.87 ± 6.26 mL, and 162.20 ± 8.39 mL, respectively, with a p-value < 0.0001 suggesting the results are statistically significant (Figure 3). The gastric fluid volume has a linear relationship with the pig weight using the following equation:

$$y=3.7304x-26.931 \quad (1)$$

where x is the gastric fluid volume and y is the pig weight (R^2=0.9935).

DISCUSSION

As the first major organ in which the drug is absorbed in the body, the amount of liquid in the stomach plays an important role in drug absorption. The gastric fluid volume is important for bioequivalence studies of swine drugs. As far as we know, there is still no gold standard to determine the volume of gastric fluid in pigs. Therefore, our results represent one of the first *in vivo* quantitative measures of the gastric fluid volume of pigs.

Concerning MRI imaging, one procedural difference between pigs and humans is that pigs must undergo anesthesia to prevent vomiting and other reactions during the scanning process. Anesthesia was also performed to prevent inhalation of food, gastric fluid, and other substances into the trachea due to dyspnea or respiratory failure. This causes difficulties in drinking water administration in pigs before MRI scans.

It is noteworthy that the amount of porcine gastric fluid during fasting could be on the conservative side. Pigs undergoing a diet or other external stimuli may have an increased gastric secretion and a higher gastric fluid volume. Hence, the equation (1) provides a relatively conservative estimation. Concerning whether the equation (1) can be extrapolated beyond the 15-50kg range, it is highly likely that when pigs reach higher body weights, the relationship between the body size and gastric fluid volume becomes curvilinear and not linear. This is due to the fact that different tissues do not increase proportionally with maturity, and the increase in weight in adult pigs is associated with body fat.

For pigs, the main form of oral drug administration is soluble powder. Therefore, the effective amount of liquid in pig stomachs is dependent on gastric fluid volume and water intake. Previous studies show that when pigs are in a neutral environment, where free water and standard dry feed are available, they consume about 2100 - 2700 mL drinking water per kg feed (Li et al., 2005; Shaw et al., 2006). Gastric emptying is another problem that cannot be ignored when calculating the effective fluid volume. These

factors need to be considered together with the fasted gastric fluid volume. Beyond its utility in determining oral drug dissolution according to the BCS, the relationship between gastric fluid volume and weight can be applied to other studies, such as the absorption of nutrients in the diet.

In future studies we will analyze the amount of gastric fluid produced and drinking water consumed over the course of a day to more accurately quantify the gastric fluid volume.

CONCLUSION

Our results show that, during fasting, the gastric fluid volume in pigs is linearly related to the body weight. This result can be used for the calculation of oral drugs solubility in pigs.

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AUTHOR CONTRIBUTIONS

HG: concept study, data acquisition, data analysis and interpretation, manuscript drafting; CW: data interpretation, manuscript drafting, drafting; ZL: image scan, image analysis, data interpretation; HG: image analysis; YL, LZ, RH, JZ and CD: contributing to experiment setup, data collection and interpretation; HH: image analysis, manuscript drafting; ZH: study concept, data interpretation, drafting of manuscript, and student supervision.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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Figures and Tables

Figure 1. A pig in a prone position for MRI scan.

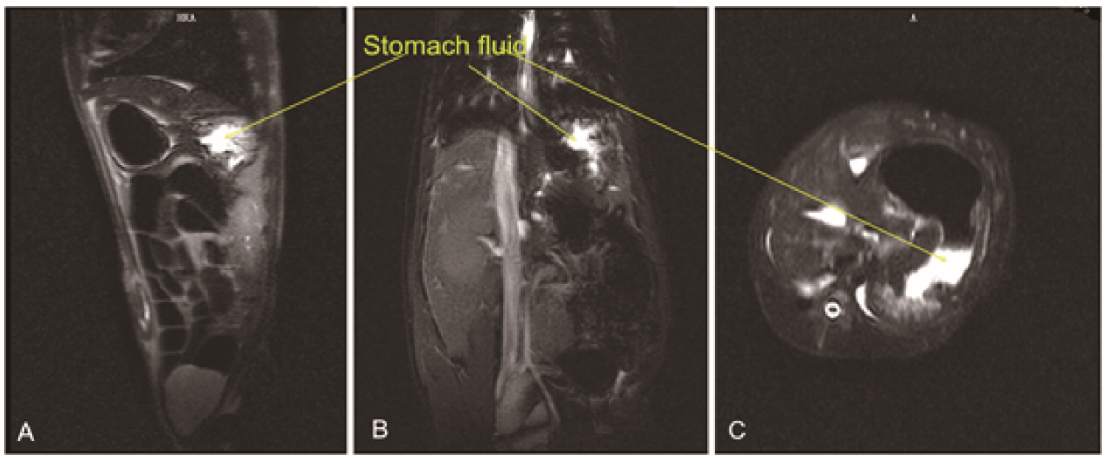

Figure 2. Scanning images of different planes: A: Sagittal, B: Coronal, and C: Transverse. The bright areas indicated by arrows are stomach fluid.

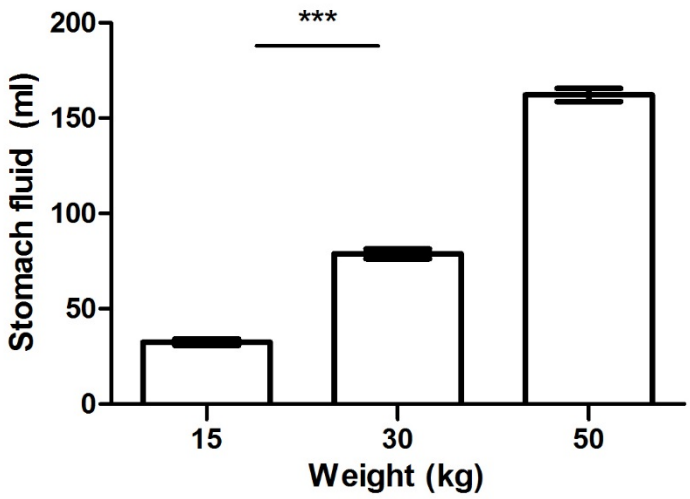

Figure 3. The relationship of weight and stomach fluid volume (P<0.0001).

Table 1. The volume of gastric fluid of 18 BamaMinipigs was measured by MRI

Weight (kg)	Stomach fluid volume (ml)						Mean±sd
	1	2	3	4	5	6	
15	28.76	34.20	31.09	29.22	31.72	40.09	32.51±4.19
30	70.61	78.87	80.23	73.81	81.09	88.63	78.87±6.26
50	159.43	148.76	165.05	172.58	168.34	159.06	162.20±8.39