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Case Report

Gastric Ulcer Model in Mini-Pig

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Abstract

Purpose: Mini-pig has been a great demand for basic and clinical research field due to easy handling, convenient size as well as its analytical and physiological similarity with human. It has been playing a huge role in several clinical research to imitate variety of diseases such as cardiovascular disease, diabetes, metabolic syndrome, bone disorders, skin diseases, and gastrointestinal diseases. It could be the most beneficial animal model for researching gastrointestinal diseases including gastric ulcer.

Materials and Methods: Sus scrofa female mini-pig weighing **around 30kg** was used in this experiment. After a week of adaptation, animals were fasted for 48h with free access to the drinking water with subsequent endoscopic intra-gastric acetic acid injection. Mini-pigs were divided into 2 groups with 2 mini-pigs in each **group**. The process of gastric ulcer development was analyzed at the time of injection, on the 7th and **on the14th day through** endoscopy. Autopsy was done **on the 14th day after drug injection followed** by stomach tissue analysis.

Results: Gastric ulcer was induced very **efficiently in mini-pig animal with clearly visible signs of** necrosis, inflammation and mineralization. The healing properties of famotidine **were** confirmed as disease **symptoms were** reduced significantly.

Conclusion: We confirmed that mini-pig can be efficient and convenient species for gastric ulcer inducement with significant resemblance to human beings, and highly applicable to further effective preclinical experiments.

Keywords: Animal Model; Endoscopy; Gastric Ulcer; Mini-Pig

Introduction

Development of drug and thorough investigation of its properties requires in vitro and in vivo experiments to be conducted in animal model before clinical test. Rodent animal models are the most frequently used as a well-established example. Despite their beneficial features, they **are not** close to human beings anatomically as well as physiologically. Thus, non-rodent animal models such as non-human primates, canines, pigs are appeared to be in demand for further evaluation of pre-clinical models. Among them, numerous laboratories are coming up to use mini-pig model as a medium and large animal, that has biochemical, anatomical and physiological resemblance to humans [1]. Although the type of epithelium is located differently, the gastric secretory function [2], glandular portions are analogous in both human and pig organism [3].

Mini-pig was developed specifically for research and has been used widely in medical field since **1980s. It usually weights 30 to 70 kg and reaches maturity earlier which prevents extensive** time consumption [4-6]. It has been playing a huge role in several clinical research studies to imitate variety of diseases such as cardiovascular disease, diabetes, eye surgery [7,8] metabolic syndrome, bone disorders [9], skin diseases, and intestinal inflammation [10-12]. **Due to their anatomical and functional structure similarities with humans, they can be very useful animal models in terms of research of gastrointestinal tract**. In the present study, we aimed to establish a useful gastric ulcer mini-pig animal model to provide further studies with efficient method of researching pathogenesis of this disease and to test potential drugs.

Materials and Methods

Animals

Sus scrofa female mini-pig, weighing **around 30 kg** was used in this experiment. The animals were fed with standard pig feed, water and kept under standard conditions: with 200-300 lux light**, t=22±3**℃ and 50±10% humidity. Breeding, maintaining and **other procedures** were conducted according to the guidelines of Medikinetics Co (Pyeongtaek, Korea) in a specific-pathogen-free room.

Chemicals

Acetic acid was used for induction of gastric ulcer. 1 ml of 40% acetic acid was injected directly into stomach by endoscope. Famotidine 20mg (Famoten Edon pharma, Seoul, Koera) was used as a positive drug for gastric ulcer.

Experimental Design

Mini-pigs were acclimatized for 1 week to adapt the laboratory conditions. They were fasted for 48hr with drinking water ad-libitum prior to the experiment. 1ml of 40% acetic acid was administered directly into the antral submucosa of stomach by endoscope. Total 4 pigs **underwent the disease inducement and they were** divided into 2 groups (G1 is vehicle control and G2 positive drug control with Famoten Tab treatment). Group 1 was used as vehicle group and was administered with 40% acetic acid (antral submucosa) at a dose of 1 ml using endoscope while group 2 **was undergoing** further treatment with Famoten Tab (20mg) given to mini-pig by mixing with food daily during whole period starting from the day of drug injection. Endoscopy was used for injection of acetic acid as well as for examination of the process of gastric ulcer development on the inner layer of a whole stomach right after drug induction, **on the 7th and on the 14th day** after imitation of procedure (Figure 1).

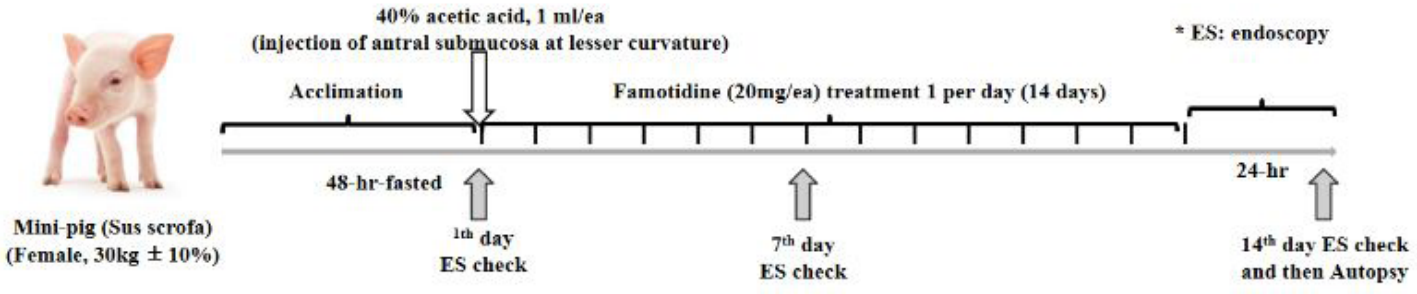


Figure 1: Experimental design and timeline. Total four mini-pigs were used in this experiment by categorizing two pigs per group. 1ml of 40% acetic acid was administered directly into the submucosa layer of gastric antrum by endoscope to induce gastric ulcer. Group 1 was used **as vehicle control group, while group 2 was used as a positive drug control group** using Famoten Tab (20mg). **Following** endoscopy was performed to observe the development of gastric ulcer immediately after injection, **on the 7th day and on the 14th day** after procedure.

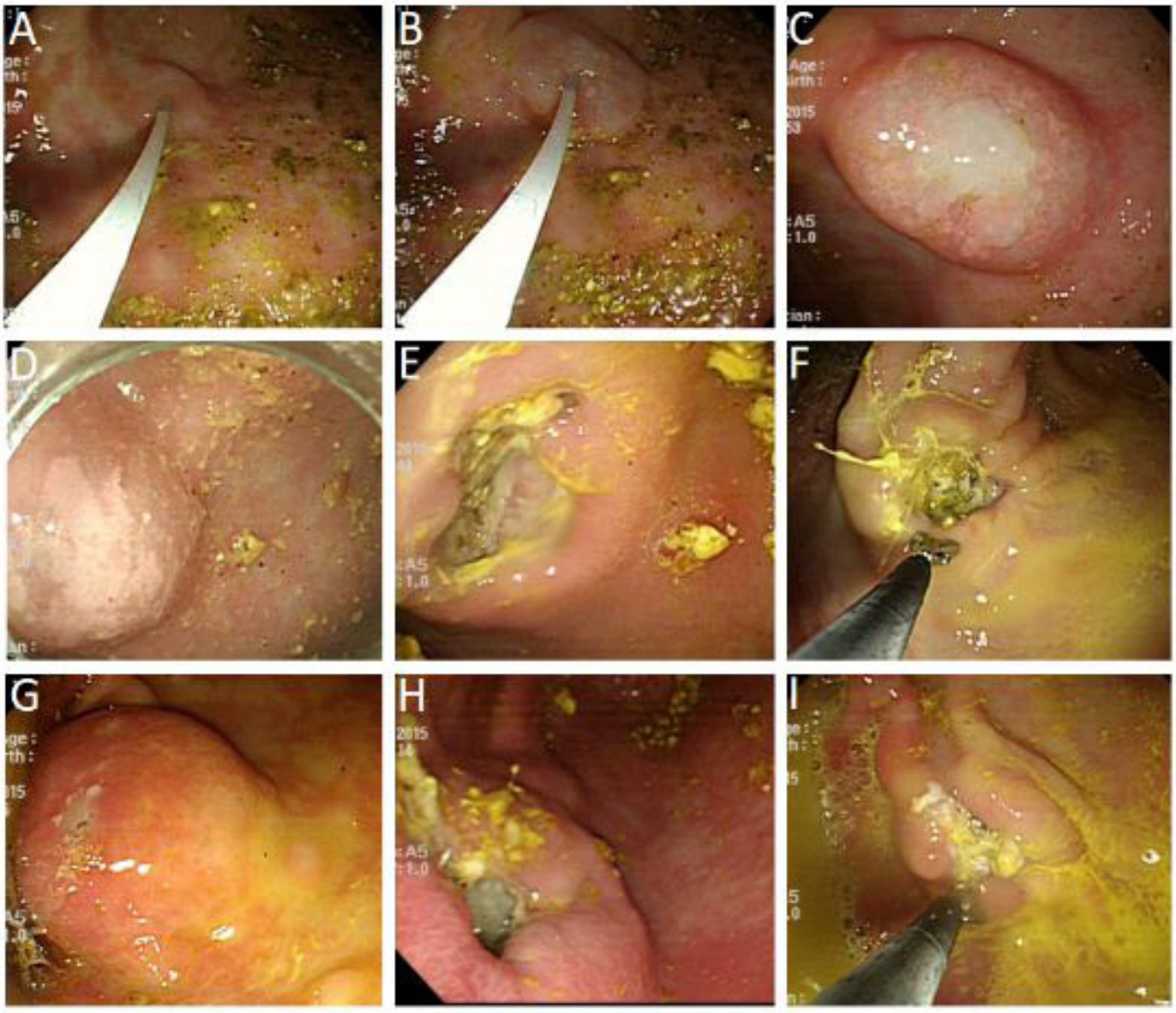
Histopathologic Analysis

Animals were sacrificed, and samples were collected **on the 14th day immediately after** endoscopic observation. The stomach tissue samples were fixed in 10% neutral buffered formaldehyde, embedded in paraffin, cut into 4~5μm thick sections and mounted on glass slides. The sections were stained with hematoxylin and eosin (H&E) to observe **signs of** necrosis, inflammation and mineralization under light microscope. The stomach images were obtained **by exploiting microscope program**.

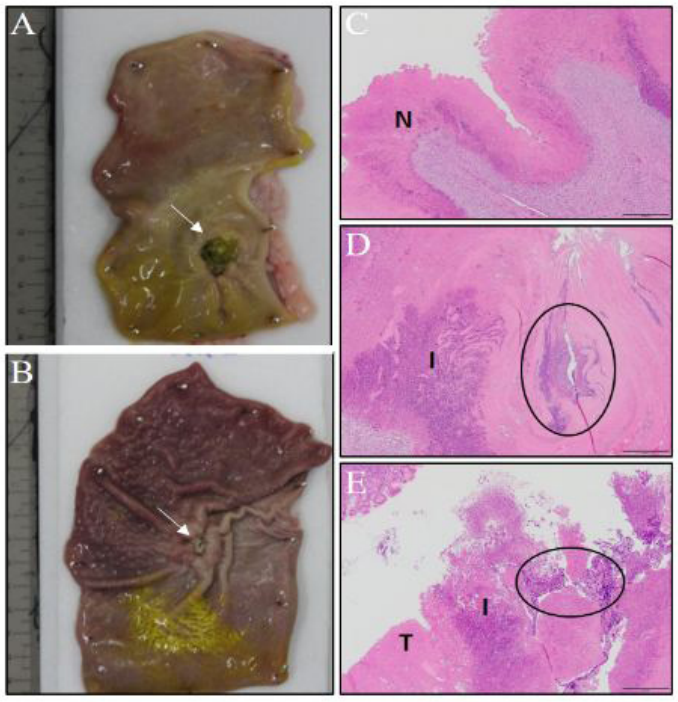
Results

Progress of Gastric Ulcer in Mini-Pig

After 1-week acclimation period, mini-pig underwent fasting for 48 hours. Drug injection and **evaluation of the** disease progress were **handled through the** endoscope, **which provided clear picture and easy access**. 1 ml of 40% acetic acid was injected into the gastric submucosal layer of mini-pigs through the endoscope. The epithelium immediately developed mucosal swelling and whitish discoloration (Figures 2A-2D). On the 7th day after administration the clear gastric ulcer was detected through endoscopy (Figure 2E), which kept developing on the 14th day in G1 group (Figure 2F). Similarly, when the inducer was administered into the G2 group, instant swelling was observed right after injection (Figure 2G) but on the7th day after it, gastric ulcer had a smaller size compared to G1 (Figure 3H). Moreover, on the 14th day, its size decreased and seemed to **be even** smaller than on the 7th day (Figure 3I).



Figures 2(A-I): Endoscopic image of drug injection and gastric ulcer development. Endoscopic image at the initiation of drug injection (A), progress(B) and right after (C). Right after injection of 40% acetic acid into the antral submucosa of stomach (D, G), **on the 7th day** (E, H) and **on the14th day** (F, I) in group 1 and 2, respectively.



Figures 3(A-E): Images of dissected stomach tissue and H&E staining in gastric ulcer mini-pig's model. Autopsy was done, and stomach tissues were collected on the 14th day right after endoscopic observation in group 1(A) and 2(B). Tissue samples were fixed in 10% neutral buffered formaldehyde, embedded in paraffin, cut into 4~5μm thick sections and stained with hematoxylin and eosin (H&E) **with subsequent observation of necrosis**, inflammation and mineralization under light microscope (original magnification, x40) in group 1 (C, D) and group 2 (E). N: severe necrosis, I: inflammation, T: mild necrosis, black circle: mineralization.

Macroscopic and Microscopic Analysis of Gastric Ulcer in Mini-Pig After Autopsy

On the 14th day after induction of **disease, mini-pigs** were sacrificed. Stomach was dissected and pictured for macroscopic evaluation. Gastric ulcer was clearly detectable in G1 group (Figure 3A). On the other hand, only small part of tissue compared to G1 group can be visible as a disease area in G2 group (Figure 3B). For microscopic evaluation, histopathologic analysis was performed. The H&E staining of dissected stomach samples of disease area demonstrated necrosis and inflammation with mucosal mineralization in both G1 and G2 group (Figures 3C-3E). However, G1 exhibited higher severity of pathological process compared to G2, that was determined to develop moderate level of gastric ulcer.

Discussion

Over the past few decades, essential knowledge about gastric mucosal defensive and aggressive factors in stomach digestive system was obtained. Continuously ongoing studies being performed throughout the world have been giving human being a chance to develop better medicine for future. One of the purposes of those studies is to reduce the gastrointestinal injury and improve the quality of ulcer healing. Even though, there are numerous studies focused on stomach protective therapies, their clinical potency remains unclear. Thus, gastric ulcer, which formation depends on several factors, needs to be investigated deeply by establishing useful gastric ulcer animal model.

Variety of animals had been exploited as a gastric disease model. However, each species possesses significant drawbacks, thereby limiting their practical application. The most commonly used animal model is rodent because of their small size, low cost, easy handling and fast reproduction rate. Majority of laboratory reagents admit rodents as most popular species for biomedical research [13]. Even though rodent and human share many common features they possess critical differences as well. Thus, rodents tend to easily obese from excess food and minimal exercise, which in turn affects their physiology and drug metabolism [14], also significant elevations in stress-related responses for experimental procedures cause alterations in obtained results [15]. Moreover, rodent stomach when compared to the anatomical equivalent of the human fundus, is lined by squamous rather than glandular epithelium. On the other hand, dogs are particularly beneficial model for the study of gastric ulcer for several reasons. They are easily accessible and have prominent status in the different cultures. However, dogs are limited in use due to **high** sensitivity and ethical issues as they are considered as family pet and companion [16]. Cats appeared to barely reach 20% disease incidence [17] while gastric inducement in hamsters led to high mortality [18].

As various species had been tried for gastric damage implementation, likewise, numerous trigger agents has been tested to find out the most efficient way of establishing gastric disease model. Among them physiological, pharmacological and surgical methods were interfered in practice: cold-water-restraint stress [19,20], **non-steroidal** anti-inflammatory drugs (NSAIDs); [21,22], ethanol [23,24], serotonin [25], reserpine [26] acetic acid [27,28], histamine-induced gastric ulcers [29], liver transplantation [30], vagotomy [31] methylene blue-induced ulcers, ischemia-reperfusion- (I-R-) induced gastric ulcers and etc. [32].

Initially, gastric ulcer model was made by using NSAIDs medicine such as indomethacin, or by using H. pylori infection in a variety of species including rats, mice [33], gerbils [34] cats [17], dogs [16] etc. Notoriously known side effect of NSAIDs **is a** gastrointestinal damage [35], which is developed due to inhibition of prostaglandin and prostaglandin protects GI tract from topical irritants accumulation in cells ion **trapping [36]**. Additionally, processes like leukocyte adherence to the vascular endothelium, microcirculatory disturbances, increase of circulating neutrophils

and superoxide radical protease release are known to be involved [37].

During experimental trials it has been clarified that acetic acid is a reliable agent [38] for gastric disease implementation. Moreover, direct injection into stomach mucosa prolongs the period of healing, while gastric damage induced through oral injection tends to recover quickly without scar formation [32]. **Similarly,** in the current experiment direct injection was playing role of a trigger agent to induce inflammation in mini-pig's stomach.

Animal models that are being used to study digestive diseases are important for understanding of pathogenesis of these disorders and testing new therapies. Thus, they should resemble with human in terms of the feasibility of anatomy, physiology as well as disease symptom itself. Nowadays increasing interests in pig as an animal model confirms the valuability of this matter despite its limitations due to its excessive weight compared to human [39]. Therefore, mini-pig is getting more attention as a preclinical model for medical research. In the present study, we exploited Sus scrofa mini-pigs, that were administered with 1 ml of 40% acetic acid through endoscopic intra-gastric injection with subsequent endoscopic evaluation at three time points (right after administration, **on the7th day and on the 14th day**). Our results demonstrated an instant swelling of mucosa right after acetic acid injection, that continuously developed into gastric ulcer throughout the whole period of experiment. The advantage of current experiment is an opportunity to estimate the disease process while its formation, through endoscopy by observing at a **certain period, whereas** in rodent model it only can be accessible after sacrifice. Additionally, severity of the inflammatory process can be regulated by the concentration of administered drug in a dose dependent manner.

Conclusion

We have confirmed that mini-pig can be efficient and convenient species for gastric ulcer inducement with significant resemblance to human being, and highly observable and applicable opportunities in term of disease progress and efficacy test of candidate drug. Future study warrants to be essential for further effective preclinical experiments.

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