



# Establishment of an Optimal Aspirin-Induced Gastric Ulcer Mouse Model for the Bio-Medical Industry

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## Abstract

Peptic ulcer disease (PUD) continues to be a source of significant morbidity and mortality worldwide. Approximately two-thirds of patients found to have PUD are asymptomatic. In symptomatic patients, the most common presenting symptom of PUD is epigastric pain, which may be associated with dyspepsia, bloating, abdominal fullness, nausea, or early satiety. Most cases of PUD are associated with *Helicobacter pylori* infection and/or the use of non-steroidal anti-inflammatory drugs (NSAIDs). Among of NSAIDs, aspirin is widely used to reduce pain and inflammation. However, aspirin-induced gastric damage remains the major limitation to its use. Therefore, the optimal establishment of an ideal and suitable gastric ulcer experimental animal model for researching the anti-PUD is very important and need. In this study, we try to seek the optimal induction dosage of aspirin to establish an ideal and suitable gastric ulcer mouse model. In the experiment I, the induction dosage, volume, and frequency of aspirin were 300 mg/kg body weight (BW) in 100  $\mu$ L sterilized water orally once daily for 10 consecutive days. In the experiment II, the induction dosage, volume, and frequency of aspirin were 500 mg/kg BW in 100  $\mu$ L sterilized water orally once daily for 10 consecutive days.

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**Keywords:** Aspirin; Establishment; Gastric ulcer; Mouse model



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Later, 500 mg/kg BW aspirin was administrated again orally once weekly for 3 consecutive weeks. According to the results of the two experiments, the average BW (ABW) of mice between D0-D9 aspirin induction is not significantly increase compared to the beginning in the experiment I. Later, the ABW of mice was continuously increase until the end of the experiment I. The various size of the superficial ulcer lesions with the needle-shaped bleeding points appeared on the gastric mucosa. Most of the GU lesions appeared randomly and sporadically in the gastric gland. The GU lesion area was calculated by ImageJ software as  $2.79 \pm 1.82 \text{ mm}^2$  (D17),  $0.84 \pm 0.34 \text{ mm}^2$  (D24),  $5.49 \pm 2.92 \text{ mm}^2$  (D38); ABW of mice between D0-D9 aspirin induction is not significantly increase compared to the beginning in the experiment II. Later, the ABW of mice was continuously increase until the end of the experiment II. The various size of the superficial ulcer lesions with the needle-shaped bleeding points appeared on the gastric mucosa. Most of the GU lesions appeared randomly and sporadically in the gastric gland. The GU lesion areas were calculated as  $122.90 \pm 51.26 \text{ mm}^2$  (D25) and  $61.55 \pm 18.36 \text{ mm}^2$  (D39). Based on the results of the GU lesion areas in two experiments. The optimal induction conditions of aspirin-induced GU mouse model are suggested according to the induction conditions of experiment II. We hope this aspirin-induced GU mouse model will be applied to the research of new anti-GU drugs in the future.

## Introduction

Peptic ulcer disease (PUD) usually occurs in the stomach and proximal duodenum of patients. The predominant causes of PUD in the United States are *Helicobacter pylori* infection and use of non-steroidal anti-inflammatory drugs (NSAIDs). Gastric ulceration is a benign lesion on the mucosal epithelium upon exposure of the stomach to excess acid and overactive pepsin activity [1-3]. However, *H. pylori* infection considered to be a cause of gastric cancer now. *H. pylori* infection is also strongly associated with gastric and duodenal ulcer disease [4,5]. The discovery of these relations has brought the long-controversial connection between peptic ulcers and gastric cancer into focus [4,5].

Clinical symptoms of PUD present epigastric discomfort, loss of appetite, and weight loss. Especially, pain relieved by food intake or antacids and pain that causes awakening at night or that occurs between meals are the predominant clinical symptoms. In the United States, approximately 500,000 persons with PUD each year. 70% PUD patients occurs between 25- to 64-year old. The annual direct and indirect health care costs of PUD are estimated at about \$10 billion. However, the incidence of peptic ulcers is declining, possibly as a result of the increasing use of proton pump inhibitors and decreasing rates of *H. pylori* infection [6]. According to the report, the prevalence of PUD is 9.4% (54 cases/527 total cases) in 2008 in Taiwan. The PUD cases (n = 54) consisted of 27 gastric ulcer (4.7%), 22 duodenal ulcer (3.9%) and 5 had both gastric ulcer and duodenal ulcer (0.9%). The independent risk factors of the patients with PUD are low education level, a high BMI, and smoker [7].

Aspirin is widely used oral analgesic drug and has profound anti-inflammatory effect. Because the association of gastric

damage with the use of aspirin remains the major limitation for use [8]. Thus, the establishment of an optimal aspirin-induced gastric ulcer (GU) mouse model for the research and development (R&D) of anti-ulcer drugs is very important in this study. We hope this aspirin-induced GU mouse model will be applied to R&D of new anti-GU drugs and therapeutic strategies as the combination therapy with aspirin and an analgesic agent possessing the anti-ulcer activity may be applicable to alleviate pain and inflammation effectively without major gastrointestinal side effects in the future.

## Materials and Methods

### Drugs and chemical preparation

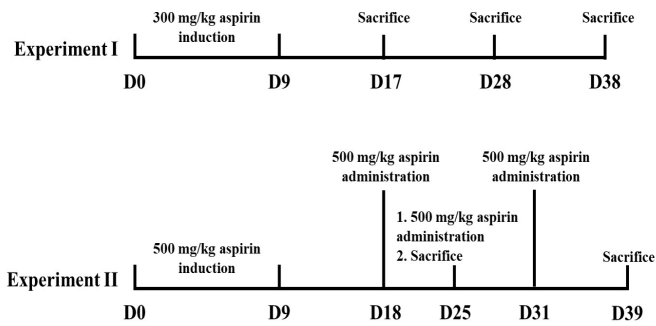
Acetylsalicylic acid ( $\text{C}_9\text{H}_8\text{O}_4$ ; Cat. No. A5376, Sigma-Aldrich), polyethylene glycol 400 (PEG; Cat. No. 8.07485; Sigma-Aldrich), Zoletil 50 (50 mg/mL; Cat. No. 5TK3, Virbac Laboratories) were used in this study. Prepare of aspirin stock was performed as 1 g aspirin was mixed and dissolved in the sterilized water (5 mL) and 5 mL PEG mixed liquid.

### Animal care and grouping

All animal experiments were complied with the ARRIVE guidelines and carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). Animal care in this study were also carried out according to previously described the guidelines of the Institutional Animal Care and Utilization Committee (IACUC) of Agricultural Technology Research Institute (ATRI), Hsinchu, Taiwan (the approval No.: 108043 and 108070). Eight-week-old male, specific pathogen free ICR mice (total of No. is 38) were obtained from BioLASCO Taiwan Co., Ltd., Taipei, Taiwan. These experimental mice were kept on a 12-h light/dark cycle at 23-25°C and 70-75% humidity in the GLP Animal Laboratories, ATRI, Hsinchu, Taiwan. Normal laboratory diet (Panlab, Barcelona, Spain) and fresh water were supplied to mice continuously ad libitum. In the experiment I, 18 ICR mice (the average body weight was 25 g/mouse) were used and grouped (n = 9/group; aspirin treatment group and normal control group). In the experiment II, 20 ICR mice (the average body weight was 25 g/mouse) were used and grouped (n = 10/group; aspirin treatment group and normal control group).

### Experimental designs

In this study, we try to seek the optimal induction dosage of aspirin to establish an ideal and suitable gastric ulcer mouse model. In the experiment I, the induction dosage, volume, and frequency of aspirin were 300 mg/kg body weight (BW) in 100  $\mu\text{L}$  sterilized water orally once daily for 10 consecutive days. In the experiment II, the induction dosage, volume, and frequency of aspirin were 500 mg/kg BW in 100  $\mu\text{L}$  sterilized water orally once daily for 10 consecutive days. Later, 500 mg/kg BW aspirin was administrated again orally once weekly for 3 consecutive weeks (Figure 1). In addition, BW of mice was detected per day in two experiments. In the experiment I, 3 mice/group were sacrificed at D17, D28, and D38 after 300 mg/kg aspirin induction; in the experiment II, 5 mice/group were sacrificed at D25 and D39 after 500 mg/kg aspirin induction (Figure 1).



**Figure 1:** Experimental designs for the establishment of aspirin-induced gastric ulcer mouse model.

**Collection of stomach and quantification of ulceration**

At the experimental design points, the collection of stomach from each sacrificed mouse was performed. The area of the gastric ulcers were scored using ImageJ software based on grading on a level 1-3 (depicting area of hemorrhagic erosions) as presented in Table 1. Scores of mucosal damage were detected by the ulcer surface area of the glandular stomach estimated in square millimeters. Mean ulcer score for each group was expressed as ulcer index (UI). The calculation equation of UI followed:

$$UI = (1 \times \text{the mouse's amount in each group belonged to level I}) + (2 \times \text{the mouse's amount in each group belonged to level II}) + (3 \times \text{the mouse's amount in each group belonged to level III}) / \text{the number of mice in each group.}$$

**Table 1:** Gastric ulcer level and score.

Level	Ulcer area	Score
I	< 1 mm <sup>2</sup>	1
II	1-3 mm <sup>2</sup>	2
III	> 3 mm <sup>2</sup>	3

**Statistical Analysis**

The GU lesion area was calculated by ImageJ software (<https://imagej.nih.gov/ij/>). The results were expressed as mean ± SD. All statistical comparisons were made with one/two-way analysis of variance. Statistical evaluation was performed using Graphpad Prism 6 (Graph Pad Software Inc.). Differences between groups were considered statistically significant at \**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001.

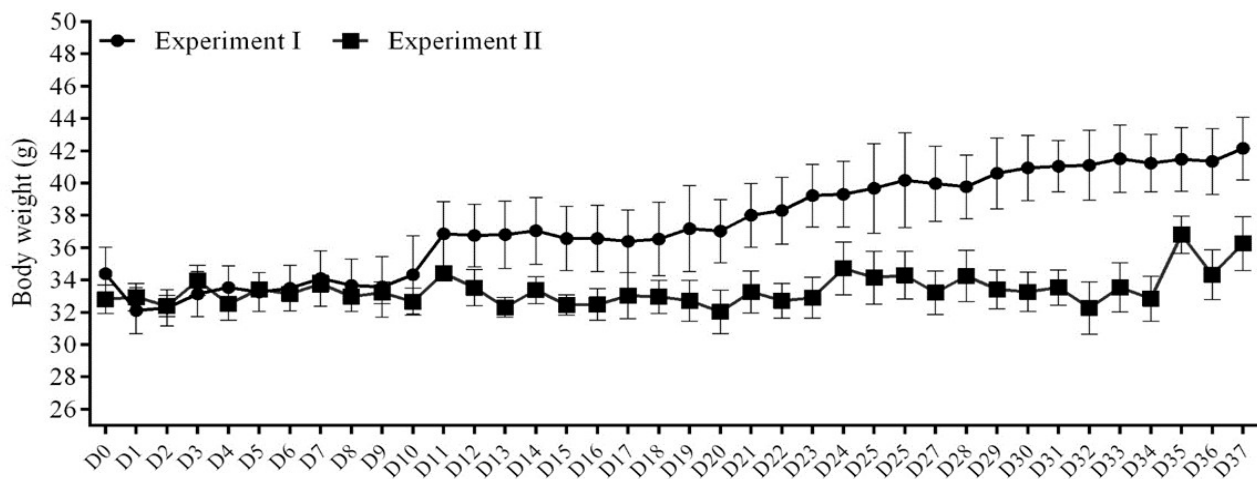
**Results**

**Change of body weight in mice**

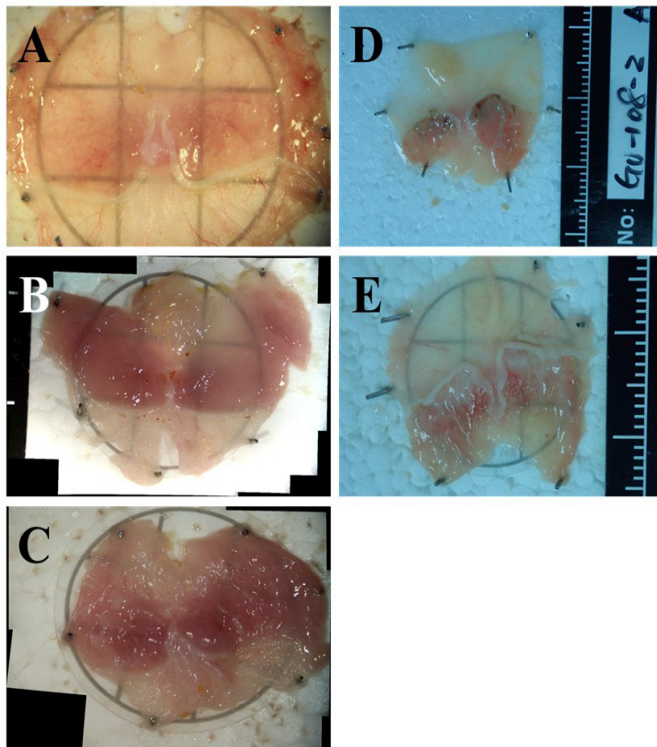
According to the results of the two experiments, the average BW (ABW) of mice between D0-D9 aspirin induction is not significantly increase compared to the beginning in the experiment I. Later, the ABW of mice was continuously increase until the end of the experiment I. On the other experiment, ABW of mice between D0-D9 aspirin induction is not also significantly increase compared to the beginning in the experiment II. Later, the ABW of mice was also continuously increase until the end of the experiment II. Furthermore, ABW in the experiment II was lower than that in the experiment I (Figure 2).

**Gross examination of the superficial ulcer lesions in stomach in mice**

The various size of the superficial ulcer lesions with the needle-shaped bleeding points appeared on the gastric mucosa. Most of the gastric ulcer (GU) lesions appeared randomly and sporadically in the gastric gland (Figure 3).



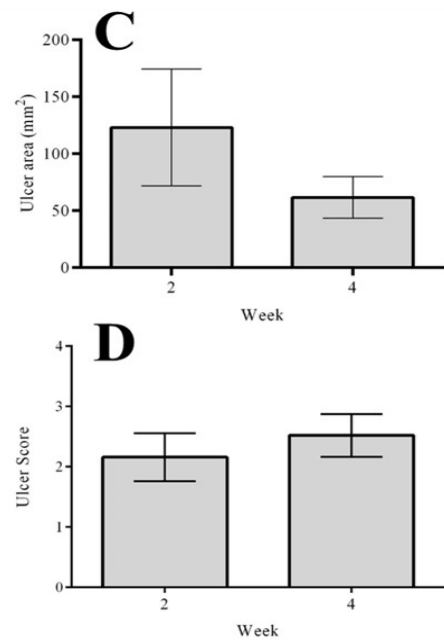
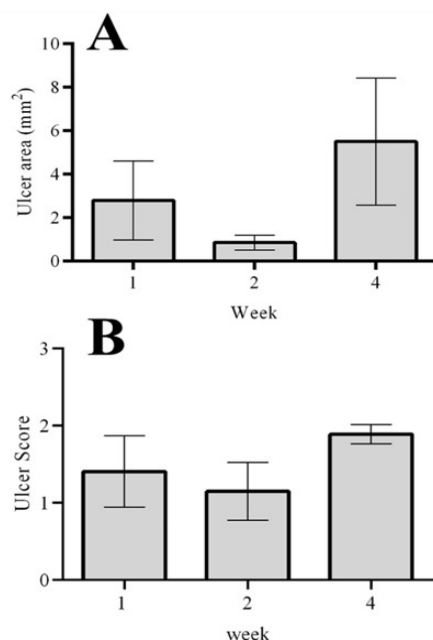
**Figure 2:** Change of mouse's body weight in two experiments in the study. 300 or 500 mg/kg BW aspirin induction at D0-D9 in two experiments. On the other aspirin administration, 500 mg/kg BW aspirin was administrated again orally once weekly at D18, D25, and D31, respectively



**Figure 3:** Gross examination of the superficial ulcer lesions in stomach in mice. (A) D17 in the experiment I. (B) D28 in the experiment I. (C) D38 in the experiment I. (D) D25 in the experiment II. (E) D39 in the experiment II

**The areas of the superficial ulcer lesions in stomach in mice**

The GU lesion area was calculated by ImageJ software as  $2.79 \pm 1.82 \text{ mm}^2$  (D17),  $0.84 \pm 0.34 \text{ mm}^2$  (D24),  $5.49 \pm 2.92 \text{ mm}^2$  (D38). On the other experiment, the various size of the superficial ulcer lesions with the needle-shaped bleeding points appeared on the gastric mucosa. Most of the GU lesions appeared randomly and sporadically in the gastric gland. The GU lesion areas were calculated as  $122.90 \pm 51.26 \text{ mm}^2$  (D25) and  $61.55 \pm 18.36 \text{ mm}^2$  (D39) (Figure 4A and 4C). Additionally, the calculation of the ulcer score was performed according to the Table 1. The highest ulcer score located at the fourth week post aspirin administration (Figure 4B & 4D).



**Figure 4:** Gross examination and calculation of the superficial ulcer lesions in stomach in mice. (A) Ulcer area in the experiment I. (B) Ulcer score in the experiment I. (C) Ulcer area in the experiment II. (D) Ulcer score in the experiment II

**Discussion**

The clinical symptoms of the patients with PUD is variable. Some patients with PUD are asymptomatic until life-threatening complications develop [7,9]. The prevalence of PUD in asymptomatic subjects is 9.4% in Taiwan [7,10]. Commonly, NSAIDs induce gastrointestinal injury, including erosions, ulceration, and hemorrhage. During long-term administration of NSAIDs, 60%-94% and 15%-31% of NSAIDs administrated patients showed mucosal damage and gastric ulcer, respectively [7,11,12]. In this study, the administration time of aspirin (300 or 500 mg/kg BW) was only 10 days. This short-term administration of NSAIDs was 100% successful induction of GU. The areas of GU lesion was dose-dependent. In symptomatic patients with PUD, the most common presenting symptom of PUD is epigastric pain, which may be associated with dyspepsia, bloating, abdominal fullness, nausea, or early satiety. Clinical symptoms of patients with PUD present epigastric discomfort, loss of appetite, and weight loss. Especially, pain relieved by food intake or antacids and pain that causes awakening at night or that occurs between meals are the predominant clinical symptoms. In this study, aspirin-induced mice presented epigastric discomfort, loss of appetite, and weight loss.

In this study, we try to seek the optimal induction dosage of aspirin to establish an ideal and suitable GU mouse model. Two experiments were performed, respectively. In the experiment I, the induction dosage and frequency of aspirin is 300 mg/kg BW once per day for ten days. In the experiment II, the induction dosage of aspirin is changed to 500 mg/kg BW. However, 500 mg/kg BW aspirin was administrated again orally once weekly for 3 consecutive weeks, which was different from that in the experiment I. According to the areas of aspirin-induced GU, the results of the experiment II is larger than those in the experiment I. The optimal induction conditions of aspirin-induced GU mouse model were suggested according to the induction conditions of experiment II. Taken these results together, the ABW of mice between D0-D9 aspirin induction was not significantly



increase compared to the beginning in the experiment I and II. The various size of the superficial ulcer lesions with the needle-shaped bleeding points appeared on the gastric mucosa in the experiment I and II. Most of the GU lesions appeared randomly and sporadically in the gastric gland in the experiment I and II. The GU lesion area in the experiment II was larger than that in the experiment I. Based on the results of the clinical symptoms, ABW, and GU lesion areas in two experiments, the optimal induction conditions of aspirin-induced GU mouse model are suggested according to the induction conditions of experiment II. In this study, we have successfully established an aspirin-induced GU mouse model. We hope this model will be applied to the research of new anti-GU drugs and therapeutic strategies in the future.

### Conclusion

In two experiments, the change of ABW in the aspirin treatment group was not found increase compare to the beginning in two experiments. Additionally, based on the results of the GU lesion areas in two experiments, the optimal induction conditions of aspirin-induced GU mouse model are suggested according to the induction conditions of experiment II. We hope this aspirin-induced GU mouse model will be applied to the research of new anti-GU drugs in the future.

### Acknowledgements

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