Lyophilized Aspirin with Trehalose May Decrease the Incidence of Gastric Injuries in Healthy Dogs

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ABSTRACT.

Trehalose has several novel anti-inflammatory and cell-protective functions. We hypothesized that lyophilized aspirin/trehalose could decrease the severity of aspirin-induced gastropathy. Thirteen dogs were assigned into aspirin, lyophilized aspirin/trehalose, and control groups, and the gastric lesions were assessed on gastroscopy with the modified Lanza scale. Another 6 dogs were used to measure the plasma aspirin concentration by high-performance liquid chromatography after aspirin or lyophilized aspirin/trehalose administration. The results indicated that lyophilized aspirin/trehalose induced less gastric ulceration than aspirin despite maintaining therapeutic concentrations of plasma aspirin in both the groups. Lyophilized aspirin/trehalose might be a solution to decrease aspirin-induced gastropathy.

KEY WORDS: aspirin, gastric ulcer, gastroscopy, trehalose.


Dogs have been suggested to be more susceptible to nonsteroidal anti-inflammatory drug (NSAID)-induced gastrointestinal toxicoses than humans. However, gastric ulcer formation resulting from NSAID administration in dogs is still controversial [23, 24, 28]. Aspirin was produced in 1897 as the first NSAID and is still widely used as an anti-inflammatory and anticoagulant drug. In 1971, inhibition of the cyclooxygenase (COX) enzyme and subsequent reduction of prostaglandin synthesis were first proposed to be the major mechanism of action of aspirin [34]. Recent studies found that both COX isoforms (COX-1 and COX-2) contribute to mucosal defense. Furthermore, there is increasing evidence that COX-independent effects, such as apoptosis, detergent-like properties, and “ion trapping,” might also be important for aspirin gastropathy; however, the contribution of these effects is still controversial [16].

Numerous strategies have been undertaken to decrease the incidence of gastropathy, including co-administration of protective substances such as sucralfate, histamine/H2-receptor antagonists, proton-pump inhibitors, and prostaglandin analogues [16]. Investigators have also tried to develop safer NSAIDs, such as selective cyclooxygenase-2 (COX-2) inhibitors [35], nitric oxide-releasing NSAIDs [38], and hydrogen sulfide-releasing NSAIDs [37].

Trehalose is a natural disaccharide and is present in a wide variety of organisms. It is widely used as a food ingredient and in the cosmetics industry [10, 41]. Recently, it has undergone several clinical trials on organ preservation during transplantation [4], inhibition of xenosis during dental treatment [22], therapy of dry eye syndrome [19], and adhesion prevention after abdominal surgery [12]. Recent studies found several novel anti-inflammatory and protective functions of trehalose. Moreover, trehalose is an important protectant of protein integrity [3] and reduces oxidative damage to cells [1]. It may also inhibit inflammatory cyto kinase production [20, 29] and suppress apoptosis [6].

When trehalose is co-lyophilized with a model compound, it would make a specific interaction mediated by hydrogen bonding with each other [31]. In our preliminary study in a rodent model, it was revealed that lyophilized aspirin/trehalose caused less gastric ulceration than aspirin alone without losing anti-inflammatory effects. In addition, this effect was not seen by using the mixture of aspirin/trehalose in this rat model. This result may be related to the structural change of aspirin by lyophilization with trehalose (data not shown).

In this study, we investigated the levels of gastric injury caused by aspirin and lyophilized aspirin/trehalose in a canine model as a preliminary study. This study was performed in accordance with the guidelines of the Committee for Animal Care, Graduate School of Agricultural and Life Sciences, the University of Tokyo. Thirteen healthy beagles (7 males and 6 females) were randomly assigned to each of 3 treatment groups: 3 dogs in
the control group, 5 dogs in the aspirin group, and 5 dogs in the lyophilized aspirin/trehalose group. The dogs received oral administration of the following drugs every 12 h before feeding for 28 consecutive days: placebo (capsules only) in the control group, 25 mg/kg aspirin in the aspirin group, and 125 mg/kg (25 mg/kg aspirin) lyophilized aspirin/trehalose (Next 21 K.K., Tokyo, Japan) in the lyophilized aspirin/trehalose group. Commercial dry food for adult dogs (Hill’sTM, Topeka, KS, U.S.A.) was given twice daily and water was available ad libitum throughout the study. Clinical signs including appetite, activity, vomiting, and diarrhea were observed daily and recorded. One dog-day of vomiting indicated that 1 dog was vomiting on a particular day, and the number of dogs displaying diarrhea or anorexia was similarly recorded.

Gastroscopy was conducted under isoflurane anesthesia, and lesion scores were recorded 7 days before drug administration (day −7) and 5, 14, and 28 days after initiation of drug administration. Under gastroscopy, 4 regions of the stomach, pyloric antrum, incisura angularis, cardia, and fundus, were photographed using an endoscopic camera (Olympus CL-VU40D/VO-2A/VQ-8143A, Tokyo, Japan). An experienced observer blinded to the treatment group scored the gastric lesions according to the modified Lanza scale (Table 1) [26, 27, 39]. Each gastric region was assessed and scored on a scale of 1–11, and the sum of the 4 regions was defined as the total lesion score. A complete blood count and serum biochemical analysis were also performed on days −7, 5, 14, and 28.

To compare blood aspirin levels between the aspirin and lyophilized aspirin/trehalose groups, blood samples were collected to measure plasma aspirin concentration by high performance liquid chromatography (HPLC). Six healthy beagles (3 males and 3 females) from 1 to 3 years of age were randomly assigned into 2 groups of 3 dogs each. A single oral dose of 25 mg/kg aspirin and 125 mg/kg (25 mg/kg aspirin) lyophilized aspirin/trehalose was administered. Food was withheld for 12 h before drug administration. Blood was collected from the cephalic vein at 1, 2, 4, 6, 8, 12, 16, 20, and 24 h after the drug administration. To avoid conversion from aspirin to salicylic acid, sample preparation for the measurement was performed within 1 h after venipuncture.

Plasma aspirin levels were determined using reversed-phase HPLC according to the method described by Cham et al. [3] with a slight modification. Briefly, p-hydroxybenzoic acid methyl ester as an internal standard and an aqueous solution of hydrochloric acid and chloroform were added to the sample, which was then centrifuged at 3,000 rpm for 5 min. The lower organic phase was collected and evaporated. HPLC was conducted using a unit (JASCO DG-2080–53/PW-2080/UV-2075, Tokyo, Japan) with a Develosil Ph column (250 × 4 mm i.d., 5 µm particle size; Nomura Chemical Co., Aichi, Japan). The detection wavelength was 270 nm. The mobile phase consisted of methanol and phosphate buffer (1:3 v/v). The flow rate was 1 ml/min. Acetylsalicylic acid (Sigma Co., St. Louis, MO, U.S.A.) was used as standard.

Lesion scores were analyzed using a Kruskal–Wallis rank-sum test. Post hoc pairwise comparisons were made using a multiple comparison test based on Kruskal–Wallis rank sums. Lesion scores for the antrum, incisura angularis, cardia, and fundus within groups at each time point were compared similarly. The Friedman test was used to compare the scores within a treatment group among time periods, and post hoc comparisons were made using a multiple comparison test.

The Mann–Whitney U test was used to compare the number of dog-days of vomiting among groups. The peak plasma aspirin concentration and the area under curve (AUC) in the aspirin and lyophilized aspirin/trehalose groups were also compared. A P value <0.05 was considered statistically significant for all statistical analyses.

The median total lesion scores of the aspirin group rapidly increased after administration to 21 (range 17–25) on day 5 and maintained high on day 14 (median 21, range 17–22). On the contrary, median total lesion scores of the lyophilized aspirin/trehalose group were significantly lower than aspirin group on day 5 (median 6, range 4–15) and on day 14 (median 7, range 6–13) (Fig. 1). Figure 2 shows typical gastroscopic findings in the aspirin and lyophilized aspirin/trehalose groups. At the fundus, multiple erosions were present in the aspirin group, and linear submucosal hemorrhage was present in the lyophilized aspirin/trehalose group.

Figure 3 shows the change in median lesion scores at each anatomical location. Scores of the aspirin group were significantly higher than those of the lyophilized aspirin/trehalose group at the fundus and pyloric antrum during the whole treatment period (days 5, 14, and 28). Significant differences were also noted at the incisura angularis (days 5 and 14) and cardia (days 5 and 28) between the 2 groups. No significant differences were observed between the control and lyophilized aspirin groups at any region during the study (data not shown).

Only 3 dog-days of vomiting were noted in the aspirin group (1 dog accounted for 2 dog-days of vomiting), and the vomiting was self-limiting. No vomiting was noted in the dogs of the lyophilized aspirin/trehalose or control groups. There were no differences among groups in the number of dog-days of vomiting. No diarrhea, anorexia, or abnormal

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>1 Submucosal hemorrhage</td>
</tr>
<tr>
<td>3</td>
<td>2–5 Submucosal</td>
</tr>
<tr>
<td>4</td>
<td>&gt;5 Submucosal</td>
</tr>
<tr>
<td>5</td>
<td>1 Erosion</td>
</tr>
<tr>
<td>6</td>
<td>2–5 Erosions</td>
</tr>
<tr>
<td>7</td>
<td>&gt;5 Erosions</td>
</tr>
<tr>
<td>8</td>
<td>1 Ulcer</td>
</tr>
<tr>
<td>9</td>
<td>2 Ulcers</td>
</tr>
<tr>
<td>10</td>
<td>3 Ulcers</td>
</tr>
<tr>
<td>11</td>
<td>Perforating ulcer</td>
</tr>
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blood chemical profiles were noted in any of the dogs during the experimental period.

The change in plasma aspirin concentration after oral administration of 25 mg/kg aspirin is shown in Fig. 4. Plasma aspirin level increased rapidly after administration and peaked 2 hr after administration. The mean peak plasma aspirin concentration was 55.6 ± 7.3 µg/ml in the aspirin group and 56.3 ± 1.5 µg/ml in the lyophilized aspirin/trehalose group, and there was no significant difference between the 2 groups. The AUC was 506.6 ± 31.8 in the aspirin group and 574.4 ± 87.9 in the lyophilized aspirin/trehalose group, and there was no significant difference between the groups.

In this study, total lesion scores in dogs receiving lyophilized aspirin/trehalose were significantly lower than those in the aspirin group on days 5 and 14. However, on day 28, there was no significant difference between the 2 groups despite the aspirin group attaining a higher lesion score. This indicates that lyophilized aspirin/trehalose may cause less gastric damage in the first 2 weeks of treatment.

Although there were no significant differences in lesion score at each time point within the aspirin group, lesion scores tended to decrease according to the period after administration. One possible explanation for the initial increase followed by a decrease in lesion scores is gastric adaptation to aspirin, in which gastric mucosa becomes more resistant to the cytotoxic effect of aspirin [13]. There have been reports on natural resolution of gastric lesions in dogs [14, 30], while conflicting studies have reported that dogs receiving aspirin treatment for up to 28 days did not show this effect [26, 27, 39].
In this study, we did not include the dogs receiving the mixture of aspirin and trehalose. This was because that our previous study using rats showed the effect of lyophilized aspirin/trehalose and that the mixture of aspirin and trehalose did not decrease the severity of gastropathy. In addition, we would like to reduce the number of experimental dogs in this study.

Duodenal lesions were not evaluated in this study. NSAIDs have been shown to cause the development of duodenal erosions or ulcers in humans and dogs after aspirin administration [2, 11, 15, 39, 40]. In our preliminary experiment in which the duodenum was evaluated, minimal lesions were observed in this region after oral administration of 25 mg/kg aspirin every 12 hr. Furthermore, attempting entry of the endoscope into the duodenum might increase the risk of iatrogenic lesions and prolong experimental procedures. Therefore, we did not evaluate duodenal lesions in this study.

In this study, a predilection site for gastric lesions was not identified, and this observation is consistent with previous studies [2, 15, 27, 39]. However, a predilection site for NSAID-induced injury is controversial. Certain human studies [36], veterinary clinical reports [9, 18], and endoscopic studies in dogs [23] have reported that the pylorus is the region most prone to NSAID-induced gastric mucosal damage. However, there is a report [39] in which the highest lesion scores were obtained in the fundus and the lowest lesion scores were obtained in the pyloric antrum region.

Despite the significant differences in lesions scores, there were no corresponding clinical signs or abnormal laboratory data. No dogs in the aspirin group showed severe gastrointestinal signs. Similar to other studies in which the investigators found gastrointestinal injuries without clinical consequences.

Fig. 3. Lesion scores of 4 regions. *P<0.05, aspirin group compared with lyophilized aspirin/trehalose group.

Fig. 4. Changes in plasma aspirin concentrations after 25 mg/kg aspirin administration. There was no significant difference in the peak plasma aspirin concentration and AUC between the aspirin and lyophilized aspirin/trehalose groups.
in a limited period (28 days), the significance of the lesions for clinical signs is unclear. Although vomiting was noted in certain dogs of the aspirin group in this study, appetite was unaffected and the signs did not correlate to the lesion scores. However, clinical signs might have been produced over a longer treatment period.

A plasma aspirin concentration of about 50 µg/ml is adequate for producing analgesic and antipyretic effects [7, 8]. In this study, the plasma aspirin concentrations in both the aspirin and lyophilized aspirin/trehalose groups exceeded the therapeutic concentration (>50 µg/ml), and there was no significant difference between the groups. This result indicates that lyophilization of aspirin and trehalose does not alter the absorption of aspirin and that comparable analgesic and anti-inflammatory effects are expected.

Aspirin has long been considered to cause gastric injuries through inhibition of COX enzymes; however, studies have increasingly shown that not only COX inhibition but also direct damage to the mucosa are required for the production of gastric lesions. Both in vitro and in vivo models [21, 32, 33], membrane permeabilization activity of drugs may be involved in the production of necrosis and apoptosis of gastric mucosal cells. Thus, molecules that protect the integrity of mucosal cells and have antiapoptotic properties were applied to prevent aspirin-induced gastric injuries [17, 25].

Although lyophilized aspirin/trehalose showed superior protection of gastric mucosa from aspirin in this study, the protective mechanism is still unknown. Previous studies have revealed that trehalose protects protein integrity, reduces oxidative damage to cells, suppresses apoptosis, and inhibits inflammatory cytokine production [1, 6, 20, 29]. Trehalose is a natural disaccharide and is a widely used food ingredient due to low price and minimal side effects. Although a further research to clarify the mechanism is warranted, lyophilized aspirin/trehalose might be a solution to decrease aspirin-induced gastropathy.

REFERENCES