Effects of pre- and postprandial aspirin on gastric bleeding based on clinical data and in vitro study

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Aspirin is a common anti-inflammatory agent. Clinical pharmacists have found an inconsistency in the timing of taking the medicine mentioned in the instructions of different brands of aspirin (100mg). To compare the effects of pre- and postprandial aspirin on gastric bleeding and assess the potential risk factors of aspirin caused gastric bleeding, Data from 100 patients taking enteric-coated aspirin tablets in the Second Hospital of Dalian Medical University were retrospectively analyzed. They included demography, medical history, brand of aspirin, time of taking medicine (pre-prandial or postprandial), gastric bleeding situation. The dissolution rates of aspirin were conducted in different pH media (1.0-6.8) by in vitro study. Clinical data showed that enteric-coated aspirin tablets had no significant changes in the incidence of gastric bleeding before or after meals (10%). The in Vitro dissolution text suggested that enteric-coated aspirin tablets had no obvious release at pH 1.0-5.0 for 2h; however, at pH >6.8, about 80% of the drug was released within 45min. The results suggest that patients with an upset stomach should be advised to take aspirin before meals to prevent aspirin release in advance.

Key Words: Aspirin; Gastric bleeding; Drug release; Medication time; Adverse

INTRODUCTION

Aspirin, discovered for more than 100 years, is an analgesic, antipyretic, and anti-inflammatory agent. It can also reduce the risk of coronary artery and cerebrovascular diseases. Emerging data suggest that aspirin also reduces the risk of multiple cancers, such as colorectal, prostate, esophageal, laryngeal, and gastric cancers. It is used preferentially for long-term primary and secondary prevention of cardiovascular diseases, that is, myocardial infarction and stroke. It is one of the most widely used medications in developed and developing countries, and more than 100 billion tablets are consumed every year. However, the use of aspirin may induce a wide range of adverse side effects in the upper gastrointestinal (GI) tract, ranging from troublesome symptoms to life-threatening peptic ulcer bleeding, perforation. The adverse effects of aspirin-induced gastric mucosal injury are mediated by both direct and indirect mechanisms. Direct gastric mucosal injury results from the trapping of high concentrations of acidic aspirin within gastric epithelial cells. The indirect effects are mediated primarily via it is a nonselective cyclooxygenase (COX) inhibitor, which both inhibit COX-1 and COX-2. It can suppress the formation of cytotoxic prostaglandins and thromboxane via COX-1 in gastric mucosa and leading to GI bleeding. Modified release formulations, like enteric-coated is a common agent used to prevent GI bleeding. Clinical pharmacists have found an inconsistency in the timing of taking the medicine mentioned in the instructions of different brands of enteric-coated aspirin. Domestic “Original®” (Shenyang, China) aspirin instructions say, “Take the medicine after meals,” while imported “Bayer®” (Leverkusen, Germany) aspirin instructions say, “Take the medicine before meals.” As we knew, Bayer company first coined aspirin as the trade name for acetylsalicylic acid. In many countries, it remains a registered trademark of this company, whereas in other aspirin has become the generic name of this substance. The “Original” enteric-coated aspirin is a generic drug product by

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Original PharmacoLabo Company in China. It was the first domestic generic drug approved by China Food and Drug Administration on May 18, 2009, for primary prevention of myocardial infarction, in addition to the original drug by Bayer, Germany. In view of this inconsistent situation, it was found that the debates were mainly about whether food or drug could affect the release of enteric-coated aspirin tablets in the GI tract in advance, leading to GI bleeding. This study aimed to compare the effects of pre- and postprandial aspirin on gastric bleeding and explore the rational administration time of enteric-coated aspirin tablets through retrospective analysis and in vitro drug dissolution test.

METHODS

Retrospective analysis of clinical cases

Clinical data of 100 patients were collected from September 2016 to November 2016 from the Second Hospital of Dalian Medical University, China. Patient data were obtained from the hospital’s information system and telephonic interviews, which included baseline information, medical history, type of aspirin (“Bayer” or “Original”), time of taking the medicine (before or after meals), and gastric hemorrhage condition. The inclusion criteria were as follows: Patients aged more than 18 years who were taking the same brand of enteric-coated aspirin tablets for more than 3 months before hospitalization, and those with GI tumors, hematological diseases, and vitamin K deficiency. The exclusion criteria were as follows: Patients with a history of any clinically significant bleeding before taking aspirin in recent 12 months; those administered with a gastric mucosal protective agent, proton pump inhibitors (PPIs), and H2 receptor antagonists; and those who did not adhere to the rules of their medication. The hemorrhage criteria included spitting blood, black stool, positive fecal occult blood tests, mucous hyperemia in the stomach and duodenum, edema, punctate and linear erosion, diffuse bleeding, or ulcers. The 100 enrolled patients were categorized into two groups: medication time and brand of aspirin. The first group was further subdivided into before-meal and after-meal groups and the second into Bayer and domestic groups.

In vitro drug dissolution test

Reagents

Standard enteric-coated aspirin tablets were purchased from Zhongke Biotechnology (Beijing, China). Bayer’s enteric-coated aspirin tablets were obtained from Bayer HealthCare Pharmaceuticals (Shanghai, China). Domestic enteric-coated aspirin tablets were purchased from Original Pharmaceutical (Shenyang, China). The high-performance liquid chromatography (HPLC)-grade acetonitrile, tetrahydrofuran, and methanol were obtained from Bo Di Chemical (Tianjin, China).

Apparatus used and HPLC

The HPLC system was equipped with a model 3000 pump, a sample injector (AS230), and an ultraviolet detector (UV230II) set at 276 nm. The column was C18 (250 × 4.6 mm²). All items were obtained from Elite (Dalian, China). The chromatogram data collection and data processing was accomplished using the EC2006 V1.80 software. The ZBS-8L dissolution tester (Tianfa Tianfa, Tianjin, China) and pH5-3C audiometer (Weiye, Shanghai, China) were used for the drug-release test. The C18 column (5 μm) was used for separation. The mobile phase consisted of 20.0% acetonitrile, 70.0% H2O, 5.0% tetrahydrofuran, and 5.0% glacial acetic acid, with a flow rate of 1.0 mL/min. The injection volume was 25-μL. The runtime for all tests was 8 min. The column oven temperature was set at 30°C.

Preparation of standard stock solution

Standard aspirin (25 mg) tablets were weighed, and 1% methanol and glacial acetic acid solution were added to dissolve the drug to obtain a concentration of 25 mg/mL stock solutions. Appropriate stock solutions were diluted with 1% methanol and glacial acetic acid solution to obtain 0, 0.05, 0.15, 0.20, and 0.25 mg/mL of aspirin standard solutions. Five solutions were prepared with the desired concentration range for calibration. The standard aspirin solutions were injected, and the chromatogram was recorded.

Drug-release test

The drug-release experiments (20 tablets) were carried out according to the Chinese Pharmacopoeia (2015 edition) using the rotating basket method in a hemispherical dissolution bath equipped with 1000-mL vessels (37°C, 100 rpm). Each condition used six groups of parallel tests to ensure accuracy. The in vivo condition was simulated using pH 1.0 Hydrochloric acid solution for 2 h, followed by pH 3.0 phosphate buffer for 2 h, then by pH 5.0 phosphate buffer for 2 h, and finally by pH 6.8 phosphate buffer for 0.75 h. The solutions were filtered using a 0.45-μm membrane filter paper and marked as Bayer’s aspirin test solution. Then, the 25-μL test solution was injected into the HPLC system and the chromatogram was recorded. The “Original” aspirin test solution was prepared in the same way. According to the linear regression equation of the standard curve, the content of aspirin in enteric-coated aspirin tablets was calculated.

Clinical statistical analysis

The Student t test and χ² test were used for comparison among groups. Statistical analyses were performed using Prism5 program (GraphPad Software Inc, CA, USA) and IBM SPSS 20.0 (IBM, IL, USA). The data were presented as mean ± standard deviation. A P value <0.05 was considered statistically significant for all analyses.
RESULTS

Retrospective analysis of clinical cases

Among the 100 patients included in this study, 49 were male and 51 were female, aged 44-87 years, with an average age of 69.03±9.60 years. Ten cases of GI bleeding occurred due to the administration of enteric-coated aspirin tablets, including four males and six females, aged 59-82 years, with an average age of 71.5±7.82 years. The bleeding rate under different clinical conditions is shown in Table 1. The average age of each group with \( P \) values is shown in Table 2.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison of aspirin-induced bleeding rate under different medication times and different brands.</th>
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<tbody>
<tr>
<td></td>
<td>Numbers</td>
</tr>
<tr>
<td>Medication time</td>
<td></td>
</tr>
<tr>
<td>Before meals</td>
<td>26</td>
</tr>
<tr>
<td>After meals</td>
<td>74</td>
</tr>
<tr>
<td>Brands of aspirin</td>
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<tr>
<td>Bayer's aspirin</td>
<td>86</td>
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<tr>
<td>Domestic aspirin</td>
<td>14</td>
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<table>
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<tr>
<th>Table 2</th>
<th>Average age of patients with ( P ) values</th>
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<tr>
<td></td>
<td>Average age</td>
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<tr>
<td>Medication time</td>
<td></td>
</tr>
<tr>
<td>Before meals</td>
<td>69.5 ± 9.75</td>
</tr>
<tr>
<td>After meals</td>
<td>68.86 ± 6.61</td>
</tr>
<tr>
<td>Brands of aspirin</td>
<td></td>
</tr>
<tr>
<td>Bayer's aspirin</td>
<td>68.73 ± 9.95</td>
</tr>
<tr>
<td>Domestic aspirin</td>
<td>70.86 ± 7.19</td>
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</table>

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Linearity data of standard aspirin</th>
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<tr>
<td></td>
<td>Concentration (mg/mL)</td>
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<tr>
<td>Peak area</td>
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</table>

In vitro release experiments

Method validation of HPLC

The system suitability test was applied to the chromatograms under optimum conditions to check various parameters such as theoretical plates (column efficiency >3000), asymmetric factor (0.95-1.05), sensitivity (≥10), and resolution (>1.5). The results ensured the adequacy of the proposed HPLC method for the routine analysis of aspirin. The system suitability results were fitted to the standard of the Chinese Pharmacopoeia. Figure 1 shows a typical chromatogram obtained from the analysis of the standard solution using the proposed method. The retention time observed was 5.86 min for aspirin. No interfering peaks and false-positive results were observed in the chromatogram.

Figure 1  Typical HPLC chromatogram of aspirin at 276 nm with a theoretical tower number of 8270.37 and retention time is 5.86.

Linearity was performed using different test concentrations. Five different standard solutions within the linear range containing 0, 0.05, 0.15, 0.20, and 0.25 mg/mL were prepared and injected into the HPLC system. The standard curve was shown in figure 2 and data were suggested in table 3. The corresponding linear regression equation was \( y=2757X+0.389 \) with the square of correlation coefficient \( R^2 \) of 0.999 for aspirin, which indicate that the peak area of aspirin ranged from 0 mg/ml ~0.2 mg/ml has better linear relation with the sample size.

Figure 2  Calibration Calibration curve of standard aspirin

The accuracy of an analytical method is the agreement between the actual value of an analysis in the sample and the value obtained by analysis. The recovery tests were carried out using three different concentration levels (0.05, 0.10, and 0.15 mg/mL). The average recoveries obtained for aspirin are 96.78%, 99.99% and 100.30%, respectively, which were satisfactory (%RSD=1.61).

The precision of the method was demonstrated by interday and intraday analyses for each concentration at three different concentration levels (0.05, 0.10, and 0.15 mg/mL). For the intraday precision, standard solutions at three concentrations were injected thrice in a day and the percent relative standard deviation (%RSD) was calculated. In the interday studies, standard solutions were injected on three consecutive days and the %RSD for intraday is 1.22, 1.06 and 0.66, respectively. The %RSD for interday precision is 0.94, 0.15 and 0.21, respectively. The data showed that the HPLC method developed was precise (%RSD<2).

The 0.05, 0.10, and 0.15 mg/mL standard solution peak areas were tested for an interval of 0, 24, 48, and 72 h at room temperature for stability. The average peak area of 0.05, 0.1, and 0.15 mg/mL standard solutions was 134.44, 275.31, and 416.77, respectively, and their RSD was 1.05%, 0.94%, and 0.56% (%RSD<2), respectively, indicating that the sample solution was stable at 72 h.
Standard aspirin (25 mg) tablets were weighed, change the solvent solvents pH 1.0 hydrochloric acid solution, pH 3.0 phosphate buffer solution, pH 5.0 phosphate buffer solution, pH 6.8 phosphate buffer solution and 1% methanol and glacial acetic acid solution, respectively, to dissolve the drug to obtain three different concentration levels (0.05, 0.10, and 0.15 mg/mL). The average peak area of 0.05, 0.1, and 0.15 mg/mL standard solutions was 136.85, 284.91, 419.22, respectively, and their RSD was 1.65%, 1.80%, 0.74% (%RSD<2), respectively, indicating that there was no significant difference for determination the content of aspirin among the five solvents mentioned above.

**Content determination**

The contents of Bayer's aspirin and domestic aspirin are depicted in Table 4.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bayer's aspirin</th>
<th>Domestic aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (mg)</td>
<td>137.5 ± 2.70</td>
<td>127.8 ± 2.51</td>
</tr>
<tr>
<td>Peak area (mv)</td>
<td>304.72 ± 6.67</td>
<td>301.13 ± 10.03</td>
</tr>
<tr>
<td>Aspirin content per tablet (mg)</td>
<td>109.25 ± 1.76</td>
<td>107.55 ± 1.82</td>
</tr>
<tr>
<td>Content uniformity (%)</td>
<td>12.51</td>
<td>12.07</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>1.70</td>
<td>1.97</td>
</tr>
</tbody>
</table>

**3.2.2 Drug-release performances**

The in vitro release experimental results were examined. At pH 1, no release of Bayer's aspirin and domestic aspirin was detected due to the special solution environment. Data are shown in Tables 5, which included the dissolution rate of Bayer's aspirin and domestic aspirin in different pH media.

**Table 5 Drug-release performances of aspirin in different pH media**

<table>
<thead>
<tr>
<th>Dissolution rate (%)</th>
<th>Bayer's aspirin(n=6)</th>
<th>Domestic aspirin(n=6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH =1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>pH =3</td>
<td>0.08 ± 0.02</td>
<td>0.06 ± 0.03</td>
<td>0.12</td>
</tr>
<tr>
<td>pH =5</td>
<td>1.13 ± 0.27</td>
<td>1.22 ± 0.22</td>
<td>0.58</td>
</tr>
<tr>
<td>pH =6.8</td>
<td>80.12 ± 7.45</td>
<td>81.37 ± 7.44</td>
<td>0.73</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The widespread use of aspirin in clinic has drawn attention owing to its side effects, especially on GI bleeding. In this retrospective analysis of clinical cases, 100 patients who fulfilled the inclusion criteria were identified. GI bleeding occurred in 10% of these patients. No statistically significant difference in age was observed between patients taking aspirin before meals or after meals (P>0.05). Previous trials have shown that up to 15-20% of patients developed gastrointestinal symptoms with aspirin monotherapy.18 And a latest retrospective Study of 612,509 Patients showed that the patients with long-term use of aspirin reported a GI bleeding incidence of 5.04% over 20 years' follow up.26 These differences may relate to race and collecting data methods. In this study, it also confirmed that Bayer's aspirin and domestic aspirin had no significant difference in the incidence of GI bleeding (P>0.05). GI bleeding caused by aspirin had no relevance with the medication time (before or after meals; P>0.05).

The question then was whether food-induced pH changes led to the release of enteric-coated aspirin tablets in advance. To further explore this, an *in vitro* experiment with two kinds of aspirin was performed. The dissolution rate of aspirin in different pH solutions was determined. Different pH media were simulated in the stomach before or after the patients had a meal to suggest the dissolution of enteric-coated aspirin tablets. However, statistical analysis results showed no significant difference between domestic and Bayer's aspirin in terms of weight variation (±7.5%), content uniformity (±15.0), and content (P>0.05). No obvious release of enteric-coated aspirin tablets was found at pH 1.0-5.0 for 2 h. However, at pH=6.8, about 80% of the drug was released within 45 min. The dissolution rates of domestic aspirin and Bayer's aspirin showed no significant statistical difference in different pH solvents (P>0.05).

The pH within the human stomach changed following the ingestion of a food substance.27 Reported in the studies on pH changes in the stomach before or after meals were analyzed thoroughly. Nokhodchi et al and Annaert et al reported that the typical median values for the gastric pH in the fasted state ranged from 1.7 to 3.3 (median of 2.5).28,29 Gardner et al suggested that median gastric pH was 1, increased to pH 4.5 with ingestion of the meal, and then returned to approximately pH 1, 3-4 h after the start of the meal.30 Ramdani et al reported that for 90% of the time, the gastric pH in the fasted state remained below 3.31 Dressman et al and Birk et al reported that pH was usually below 3 in the normal stomach, and after a moderate meal, it increased to approximately 5.0.32,33 Annaert's report also suggested that after ingesting a meal, the gastric pH in the food state ranged from 2.7 to 6.4.34 Therefore, it was concluded that the gastric pH in the fasted state was around 1-3, and increases to approximately 5.0 after a meal. These findings suggested that no obvious release of enteric-coated aspirin tablets occurred in the fasted state. However, after a meal the pH in stomach increased, when it increased to 6.8, about 80% of the drug released.

PPIs, which inhibit the function of the proton pump responsible for the terminal step in gastric acid secretion, are considered to be the most effective medical treatment for patients with acid-related diseases such as peptic ulcers.34,35 Concomitant use of aspirin and proton-pump inhibitors (PPIs) has been recommended in patients with a history of GI hemorrhage, especially for the patients who take aspirin for secondary CV protection.36,37,38 Humorous studies have shown that these agents significantly reduce the risk of upper GI adverse events in aspirin-treated patients.37,41 PPIs inhibit gastric acid secretion, which increases gastric pH and can alter the pharmacokinetics of aspirin. This might account for a decline in bioavailability and therapeutic effects of combination therapy with aspirin and PPIs.34-47 However, previous studies have found that PPIs can alter the acidic environment of the stomach rapidly (pH>4) and some of them can raise the pH approximately 7.31,35,48-50 This means that administering PPIs and alkaline food may lead to the release of enteric-coated aspirin tablets in advance and increase the possibility of GI bleeding. Several published studies revealed that 4-12 h after taking PPIs, the pH in the stomach decreased to less than 4.5.49,53-55 Therefore, according to the study we suggest patients avoid taking PPI and aspirin simultaneously. Instead, they should take aspirin 12 h after taking the PPI to avoid aggravating.
the effect of aspirin in the GI tract.

CONCLUSIONS

Postprandial aspirin has a risk of gastric bleeding. Some food or drug can alter pH in the stomach dramatically. Therefore, patients with upset stomach should be advised to take aspirin before meals to prevent aspirin release in advance, and reduce damage to the stomach.

REFERENCES


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