Preparation and Evaluation of Oral Dosage Form Using Acylglycerols. II.\(^1\) Effect of Food Ingestion on Dissolution and Absorption of Aspirin from the Granules Prepared by Acylglycerols in Human Subjects\(^2\)

Yoshiteru Watanabe,\(^3\)\(^,\)\(^4\) Motoshi Suda,\(^5\) Yoshiaki Matsumoto,\(^6\) Kazuo Takayama,\(^5\) Mitsuo Matsumoto\(^6\) and Wen Zhao\(^6\)

Department of Pharmaceutics, Showa College of Pharmaceutical Sciences,\(^3\) 3-3165, Higashi-Tamagawagakuen, Machida, Tokyo 194, Japan and China Tianjin Pharmaceutical Research Institute,\(^6\) Nan Kai, Tianjin 300090, China. Received February 4, 1991

The dissolution behavior of the aspirin enteric granule prepared using acylglycerols, glyceryl monostearate (GMS) and glyceryl triurate (GTL), was investigated in vitro and in human subjects in a fasting or non-fasting state. Aspirin was slowly released from the granule in vitro at pH 1.2. No acceleration of the aspirin dissolution rate in the medium without lipase and cholic acid was observed when the pH level of the medium increased to a neutral region (pH 6.4). However, the dissolution of aspirin was significantly increased by increasing the concentrations of lipase and cholic acid in the medium. Lipase appears to play an essential role in the dissolution process of aspirin granules. In human subjects, the average levels of the cumulative amount of total salicylate excreted in a urine-time curve, and the mean residence time (MRT) obtained after oral administration of a granule in the fasting state were markedly delayed in comparison with the results observed using an aqueous solution and a crystalline form of aspirin. In comparing the fasting condition with the non-fasting condition (after food ingestion), no significant difference was recognized in the total amount of salicylate excreted in urine to an infinite time \(A_t(x))\), whether the MRT was obtained by granule, crystalline form or aqueous solution. It can be concluded that aspirin granule prepared by GMS and GTL has a property of pancreatic lipase-sensitive dissolution, and its bioavailability is unaffected by food intake.

Keywords aspirin enteric granule; mean residence time; food ingestion effect; acylglycerol; glyceryl monostearate; glyceryl triurate; human subject; gastrointestinal absorption

Introduction

An enteric preparation, which has a pH-independent dissolution property, may offer promising drug release in patients with low gastric acidity, e.g. anacidity and achyria. Recently, we reported that pH-independent enteric granules containing aspirin could be prepared by combining aspirin and glyceryl monostearate (GMS) mass coated with melted glyceryl triurate (GTL) using a centrifugal rotating mixer.\(^1\) These acylglycerols, edible and safe pharmaceutical ingredients, are digested by lipase and bile salts in intestinal juice. Consequently, acylglycerols provide a useful means of preparing a pH-independent (pancreatic lipase-sensitive) enteric granule containing aspirin. In a dissolution test in vitro, aspirin could not be released (dissolution percentage, below 10% within 2 h) from the prepared granule in a pH 1.2 aqueous solution, whereas the dissolution percentage of aspirin was increased (about 80% at 2 h) in a pH 6.4 phosphate buffer solution (PBS) containing lipase and cholic acid.

In the present investigation, to better understand the dissolution behavior of aspirin granules prepared using acylglycerols, absorption of aspirin from the granules was evaluated in healthy human subjects after oral administration. The aim of this study was to compare absorption from the aspirin granule with that from a crystal form in an aqueous solution, under a fasting condition as well as when taken with a standardized meal (food ingestion).

Experimental

Materials Aspirin (JP XI) was obtained from Iwaki Seiyaku (Tokyo, Japan). Acylglycerol: GMS (Riken Vitamin, Tokyo, Japan) was used after the removal of free fatty acids and glycerol according to the method described in our previous report,\(^1\) and GTL (Tokyo Kasei Kogyo, Tokyo, Japan) was used. Lipase (triacetylgllycerol lipase, EC 3.1.1.3) and cholic acid (sodium salt) were purchased from Sigma (St. Louis, MO, U.S.A.). Other chemicals obtained were of commercial analytical grades.

Preparation of Aspirin Granules Aspirin granules using GMS and GTL were prepared by the two-step dry mixing method, as described in our previous paper.\(^1\) Aspirin powder (80—100 mesh) was aggregated by melted GMS; the aspirin-GMS mass was formed. Subsequently, the aspirin granule (5.5—12 mesh) coated with melted GTL was prepared using crushed aspirin-GMS masses of 12—42 mesh. After GTL coating, free GTL which did not adhere to the surface of crushed aspirin-GMS mass was removed by sieving. The content-ratio of aspirin (50%), GMS (25%) and GTL (25%) in granules was determined by the spectrophotometric method described in our previous report.\(^1\)

In Vitro Dissolution Test

The dissolution rates of aspirin from granules were determined in a manner similar to the paddle method described in JP XI, using a dissolution test apparatus (model NTS-2S, Toyama Sangyo, Osaka, Japan). A sample of 200 mg calculated as aspirin was tested. Dissolution media were 500 ml of the following aqueous solution: pH 1.2 solution (the first fluid for disintegration test, JP XI), pH 6.4 PBS\(^7\) and PBS containing lipase and cholic acid, at a constant temperature (37±0.1°C). The dissolution medium was stirred at 100 rpm with a paddle stirring element. At predetermined time intervals, an aliquot of 4 ml of the dissolution medium was taken, and the medium was replenished with the same volume of each solution. Aspirin was not hydrolyzed by lipase and cholic acid in the dissolution medium during the experimental period.

The dispersion was immediately filtered through a 0.2 \(\mu\)m cellulose nitrate membrane filter (Dismic-25, Toyoshii, Tokyo, Japan) to remove the particles. The concentration of salicylic acid was assayed by measuring the absorbance (300 nm) on an ultraviolet (UV)-visible spectrophotometer (model UV-240, Shimadzu Seisakusho, Kyoto, Japan) after hydrolysis of aspirin with 1 N NaOH. The absorbance of salicylic acid was determined by subtracting the absorbance of lipase and cholic acid in the dissolution medium without salicylic acid (blank test) from observed absorbance in the dissolution medium.

In Vivo Studies Male volunteers (21 to 25 years old, weighing 60 to 70 kg), from whom informed consent was obtained, participated in this study. All subjects were in good health and none were taking any other medication. After overnight fasting, the subjects received single oral administration of 500 mg of aspirin crystal (12—42 mesh) or granule containing 500 mg of aspirin together with 200 ml of water in the fasting state. Participants in the control group received 200 ml of aqueous solution containing 500 mg of aspirin instead of the crystalline form or granules. The subjects in the non-fasting state received the same preparations as those subjects in the fasting state 30 min after a

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standard breakfast. The standard breakfast consisted of 100 g of bread, 20 g of butter, a boiled egg, 35 g of cucumber and 200 ml of milk. All subjects abstained from any other food or liquid until 6 h after dosing, and then took food and drink ad libitum. Each dosage was separated by at least a week.

Urine samples were collected immediately before drug administration and at predetermined time intervals following administration. After the urine volume was measured, urine samples were kept frozen until analysis. With respect to the observation of bioavailability of aspirin dosage forms such as powder, tablet and enteric-coated preparations in human subjects, the determination of total salicylate in urine has been reported. In this study, therefore, total salicylate excreted in urine was determined by the colorimetric method.

Data Analysis

The statistical moments for the urinary excretion rate-time curve are defined as the following equations:

\[ A_e(x) = \int_0^x (dA_e/dt)dt \]  

(1)

\[ MRT = \frac{\int_0^x t(dA_e/dt)dt}{\int_0^x (dA_e/dt)dt} \]  

(2)

\[ dA_e/dt \] is the urinary excretion rate, \( A_e(x) \) is total amount of drug excreted in urine to an infinite time, and \( MRT \) is the mean residence time. These symbols are in accord with the proposal of Rowland and Tucker. \( A_e(x) \) and \( MRT \) were calculated by linear trapezoidal integration with extrapolation of the time course curve to infinite time according to a monoexponential equation.

The one-way ANOVA and Dunnett's tests were employed for statistical evaluation. Differences were considered to be significant at the \( p < 0.05 \) level for all experiments.

Results and Discussion

Dissolution of Aspirin from the Granules Prepared by Acylglycerols in Vitro

Figure 1 illustrates the mean dissolution percentage–time curves of aspirin from granules at pH 1.2 (shown as unfilled triangles) and 6.4 (shown as filled triangles). Aspirin crystal rapidly dissolved in an aqueous solution at pH 1.2 (dissolution percentage: 100% within 30 min). However, aspirin was slowly released from granules in the dissolution medium at pH 1.2. Consequently, a very low dissolution percentage (less than 10%) was obtained at 2 h. When the pH value was increased to a neutral region (pH 6.4), the dissolution percentage did not increase remarkably (approximately 20% or below at 2 h) in the dissolution medium without lipase and cholic acid (represented by the broken line with filled triangles). These results show that the dissolution of aspirin from granules is not influenced by changes in the pH level.

When lipase and cholic acid were added to the dissolution medium at pH 6.4 (represented by the straight line with filled triangles), the dissolution of aspirin from granules increased significantly. The dissolution percentage (ca. 75%) at 2 h was approximately 4 times higher than at 2 h in the medium without lipase and cholic acid. Aspirin was released more efficiently from granules in the dissolution medium containing lipase and cholic acid than in the medium without both materials. However, it is difficult to define the optimal concentration of pancreatic lipase and cholic acid in the in vitro dissolution test, since the secretion of both materials is changed by various physiological conditions, for instance, following the consumption of food. The maximal effect of lipase and gall powder on the lipid digestion of the triglyceride spheres containing sulfamethizole at a concentration of 0.4% has been reported. In our preliminary observation, the effective concentrations of lipase and cholic acid on the digestion of prepared aspirin granules were obtained at 0.6—1.0% and 0.1—0.2%, respectively. Concerning the effectiveness of lipase and cholic acid on dissolution, lipase appears to play an essential role in the action. It was confirmed that the aspirin is dissolved from granules prepared using GMS and GTL by lipase.

Dissolution Behavior and Absorption of Aspirin from Granules in Vitro

The aspirin granules prepared using
GMS and GTL are digested by pancreatic lipase. Intake of food may influence the dissolution of aspirin from these granules. To clarify the effect of food ingestion on the dissolution behavior and the gastrointestinal absorption of aspirin from granules, the cumulative amount of total salicylate excreted in urine after oral administration of aspirin granules was evaluated in human subjects in the fasting state or non-fasting state (after meals). Aspirin in an aqueous solution or in crystalline form was tested as the reference preparation.

The mean cumulative amount of urinary excretion–time curves after oral administration of aspirin solution, powder and granule in a fasting state (shown as unfilled symbols) and non-fasting state (shown as filled symbols) are illustrated in Figs. 2, 3 and 4, respectively. $A_i(x)$ and MRT obtained after administration of aspirin preparations are summarized in Table I. In the case of aqueous solution, aspirin was rapidly absorbed from the gastrointestinal tract in the non-fasting condition as well as the fasting condition. The rapid absorption of aspirin observed in our study is in general agreement with the results of Levy et al. No significant difference in the cumulative amount of urinary excretion–time curves between the two conditions was found (Fig. 2). The elimination rate constants ($0.230 \pm 0.083 \text{ h}^{-1}$ in the fasting state; $0.306 \pm 0.120 \text{ h}^{-1}$ in the non-fasting state) of total salicylate obtained by the calculation method of Levy were not significant. These results suggest that gastrointestinal absorption and elimination of aspirin from an aqueous solution is not influenced by foods.

When aspirin was administered in a crystalline form, aspirin was also efficiently absorbed. The average levels of the cumulative amount of urinary excretion from a crystalline form (Fig. 3) were not lower than those from an aqueous solution (Fig. 2), and no significant difference was recognized in $A_i(x)$ and MRT between both forms in the fasting condition (Table I). Therefore, aspirin could dissolve rapidly in gastrointestinal fluid. After food ingestion, mean values of $A_i(x)$ (466 ± 27 mg) and MRT ($6.7 \pm 1.5 \text{ h}$) were similar to those ($A_i(x)$, 437 ± 11 mg; MRT, 7.2 ± 1.0 h) obtained in the fasting condition. It can therefore be presumed that foods do not induce a change of aspirin dissolution from the crystalline form in gastrointestinal fluid.

It is generally accepted that the gastric emptying rate of pharmaceutical preparations is influenced by coadministered drugs or food intake. The effect of food ingestion on the gastric emptying rate of aspirin in aqueous solution or crystalline form may be ignored in evaluating the bioavailability of aspirin in human subjects since similar results of $A_i(x)$ and MRT were obtained between the fasting and non-fasting states.
The aspirin granule significantly delayed the urinary excretion until 12 h after administration (Fig. 4) and increased MRT values in the fasting state (10.2 ± 2.2 h) as well as in the non-fasting state (10.4 ± 3.3 h). These results suggest that aspirin in granules dissolved slowly in the gastrointestinal tract. However, no significant difference of \( A_{\text{d}}(\infty) \) values from granules between the two conditions was found.

Considerable attention has been paid to the effect of foods on the absorption characteristics of aspirin from various oral preparations such as conventional tablets, enteric-coated tablets, and granules, etc., in volunteers. Furthermore, the influence of food ingestion on the gastric emptying rate of an oral dosage form of aspirin has been studied in humans. Taken with food, the enteric-coated tablets gave a much lower absorption than the enteric-coated granules which were not influenced by the intake of food. Moreover, the dosage form with a larger size was found to be more slowly emptied from stomach, and the gastric emptying rate of aspirin granules (diameter, 1 mm) was less affected by food than that of tablets (diameter, 4—8 mm). These findings suggest that aspirin granules permit more reproducible absorption than tablets.

The observation made in this study is that the aspirin granules prepared using GMS and GTL show delayed absorption compared with the aqueous solution or crystalline form. However, there was no detectable influence of food on aspirin absorption from granules. The granules were obtained by sieving the product through 5.5—12 mesh (diameter, 3.35—1.40 mm). Probably, the gastric emptying rate of these granules is not affected by food ingestion.

Generally, intake of food enhances the secretion of lipase and bile salts. However, it is impossible to determine the exact concentrations of pancreatic lipase and cholic acid in intestinal juice during the fasting or non-fasting condition. Although the difference of concentration of these materials between two conditions may influence the dissolution of aspirin from the prepared granules in the small intestine, aspirin can dissolve from granules at a low concentration (only less than 1%) of lipase. Thus, this may be one of the reason why significant differences of \( A_{\text{d}}(\infty) \) and MRT were not observed between the fasting and non-fasting states.

In conclusion, the aspirin granules prepared by GMS and GTL have a property of pancreatic lipase-sensitive dissolution, and its bioavailability is unaffected by food intake. This aspirin granule is a useful dosage form of a delayed-release preparation having a pH-independent dissolution.

References and Notes

2. Part of the work described was presented at the 110th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, August, 1990.