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Research Article

Changes in the Amounts of Amoxicillin and Metronidazole Used for *Helicobacter pylori* Eradication Therapy in the Stomach after Their Oral Administration to Rats

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Abstract: In the eradication therapy of *Helicobacter pylori*, the changes of the amounts and concentrations of antibiotics in the stomach after oral administration were unclear. Amoxicillin (AMX) and metronidazole (MTZ), which are used for such therapy, were selected for this study. The levels of these drugs in homogenate of the stomach after their oral administration to rats were determined by HPLC. The doses of AMX and MTZ to the rats were 15 mg/kg and 5 mg/kg, respectively. The proportions of these drugs remaining in the stomach decreased according to first-order kinetics. The rate constants for AMX and MTZ were 0.512 and 0.436 h^{-1}, respectively. The decrease of the stomach volume was also expressed as first-order kinetics, with a rate constant of 0.184 h^{-1}. These data are useful for the design of intragastric buoyant sustained-release preparations for the eradication therapy of *H. pylori*.
Keywords: Metronidazole, Amoxicillin, HPLC, Stomach, Rat, Proportion remaining.

INTRODUCTION

Helicobacter pylori is often observed to adhere to the antral epithelium of the human stomach and gastric metaplasia in the duodenum. Recent studies have provided evidence that cytotoxin-associated gene A product (cagA)-positive *H. pylori* plays a causal role in the development of gastric carcinoma [1-3]. Therefore, the eradication of *H. pylori* is considered to be very important. The primary eradication therapy for *H. pylori* in Japan, which is a triple therapy using amoxicillin, clarithromycin, and proton pump inhibitor, was started in 2000, with this therapy being approved for health insurance coverage [4]. However, because of the increase of incomplete eradication, second-line eradication therapy was approved in 2007 [4]. In this eradication therapy, metronidazole (MTZ) is used in the place of clarithromycin. As the prevalence of *H. pylori* strains with clarithromycin resistance continues to increase markedly, the importance of second-line eradication therapy is also increasing [4]. The pharmaceutical preparations used in eradication therapy were not originally developed for this purpose. For example, in Japan, the pharmaceutical preparations Pasetocin Tablets 250 for amoxicillin and Flagyl for MTZ were developed for various bacterial infections in 1981 and 1963, respectively. These preparations were designed to act through the blood circulation after GI absorption. However, for *H. pylori* eradication therapy, an intragastric local attack is expected [5]. From the point of view of pharmaceutical preparation design, the preparations used are thus not optimal [6].

In order to design pharmaceutical preparations, information about the amounts and/or concentrations of antibiotics in the stomach after their oral administration is very important. If such information could be obtained, it would be possible to design a dissolution profile of the antibiotics. Against this background, we previously reported a typical type of intragastric buoyant sustained-release tablet containing AMX [7] and a method to simulate the amount of antibiotics in the stomach based on the data of dissolution studies and the gastric emptying rate of drug [6]. However, the information about the amounts and/or concentrations of drugs in the stomach after oral administration of the antibiotics used for eradication therapy was insufficient for both humans and experimental animals. We previously reported a simple high-performance liquid chromatographic (HPLC) procedure for determining the intragastric amount of MTZ in rats, and the results of a preliminary study on its intragastric behavior in rats [8].

This paper describes the intragastric behavior of AMX and MTZ after their oral administration to rats.

MATERIALS AND METHODS

Chemicals and solvents

Amoxicillin (AMX) and metronidazole (MTZ) were purchased from Sigma Chemical Company (St. Louis, MO, USA) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan), respectively. Other chemicals were of special reagent grade or HPLC grade.

Apparatus and chromatographic conditions

The HPLC system consisted of a Model LC-10AS pump, equipped with a Model SCL-10A system controller, a Model SPD-10A UV spectrophotometric detector, a Model CTO-10A column oven, a Model C-R7A Chromatopac, and a Model SIL-10A autoinjector, all from Shimadzu Corporation (Kyoto, Japan). The mobile phases were methanol-water-perchloric acid (60%)-sodium perchlorate monohydrate=50:950:1:5, (V/V/V/W) for MTZ and 120:880:1:5 for AMX. The chromatographic column was a YMC Pack AM12S05 ODS (150 mm x 6 mm I.D., particle diameter 5 μm) obtained from YMC Co. Ltd. (Kyoto, Japan). The flow rate and temperature of the column were 1 mL/min and 40°C. The wavelengths for determination for AMX and MTZ were 254 and 370 nm, respectively.

Animal study and preparation of stomach homogenate

Male Sprague-Dawley rats were used. All rats (270-330 g body weight) were allowed free access to water and food. AMX and MTZ were dissolved in a 0.5% methylcellulose solution to make solutions with concentrations of 25 mg/mL MTZ and 75 mg/mL AMX. Each solution at 0.2 mL/kg was administered orally at 10:00 (9:40-10:20) a.m. on the experimental day. In accordance with the method described in a previous report [8], the stomachs were withdrawn from the abdomen after the rats had been anesthetized with ether. Once the stomachs had been withdrawn, the esophagus on the upper side of the stomach and the duodenum on the lower side of the stomach were ligated. The withdrawn stomachs were then washed out with saline and weighed. Saline equivalent to five times the weight of each stomach was added to the stomach, with the mixture then being homogenized. All animal experiments were carried out in accordance with the 'Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University'.

Preparation of standards

Rat stomachs not administered the drugs were withdrawn, washed out with saline, and weighed. The drug-free homogenates were prepared in accordance with the method described above. AMX and MTZ at 100 mg were dissolved in 50 mL of the drug-free homogenate. These homogenates were used as the standard homogenates. The standard homogenates were diluted with the drug-free homogenate, and standard curves were prepared [8].

Assay procedures

Stomach homogenates at 1.0 mL or 2.5 mL were added to glass tubes, and the weight of the homogenates was measured. Exactly two volumes of methanol, 2.0 or 5.0 mL, equivalent to the volume of the stomach homogenates, was added to each tube cooled in an ice-bath. The mixture was stirred on a vortex mixer for 10 sec and centrifuged at 3,000 rpm for 10 min; 10 μL of the supernatant was injected into a chromatograph. The animal study and assay were performed on the same day [8].

Calculations

The peak areas of AMX and MTZ in the HPLC profiles were measured. Calibration curves were established by comparing the peak area with the concentrations of AMX and MTZ in the standards. The slope and intercept of the curves were calculated using weighted ($1/Y^2$) linear regression. The concentration of MTZ in the experimental samples was calculated using the following equation: $X\ (\mu g/mL) = (Y - b)/a$, where Y is the peak area of AMX or MTZ in an experimental sample and *b* (intercept) and *a* (slope) are constants generated by linear regression analysis of the calibration curve data. The validation data for AMX were previously reported (8), and the data for MTZ were as follows: This method was linear over the standard curve range from 5 to 2000 μg/ml. The inter-day precision and accuracy from 5 to 2000 μg/ml (n=5) ranged from 1.4 to 5.0% and from -4.6 to 4.9%, respectively. The quantification limit for this method was 5 μg/ml based on the validation data.

Calculations of AMX and MTZ in rat stomach

Concentrations of AMX and MTZ in the homogenates were calculated from the calibration curves. After calculating these concentrations, the amounts of the drugs in the stomach were calculated from the weight of homogenates used for the assay, and the weight of the homogenates prepared. The amounts of drugs in the homogenates prepared were equal to their amounts in the rat stomach. The proportion of drug remaining (%) was calculated from the drug in the rat stomach and the oral dose of the drug (mg) for each rat.

RESULTS AND DISCUSSION

Proportion of AMX remaining in the stomach

The amounts of AMX found in the stomach at 5 min, 1 h, 3 h, and 5 h after oral administration were 4.5 ± 0.2 (mean ± SD, n=3-6), 2.1 ± 1.1, 1.2 ± 0.5, and 0.6 ± 0.3 mg, respectively. The proportions of AMX remaining calculated from the amount in the stomach at each time after oral administration to rats at a dose of 15.0 mg/kg are shown in Fig. 1. All values are the mean ± SD of 3-6 rats. The proportions remaining at 5 min, 1 h, 3 h, and 5 h were 98.8 ± 3.4%, 43.4 ± 22.4%, 24.2 ± 9.8%, and 12.5 ± 5.8%, respectively. The decrease of AMX in the stomach was considered to be a

first-order-type process. However, in order to calculate the first-order rate constant, the assumption was made that the proportion remaining at 0 h was 100% because the data had a large scatter. This assumption is considered to be acceptable because the AMX remaining at 5 min was 98.8 ± 3.4%. Calculating an approximation curve, a good first-order equation, $Y=100.0\ e^{-0.512t}$, r=0.980, was obtained. The emptying rate constant of AMX in the stomach was found to be 0.512 h^{-1}.

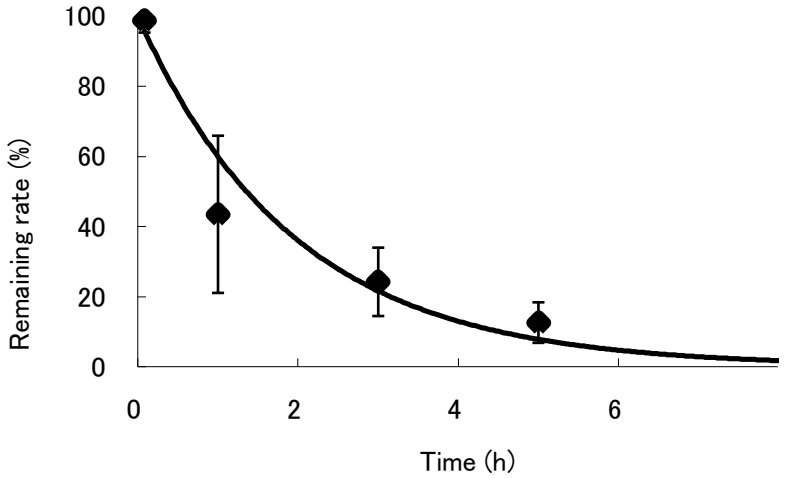

Fig-1:The proportion of amoxicillin remaining in the stomach of rats after its oral administration at a dose of 15 mg/kg. Each point represents the mean ± SD (n=3-6).

Proportion of MTZ remaining in the stomach of rats

Figure 2 shows the proportion of MTZ remaining after oral administration to rats at a dose of 5.0 mg/kg. All values are the mean ± SD of 3-6 rats. Data reported previously as the results of a preliminary study [8] are included in the data of Fig. 2. The proportions remaining at 5 min, 1 h, 3 h, and 5 h were 95.7 ± 5.2%, 46.5 ± 14.2%, 31.7 ± 16.1%, and 19.2 ± 15.6%, respectively. The decrease of MTZ in the stomach was considered to be a first-order-type process. However, in order to calculate the first-order rate constant, the same as for AMX, the assumption was made that the proportion remaining at 0 h was 100% because the data had a large scatter. The rate constant was calculated based on the same assumption as for AMX. The approximation curve of a good first-order equation, $Y=100.0\ e^{-0.436t}$, r=0.967, was obtained. Therefore, the emptying rate constant of MTZ was calculated to be 0.436 h^{-1}.

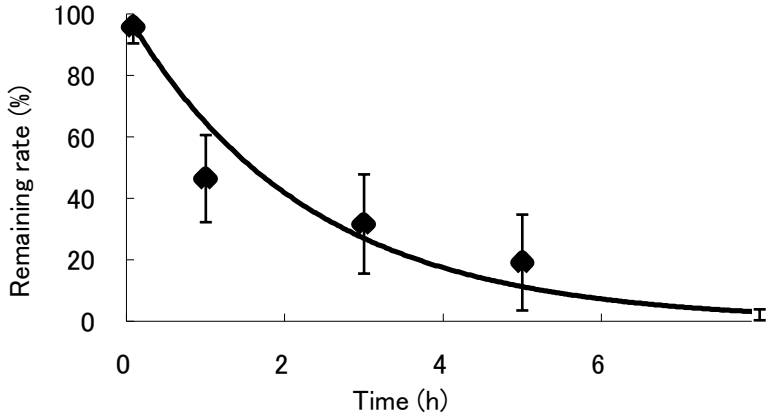

Fig-2: The proportion of metronidazole remaining in the stomach of rats after its oral administration at a dose of 5 mg/kg. Each point represents the mean ± SD (n=3-6).

Changing weight of the stomach in rats

Fig. 3 shows the changing weight of the stomach of rats, using the whole weight of the stomach as described in section 1.3 and the weight of an empty stomach, 1.4 ± 0.1 g (mean ± SD, n=3). The weights at 5 min, 1 h, 3 h, 5 h, and 8 h were 4.7 ± 3.1, 5.9 ± 1.7, 3.4 ± 1.8, 2.9 ± 1.2, and 1.2 ± 0.5 g, respectively. The decrease of the weight was considered to be a first-order-type process. The approximation curve, $Y=6.6\ e^{-0.184t}$, was obtained. The gastric substance is considered to be almost all water. The specific gravity of the substance can be assumed to be 1. Considering this, the stomach capacity change constant was also 0.184 h^{-1}. From these results, we found that the stomach capacity

can be dealt with as a linear equation. This is in agreement with the reported finding that the gastric emptying rate is a first-order process.

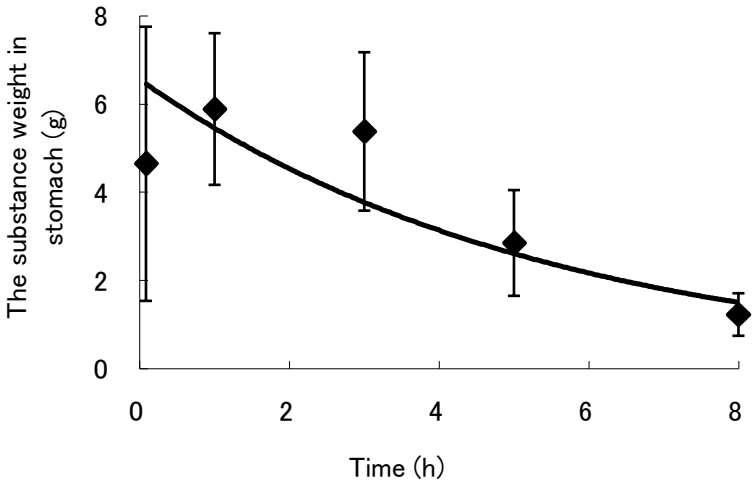

Fig-3: The substance weight change in the stomach of rats after oral administration of drugs. Each point represents the mean ± SD (n=6-12).

CONCLUSION

The amounts of AMX and MTZ in the stomach after their oral administration to rats were determined by HPLC. The data showed that AMX and MTZ in the stomach decreased with time according to first-order kinetics, and the rate constants for AMX and MTZ were 0.512 and 0.436 h^{-1}, respectively. A method of simulating intragastric drug concentrations based on the dissolution data was previously reported [6]. In this method, the gastric emptying rate of drugs is the essential parameter, the acceptableness of which had not been reported. The rate constants in rats were found in this study. These data are considered to be useful for the design of intragastric buoyant sustained-release preparations for the eradication therapy of *Helicobacter pylori*.

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