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Multiple Plasma Peaks of Acetaminophen and Ranitidine after Simultaneous Oral Administration to Rats

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Abstract □ Acetaminophen (AAP) and ranitidine (RT) were coadministered orally to nine rats, and the possible contribution of the gastric emptying to the plasma concentration profiles of them was examined. The drugs showed multiple plasma peaks similar to the respective ones after separated administration of each durg. It implies that there is no significant interaction between AAP and RT in terms of the gastric emptying or drug absorption. There were no significant linear correlations of the peak patterns (peak height and peak time) between AAP andd RT. It is contrary to the expectation from the biphasic gastric emptying (BGE) theory previously suggested for AAP and RT. The BGE theory, therefore, seemed to have some draw-backs in explaining satisfactorily the multiple plasma peaks of AAP and RT. Two more doubts raised previously against the BGE theory were also discussed.

Keywords □ Multiple plasma peaks, acetaminophen (AAP), ranitidine (RT), biphasic gastric emptying (BGE), simultaneous administration

Multiple peaks in the plasma concentration-time curves of acetaminophen (AAP)[1-5] and ranitidine (RT)[6,7] following oral administration to human subjects and animals have been reported. The multiple peaks have often been attributed to the biphasic GI absorption due to biphasic gastric emptying (BGE) for AAP[1,2], and to the enterohepatic recycling[8] or release to plasma pool of temporarily deposited drug in some tissues for RT[8]. For the multiple peaks of some other drugs, several different mechanisms including absorption of drugs from the two different sites in the GI tracts[9-15], multifractional absorption due to fractional dissolution from the dosage-form in the GI tract[16-18] and release to plasma pool of temporarily deposited in some tissues[8,19,20] have also been postulated as the possible causes.

Among the above mechanisms, enterohepatic recycling and two sites-absorption did not contribute significantly to the multiple peaks of AAP[4] and RT[7]. Multifractional absorption would be excluded from the consideration if the experiment is performed using a solution dosage-form. Release to plasma pool from the tissue depot has not been tested experimentally.

On the other hand, the BGE-theory seemed most plausible for AAP and RT, since the second plasma peaks of them were highly reproducible when the drugs were administered twice diretly to the duodenum of rats with appropriate dose ratio and dose interval[21].

In this study, we tried to re-examine the BGE-theory for its validity as a mechanism of the multiple peaks of AAP and RT in rats. If BGE predominates the GI absorption and plasma profiles of oral drugs, plasma profiles of the drugs adminstered simultaneously will be influenced equally by the gastric emptying patterns: The ratio of the plasma concentrations of the first peak (C_{max1}) to the second peak (C_{max2}) will depend on the ratio of the fractions emptied at the first emptying to the sec-

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ond emptying from the stomach. The time interval between the time to reach the first peak (T_{max1}) and the second peak (T_{max2}) will also depend on the time lag between the first and second emptying as long as there is no drug interaction between AAP and RT.

The patterns of the BGE, i.e., the ratio of fractions emptied and the time lag, may vary among subjects. However, they will affect the C_{max1}, C_{max2}, T_{max1} and T_{max2} of the co-administered drugs equally in each rat. As a consequence, linear relationships of C_{max1}, C_{max2}, T_{max1} and T_{max2} between the drugs among rats is expected.

In this study, the possible contribution of BGE on the plasma profiles of AAP and RT was examined after simultaneous oral administration of the drugs to rats.

EXPERIMENTAL

Chemicals and animals

Acetaminophen (AAP) and ranitidine (RT) were purchased as powders from Hong Sung Pharm. Ind. Co. (Seoul, Korea) and Il-Dong Pharm. Ind. Co. (Seoul, Korea), respectively. All other reagents were of analytical or HPLC grade. Before the experiment, male Wistar rats (Experimental Animal Center, Seoul National University) weighing 230-250g were fasted for 12 hr.

Oral administration of AAP and RT to rats

Under light ether anesthesia, the femoral artery of a rat was cannulated with polyethylene tubing (PE-50, Intramedic, Clay Adams, USA) for blood sampling. After complete recovery from the anesthesia, AAP and RT were adminstered orally as a solution at a dose of 2 ml/kg (40 mg/kg and 20 mg/kg, respectively) using an oral feeding tubing. The solution was prepared by disssolving AAP and RT with 30% (v/v) propylene glycol in saline to yield 1 and 2% (w/v) of them, respectively. The rats were kept at supine position during the experiment, and their body temperatures were maintained with a light lamp. Nine rats were used in this study. Blood samples (0.15 ml) were withdrawn through the catheter at 0, 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 60, 90, 120, 180, 240 and 360 min. After centrifugation, the resultant plasma samples were frozen at −20℃ until assay for AAP and RT.

HPLC assay of AAP and RT in plasma

To 50 μl of plasma sample, 1 ml of 1 : 1 (v/v) mixture of acetonitrile and isopropanol containing 0.05% (w/v) procaine hydrochloride as an internal standard was added and mixed. After vortexing for 1 min and centrifuging (6000×g) for 10 min, 800 μl of the supernatant was transferred to the other test tube and evaporated to dryness under N_2 stream at 40℃. To the residue, 100 μl of the deionized distiled water was added and vortexed.

For AAP, a 15 μl aliquot of the resultant solution was injected onto the HPLC column (30×0.39 cm i.d., stainless steel) containing 10 μ μ-Bondapak C_{18} reversed-phase material. The pump and UV detector were from Spectraphysics (model SP 8810) and Applied Biosystems (model 757), respectively. The mobile phase was a 11 : 89 (v/v) mixture of acetonitrile and water. The flow rate was 1.0 ml/min, and the mean operating pressure was approximately 60 bar. AAP was monitored at 245 nm. Inter- and intra-assay coefficients of variation were 7.8 and 4.5%, respectively.

For RT, another 15 μl aliquot of the solution was injected onto the same HPLC column. The mobile phase was a 50 : 50 (v/v) mixture of 0.2 N-phosphate buffer (pH 6.8) and methanol. The flow rate was 1.0 ml/min and the mean operating pressure was approximately 80 bar. RT was monitored at 313 nm. Inter- and intra-assay coefficients of variation were 7.4 and 5.7%, respectively.

AAP and RT concentrations in the plasma were determined by the peak height ratio of each drug against the internal standard using respective standard calibration curve prepared by adding known amounts of AP or RT to the blank plasma. Linearity of the calibration curve was found in the range of 0.5-200 μg/ml for AAP and 0.05-33.0 μg/ml for RT, respectively.

Pharmacokinetic analysis

Plasma concentrations of the first (C_{max1}) and second (C_{max2}) peaks, and the time to reach the first (T_{max1}) and second (T_{max2}) peaks were read directly from the experimental data.

RESULTS AND DISCUSSION

Plasma concentration-time curves of AAP and

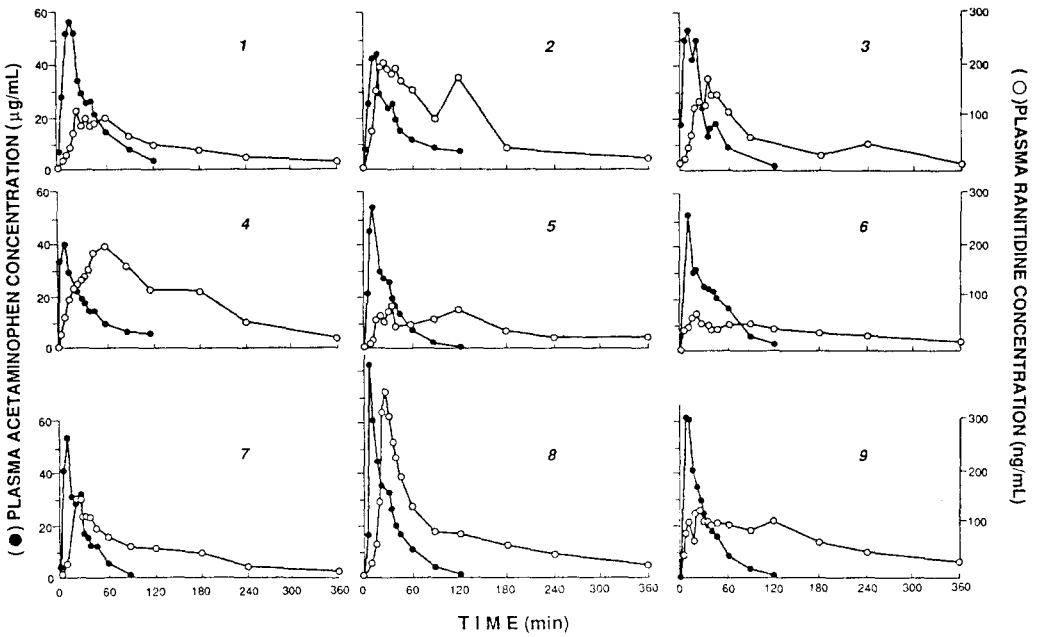

Fig. 1. Plasma concentration-time profiles of acetaminophen (●) and ranitidine (○) after simultaneous oral administration to rats.

RT after simultaneous oral administration of them are shown for each rat in Fig. 1. The plasma concentration profiles of AAP and RT showed multiple peaks in most rats. The multiple plasma peak phenomenon was more distinct for RT than for AAP.

In the case of AAP, the C_{max2} was much lower than the C_{max1}. The first peak of AAP appeared at approximately 15 min after the administration. Due to large intersubject variation of the T_{max2} and rather low C_{max2} in each rat, no notable second peaks of AAP appeared in the mean plasma concentration-time curve of nine rats (not shown as figure).

In the case of RT, the mean T_{max1}, T_{max2}, C_{max1} and C_{max2} of nine rats were 30 min, 120 min, 1.4 μg/m*l* and 0.8 μg/m*l*, respectively. Due to relatively high values of C_{max2} in each rat, a distinct second peak appeared in the mean plasma concentration-time curve of RT (not shown as figure).

The multiple peak patterns of AAP and RT in the present study were not different from the respective multiple peaks of them following separated administration[5,7,9], which implies no interactions between AAP and RT in terms of the gastric emptying or drug disposition in the body.

Several different mechanisms have been postulated as the possible causes of the multiple peaks of some drugs. Among them, the enterohepatic recycling for cimetidine[22], the absorption from the two sites in the GI tract for cimetidine[9], and the multfiractional absorption for cimetidine[18] could be ruled out for AAP and RT, and the BGE theory[1] seemed most plausible for AAP[4,5] and RT[7,21].

It should be noted that the multiple plasma peak profiles of drugs can vary considerably according not only to the gastric emptying patterns, but also to the pharmacokinetics of each drug, such as first-pass elimination, total body clearance (CL_t) and/or absorption rate constant (K_a)[7]. For example, if the stomach evacuates most of its content containing durgs at the first emptying and the rest of it at the second evacuation, the C_{max2} of the drugs which suffer severe first-pass elimination of Michaelis-Menten type will become very low compared with C_{max1}. AAP suffers the frist-pass elimination considerably[23], while RT does not[24]. It may explain why the C_{max2}/C_{max1} ratio of AAP are smaller than those of RT in each rat in Fig. 1.

The time lag between the first and second empty-

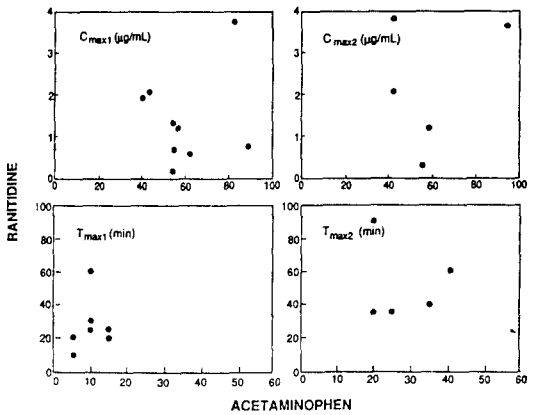

Fig. 2. Correlation of plasma profiles between acetaminophen and ranitidine in each rat. Each data point were read from Fig. 1. For the meaning of C_{max1}, C_{max2}, T_{max1} and T_{max2}, see text.

ing as well as CL_t and K_a of drugs will also affect the peak profiles (C_{max1}, C_{max2}, T_{max1} and T_{max2} of drugs[7-21]). If BGE is a true cause of the multiple peaks of AAP and RT in this study, the plasma profiles of the two drugs will be almost parallel with each other in each rat as long as the drugs are absorbed at the same site in the GI tract and the K_a of the two drugs are comparable with each other, and unless the CL_t and K_a of the drugs fluctuates considerably in each rat.

However, the results were not consistent with this expectation. Fig. 1 clearly shows that T_{max2} of the two drugs were not parallel in each rat, especially in rats 2, 5 and 9: a distinct second or third peak of RT appeared without any notable peak of AAP at 120 min. As a consequence, any significant linear correlation of T_{max1} or T_{max2} was not found between AAP and RT among the rats (Fig. 2). In this respect, the BGE theory seems to have some draw-backs in explanining the multiple peaks of AAP and RT despite the excellent explanation by Clement *et al.*[1].

Two more doubts could be raised against the BGE theory for AAP and RT. First, the multiple plasma peaks of AAP were found in one rat among ten rats which received *iv* AAP (40 mg/kg)[5]. Similar results were also reported for *iv* RT[7,24]. These facts imply that the multiple peaks are not necessarily associated with GI absorption process. Release to plasma pool of temporarily deposited drug in some tissue[19,20] can not be excluded from the mechanism of the multiple plasma peaks after *iv* administration. Second, our recent work on oral RT in man[6] showed an extremely small intrasubject variability of the multiple-peak patterns of RT over a period of one week. It seems quite unnatural considering the large inter- and intrasubject variabilities in duration of each phase of the interdigestive migrating motor complex (IMMC) and inter-IMMC interval[25].

Possible contribution of new mechanisms other than the BGE theory could not be ruled out completely for the multiple peaks of AAP and RT. However, more studies on the GI physiology and the pharmacokinetic parameters of the drugs should be accumulated before an appropriate criticism against the BGE theory is made.

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