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Notes

Noncontribution of enterohepatic recycling to multiple plasma peaks of acetaminophen in rats

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Summary

Multiple plasma peaks of acetaminophen (AAP) were observed following oral administration of an AAP (40 mg/kg) suspension to fasted rats. The multiple peaks were not diminished when the AAP suspension was administered to enterohepatic recycling-blocked (ERB) rats. Therefore, enterohepatic recycling does not contribute significantly to the multiple peaks of orally administered AAP in rats. After intravenous administration of AAP (40 mg/kg) solution to 10 rats, 10% of the rats showed distinct multiple plasma peaks. This indicates that multiple peaks may be unrelated to the gastrointestinal absorption process for some drugs.

Multiple peaks in the plasma concentration-time curves of some drugs following oral administration to human subjects have been reported. They include acetaminophen (AAP) (Clements et al., 1978; Shim et al., 1990), cimetidine (Ziemniak et al., 1986), ranitidine (Shim and Hong, 1989), furosemide (Hammarlund et al., 1984), veralipride (Staveris et al., 1985), naltrexone (Shepard et al., 1985), doxycycline (Pedersen and Miller, 1980a), piretanide (Brockmeier et al., 1986), sobrerol (Schkla, 1985), aspirin (Lui et al., 1986) and penicillamine (Wagner, 1984).

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The multiple peaks of drugs have often been attributed to one of the following mechanisms: (1) biphasic gastrointestinal (GI) absorption due to biphasic gastric emptying for AAP (Clements et al., 1978), cimetidine (Logan et al., 1978; Gugler et al., 1981; Kanto et al., 1981; Oberle and Amidon, 1987) and aspirin (Lui et al., 1986); (2) enterohepatic recycling (ER) for cimetidine (Grahnen et al., 1979; Ziemniak et al., 1986), furosemide (Smith et al., 1980; Waller et al., 1982), naltrexone (Shepard et al., 1985) and doxycycline (Pedersen and Miller, 1980a); (3) the presence of two different absorption sites in the GI tract or discontinuous absorption for cimetidine (Griffiths et al., 1977; Bodemar et al., 1979), furosemide (Chungi et al., 1979; Waller et al., 1982), veralipride (Staveris et al., 1985; Plusquel-

lec et al., 1987) and piretanide (Brockmeier et al., 1986); (4) multi-fractional absorption due to fractional dissolution of the dosage form in the GI tract for cimetidine (Funaki et al., 1986; Murata et al., 1989) and general drugs (Murata et al., 1987); and (5) release into the plasma pool of temporarily deposited drug in some tissues for cimetidine (Pedersen and Miller, 1980b; Pedersen, 1981) and ranitidine (Miller, 1984).

In spite of the various theories suggested thus far, the detailed mechanisms of the multiple peak phenomena of drugs remain to be elucidated. Several different mechanisms have been postulated as a possible cause of the multiple peaks of a drug. For example, as many as five mechanisms were suggested for cimetidine, and two for furosemide as cited above.

In a recent study (Shim et al., 1990), AAP showed very distinct second plasma peaks in all the male subjects ($n = 4$) on oral administration of AAP tablets (500 mg). Clements et al. (1978) suggested biphasic gastric emptying as the most probable mechanism of the multiple peaks of AAP in man. Their suggestion was based on the very strong correlation between gastric emptying patterns and plasma AAP profiles. However, their proposal itself does not mean enterohepatic recycling (ER) did not contribute to the second or third plasma peaks of AAP. Considering the fact that up to 20% of oral AAP is excreted into bile as glucuronides (Hjelle and Klassen, 1983), and that the parent drug might be reabsorbed from the small intestine after hydrolysis of the glucuronides to AAP, the possible contribution of ER to the multiple peaks appears to be worthy of examination.

Thus, the first objective of the present study was to determine how much ER could contribute to the multiple peaks of AAP in rats. If ER is a predominant cause of the multiple peaks, the second or third peaks of AAP are most likely to disappear and the area under the plasma AAP concentration-time curve (AUC) will also be decreased by blocking ER.

For this purpose, two male Wistar rats (230–250 g, 12 h fasted) were used simultaneously with one for the ER-blocked (ERB) rat and the other for the bile juice-donor rat. Under light ether anesthesia, the femoral artery of a rat was cannulated with PE-50 polyethylene tubing (Intramedic, Clay Adams, U.S.A.) for blood sampling. Then the abdomen of the rat was opened through a midline incision (3 cm) and two PE-10 tubings were inserted into the bile duct, one being upward toward the liver and the other downward toward the duodenum. This rat served as an ERB rat. Bile flow of the ERB rat was eliminated from the body through the catheter, and the eliminated bile flow was replenished from the bile juice-donor rat through the catheter. Replenishment was performed in order to avoid any possible change in the pharmacokinetics of AAP in the ERB rats due to the depletion of bile juice. After confirmation of spontaneous bile flow, the abdomens were sutured. The femoral artery of the ERB rat was cannulated with PE-50 tubing for blood sampling. After complete recovery from anesthesia, an AAP suspension in 0.2% (w/w) gum tragacanth (20 mg/ml of AAP) was administered orally at a dose of 40 mg/kg by gavage. A suspension was selected instead of a solution because of the poor water solubility of AAP. The ERB rats were kept in a supine position during the experiment. The body temperature of the rats was maintained at 37°C with a heat lamp. Blood samples (0.15 ml) were withdrawn through the catheter at 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 60, 90 and 120 min. After centrifugation, the plasma samples were frozen at -20°C until assay for AAP.

Sham operation was also performed on the rats to serve as a control group. The abdomen of the rat was opened and two PE-10 catheters were inserted into the bile duct with one toward the duodenum and the other toward the liver. Then the exposed ends of the two catheters were connected to each other to allow for bile flow toward the duodenum. After suture of the abdomen and recovery from anesthesia, an AAP suspension (40 mg/kg) was administered orally in the same manner as mentioned for ERB rats. Other procedures were identical to those described above. Four ERB rats and four control rats were used in this study.

AAP in plasma was assayed as follows. To 50 μl of plasma sample, 100 μl of 0.3 N barium hydroxide was added. After vortexing for 2 min,

100 μl of 5% (w/v) zinc sulfate was added and mixed. After vortexing for 1 min and centrifugation ($6000 \times g$) for 10 min, 20 μl of the supernatant was injected onto the HPLC column (30×0.39 cm i.d., stainless steel) containing 10 μm μ-Bondapak C18 reversed-phase material. The pump and UV detector (245 nm) were from Spectraphysics (model SP 8810) and Applied Biosystems (model 757), respectively. The mobile phase was an 11:89 mixture of acetonitrile and water. The flow rate was 1.0 ml/min and mean operating pressure was 60 bar. The AAP concentration in the plasma was determined from the peak height of AAP using a standard calibration curve prepared by adding known amounts of AAP to blank plasma. Linearity of the calibration curve was observed over the range 0.5–200 μg/ml, and the detection limit was 0.1 μg/ml. Recovery of AAP from the plasma sample amounted to 92.5%, and the intra- and interassay coefficients of variation were 5.5 and 6.7%, respectively.

AUC from time zero to 120 min was calculated according to the trapezoidal rule. 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.

Figs 1 and 2 show the plasma AAP concentration-time curves after oral administration of AAP suspension (40 mg/kg) to sham-operated (Fig. 1) and ERB rats (Fig. 2). Significant multiple peaks appeared in both groups of rats. This observation is consistent with our previous results in man (Shim et al., 1990), where all the subjects ($n = 4$) showed distinct double peaks in the plasma and saliva profiles of AAP. One rat in the control group and two in the ERB group showed third plasma peaks. Parameters relevant to the plasma peak-profiles of AAP in both groups are summarized in Table 1.

Contrary to expectation from ER theory, there were no significant differences between the control and ERB rats in the peak patterns (C_{max1}, C_{max2}, T_{max1}, T_{max2}) and AUC of AAP (Table 1). Therefore, it was concluded that ER does not contribute significantly to the multiple peaks of AAP. Furthermore, Staveris et al. (1985) also insisted that ER did not contribute to the second

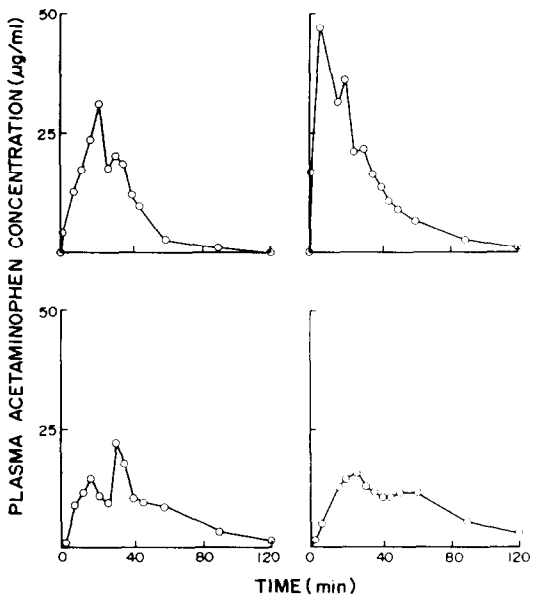

Fig. 1. Plasma concentration-time curves after oral administration of acetaminophen suspension (AAP 40 mg/kg) to control (sham-operated) rats.

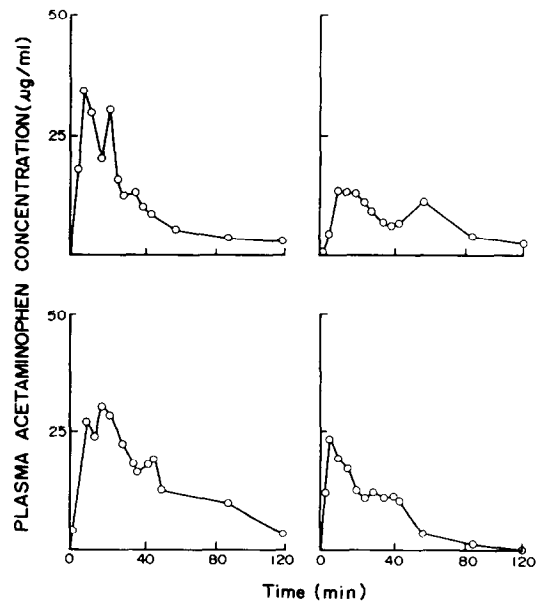

Fig. 2. Plasma concentration-time curves after oral administration of acetaminophen suspension (AAP 40 mg/kg) to enterohepatic recycling-blocked (ERB) rats.

peak of veralipride. Similar results have been reported for oral ranitidine in rats (Shim and Kim, 1991). On the other hand, the second plasma peak of [^{14}C]cimetidine disappeared in bile-cannulated rats (Ziemniak et al., 1986). The data are inadequate in that they represent only total radioactivity, and a possible contribution of ER cannot be ruled out. Therefore, it would appear most appropriate to conclude that ER does not contribute to the multiple peaks of AAP, ranitidine and veralipride, however, extrapolation of this conclusion to other drugs should be made with a great deal of caution and must entail further research.

The second objective of the present study was to ascertain whether the multiple peaks are correlated to GI absorption processes. Under light ether anesthesia, the femoral vein and artery of 10 rats were cannulated with polyethylene tubing (PE-50) for drug injection and blood sampling, respectively. After complete recovery from anesthesia, an AAP solution (10 mg/ml) in propylene glycol-saline (4 : 6) was injected through the femoral venous catheter at a dose of 4 ml/kg (40 mg/kg). After drug injection, blood samples (0.15 ml) were withdrawn through the femoral arterial catheter at 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 60 and 90 min. They were then centrifuged and the resultant plasma samples were stored frozen at −20°C until assay for AAP. Rats were kept in a supine position during the experiment and their body temperature was maintained at 37°C with a heat lamp. All other procedures were identical to those of the study in the ERB rats.

TABLE 1

Effect of enterohepatic recycling-blocking (ERB) on the multiple plasma peaks of acetaminophen in rats [a]

	C_{max1} (μg ml^{-1})	C_{max2} (μg ml^{-1})	T_{max1} (min)	T_{max2} (min)	AUC (μg min ml^{-1})
Control	26.8	22.2	15.0	30.0	1200.0
	(15.2)	(10.1)	(7.1)	(12.2)	(337.5)
ERB	19.5	15.7	6.3	31.3	1247.7
	(7.5)	(10.0)	(2.5)	(20.2)	(491.4)

[a] AAP (40 mg/kg) was orally administered as a suspension to the control ($n = 4$) and ERB ($n = 4$) rats. Each value is expressed as the mean + SD of four experiments.

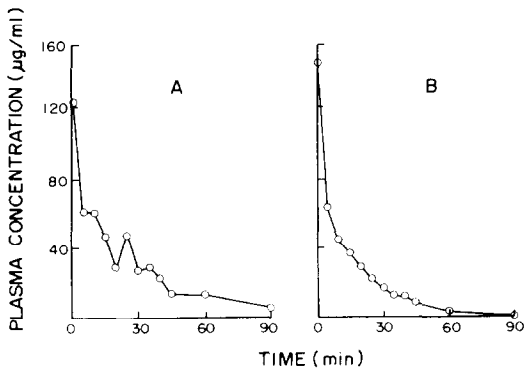

Fig. 3. Plasma concentration-time curves after i.v. administration of acetaminophen solution (AAP 40 mg/kg) to normal rats. (A) Data from the single rat among 10; (B) results from the remaining nine rats.

Interestingly, one of 10 rats showed a distinct plasma peak at 25 min. Fig. 3 depicts the result from this rat together with representative data on the remaining nine. The latter rats showed no distinct plasma peaks. The concentration of the peak was approx. 15 μg/ml higher than that of the preceding sampling point, indicating that the peak did not originate from assay error. This is consistent with a study on furosemide (Waller et al., 1982), where two out of 18 subjects who received i.v. furosemide showed peaks in their plasma concentration profiles of furosemide at 5–6 h. Similar results have also been reported for i.v. ranitidine (Lebert et al., 1981). These facts imply that the multiple peaks of certain drugs are not necessarily associated with GI absorption processes.

In conclusion, this study shows that (1) ER is not the major cause of the multiple peaks of oral AAP in rats, and (2) the multiple peaks of AAP can be produced in rats after i.v. administration. Extrapolation of these conclusions to other drugs or to man cannot be taken to be valid in every case. Further studies should be conducted to elucidate the exact mechanism of the multiple peaks of AAP.

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