J. Pharm. Pharmacol. 2000, 52: 957–961
Received November 2, 1999
Accepted April 20, 2000

Estimation of Oral Bioavailability in the Rat by the Accelerated Infusion Technique

ZUYU GUO, YONGYI LUO, GLENN L. DOBSON, MICHAEL P. MARIETTA, GERALD R. RHODES AND ISMAEL J. HIDALGO*

*Department of Drug Metabolism and Pharmacokinetics, Rhone-Poulenc Rorer Central Research, Collegeville, PA 19426-0107 and *Absorption Systems LP, Exton, PA 19341-2554, USA*

Short Communication

Determination of oral bioavailability is essential in drug discovery and drug development. The oral bioavailability of drugs is routinely determined as the ratio of the normalized area under plasma concentration–time curves (AUCs) after oral and intravenous dosing, although bioavailability determined in this way is usually very variable. Although inter-subject variability in estimates of bioavailability can be reduced by use of crossover study designs, this has the disadvantage of requiring several series of observations, which might be difficult to achieve with small laboratory animals. The quality of bioavailability values depends on the accuracy with which oral and intravenous AUCs are determined. Intravenous AUC can be determined with reasonable reproducibility only by taking many plasma samples during the early (distribution) phase. In contrast, selection of optimum sampling times after oral dosing is very difficult, especially during the discovery phase, when the pharmacokinetics of compounds is not well characterized.

Given the difficulties of obtaining good estimates of oral bioavailability by the conventional AUC-ratio method in small rodents, we decided to examine the possibility of estimating this parameter by use of the accelerated infusion method (Li et al 1997), a relatively new technique in which a pump controlled by a computer program delivers a dosing solution at constant acceleration. This mode of delivery enables better control on the output (observations). Because of reduced experimental variability, fewer animals, experiments and samples are required. A further reduction in the number of samples can be achieved by omitting sample collection in the initial equilibration period of the infusion. Higher drug concentrations, because of the nature of the drug delivery, greatly facilitate sample analysis.

Correspondence: I. J. Hidalgo, Absorption Systems LP, 440 Creamery Way, Suite C, Exton, PA 19341-2554, USA.

In an early study we showed that accelerated infusion could be used to obtain important pharmacokinetic information, such as systemic clearance, pharmacokinetic linear plasma concentration range, mechanisms for non-linearity, and organ extraction ratios (Li et al 1997). The objective of this study was to assess the feasibility of using accelerated infusion to estimate intrinsic oral bioavailability. The method might be only limited to the study of intrinsic bioavailability, i.e. compounds in solution and independent of gastric emptying. Conventional bolus dosing would have to be used to study the relative bioavailabilities of different formulations. Because the accelerated infusion method requires only the calculation of the slope of the straight lines (e.g. after oral and intravenous infusion), it is possible that this method can provide a more reproducible estimate of intrinsic bioavailability than the conventional AUC-ratio method.

Estimation of oral bioavailability

The conventional method of oral bioavailability determination entails comparison of dose-normalized AUCs after intragastric (i.g.) or intraduodenal (i.d.) dosing with that observed after intravenous (i.v.) bolus dosing. Thus, oral bioavailability (F) can be defined as in equation 1.

$$F = (AUC_{i.g.} \times Dose_{i.v.})/(AUC_{i.v.} \times Dose_{i.g.}) \text{ or}$$
$$F = (AUC_{i.d.} \times Dose_{i.v.})/(AUC_{i.v.} \times Dose_{i.d.}) \quad (1)$$

The methods used to determine oral drug bioavailability require administration of drug to the gastrointestinal tract and measurement of drug concentration in blood or plasma. Low oral bioavailability might be because of poor intestinal absorption or first-pass metabolism in the intestinal mucosa or the liver, or all of these (Figure 1).

After infusion into the rat duodenum, some of the drug might reach the systemic circulation after passing through the intestine and liver. The rate at

which a drug is delivered to the duodenum, i.e. the pump rate, R_{in}, is given by equation 2.

$$R_{in} = K_{acc} \times t$$

Under the assumed sink conditions, the rate at which a drug leaves the intestinal mucosa and enters the liver is given by $K_{acc} \times t \times Fabs$, where Fabs is the fraction of the dose absorbed. Finally, the rate at which a drug leaves the liver and enters the systemic circulation is $K_{acc} \times t \times F$, where F is the bioavailability. Systemic clearance (CL_s) can be determined from the slopes of plasma concentration–infusion time curves (Li et al 1997). For intravenous infusion, the slope of the straight portion of the curve is K_{acc}/CL_s. For intraduodenal infusion the slope of the straight portion of the curve is $K_{acc} \times F/Cl_s$. The bioavailability (F) can then be calculated as the ratio of the slopes after intraduodenal and intravenous infusion (Figure 2).

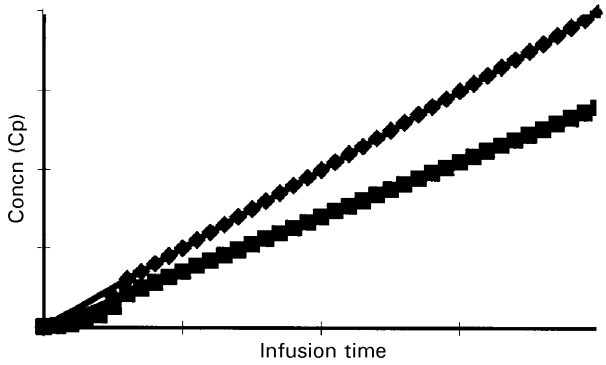

Figure 2. Simulated drug plasma concentration–infusion time profiles after intravenous (◆) and intraduodenal (■) infusion.

Materials and Methods

Chemicals

Paracetamol, aminopyrine, penicillin G, penicillin V, sulphamerazine, cephalexin and cefoxitin were purchased from Sigma (St Louis, MO). Solvents were HPLC grade from standard sources.

Accelerated infusion

Set-up and validation of the accelerated infusion system was performed as described elsewhere (Li et al 1997).

Each test drug was administered intravenously to rats (groups of four) by bolus dosing at a dose of $30\,mg\,kg^{-1}$. For oral bolus dosing the dose was $30\,mg\,kg^{-1}$ for paracetamol, cephalexin, and aminopyrine and $150\,mg\,kg^{-1}$ for penicillin V and penicillin G. Intravenous accelerated infusion was performed for all compounds with an acceleration constant of $2{\cdot}4\,mg\,h^{-2}$. Intraduodenal accelerated infusion was performed for paracetamol, cephalexin, and aminopyrine with an acceleration constant of $2{\cdot}4\,mg\,h^{-2}$ and for penicillin V and penicillin G with an acceleration constant of $12{\cdot}0\,mg\,h^{-2}$. Drugs were given intraduodenally and intravenously on separate occasions. After bolus dosing blood samples (0·25 mL, approx.) were collected from the jugular vein after 0 (pre-dose), 2, 5, 15, 30 and 45 min and 1, 2, 4, and 6 h. For accelerated infusion, blood samples (0·25 mL, approx.) were collected before infusion and every 30 min up to 3 h (paracetamol, cephalexin, and aminopyrine) or 4 h (penicillin V and penicillin G). Plasma samples were prepared by centrifugation of the blood samples at 15 000 *g* for 5 min.

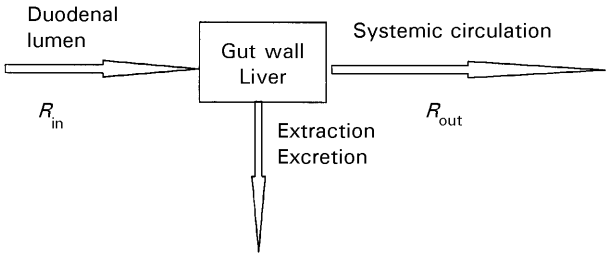

Figure 1. Infusion into the duodenum.

Compound selection

To assess the utility of the accelerated infusion technique for estimation of intrinsic oral bioavailability in the rat, paracetamol, cephalexin, aminopyrine, penicillin G, and penicillin V were studied. Paracetamol is highly absorbed and metabolized (Gaynall et al 1979; Gembory & Mudge 1981). Cephalexin is highly absorbed, and excreted, mainly renally, as unchanged drug (Kwan & Rogers 1983). Aminopyrine is well absorbed in rat and its absorption is apparently dependent on gastric emptying (Tsuzuki et al 1974). Penicillin G and penicillin V are poorly and intermediately bioavailable, respectively (Kwan & Rogers 1983). Both drugs are mainly excreted in the urine.

Animal model

Sprague–Dawley male rats, 350 g (approx.), were purchased from Hilltop Lab Animals (Scottdale, PA). The rats had one cannula placed into the jugular vein for blood collection and another either into the duodenum or the inferior vena cava for drug administration. During the process of infusion, rats were conscious and able to move freely in large

bowls (BAS/Carnegie Medicin, West Lafayette, IN). Drug solutions were infused through silastic tubing (0·02 inches i.d.; Dow Corning, Midland, MI) connected to the cannulae.

Sample analysis

Cephalexin was analysed with cefoxitin as internal standard according to a published procedure (Charles & Ravenscroft 1984). Paracetamol was analysed essentially according to a published procedure (Colin & Sirois 1987).

For aminopyrine analysis, plasma (100 μL) was mixed with internal standard solution (sulphamerazine in acetonitrile, 6·25 μg mL^{-1}; 200 μL). After vortex-mixing for 15 s and centrifugation at 1000 *g* for 5 min, the clear solution was transferred into a glass test tube (12 mm × 75 mm) and dried under nitrogen at 30°C. The residue was reconstituted in 20% acetonitrile (150 μL) and the resulting solution (100 μL) was injected for quantitation by reverse-phase HPLC on a 250 mm × 4·6 mm Inertsil ODS-3 column, at 25°C, with a guard column of the same packing. The mobile phase, 68 : 22 : 10 (v/v) water–acetonitrile–phosphate buffer (pH 6·0, 0·1 M) was delivered at 1·0 mL min^{-1}. UV detection was conducted at 250 nm. The total run time was 20 min, and the retention times of internal standard and drug were 7·9 and 16·1 min, respectively. The limit of quantitation was 0·1 μg mL^{-1}.

For penicillin G and penicillin V analysis the compounds were used as internal standards for each other. Plasma (100 μL) was mixed with acetonitrile (0·3 mL) and internal standard solution (10·0 μg mL^{-1} in 50 mM phosphate buffer, pH 6·8; 50 μL). After vortex-mixing for 15 s and centrifugation at 1000 *g* for 5 min the clear solution was transferred into a glass tube (12 mm × 75 mm) and dried under nitrogen at 30°C. The residue was reconstituted in phosphate buffer (50 mM, pH 6·8; 150 μL) and the resulting solution (100 μL) was injected for quantitation by reverse-phase HPLC on a 250 mm × 4·6 mm Inertsil ODS-3 column, at 25°C, with a guard column of the same packing.

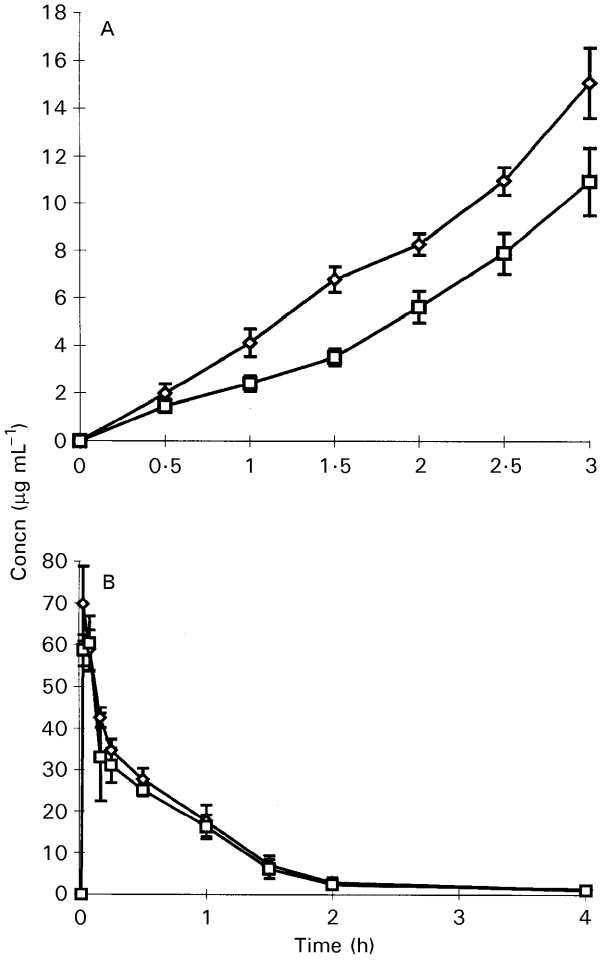

Figure 3. Plasma concentrations of paracetamol during intravenous (◇) and intraduodenal (□) accelerated infusion (A) and bolus dosing (30 mg kg^{-1}) (B).

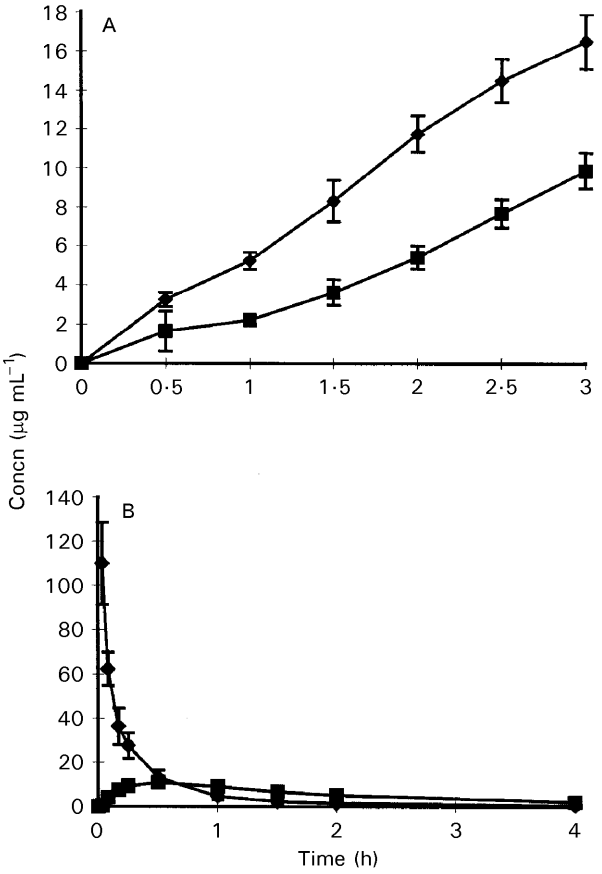

Figure 4. Plasma concentrations of cephalexin during intravenous (◆) and intraduodenal (■) accelerated infusion (A) and bolus dosing (30 mg kg^{-1}) (B).

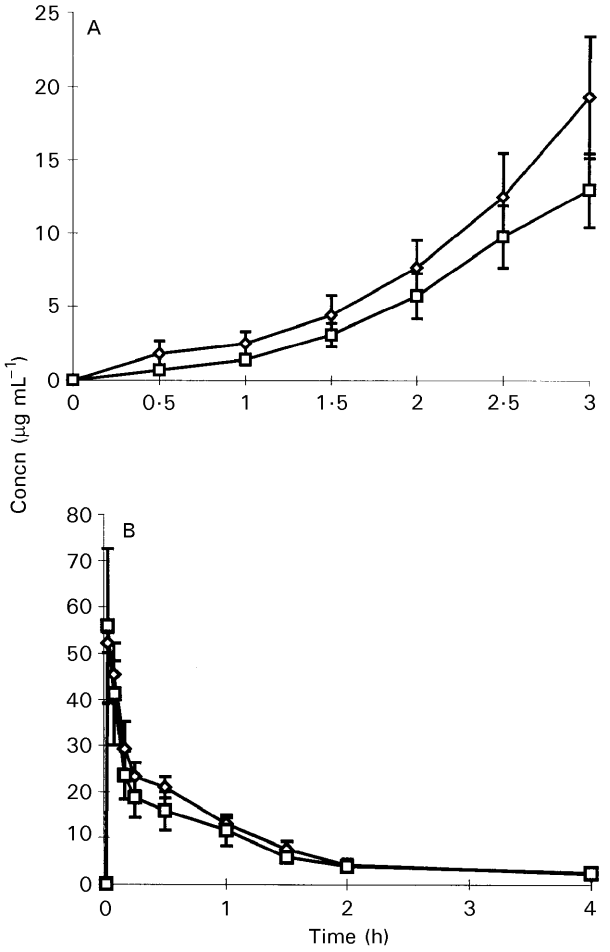

Figure 5. Plasma concentrations of aminopyrine during intravenous (◇) and intraduodenal (□) accelerated infusion (A) and bolus dosing (30 mg kg^{-1}) (B).

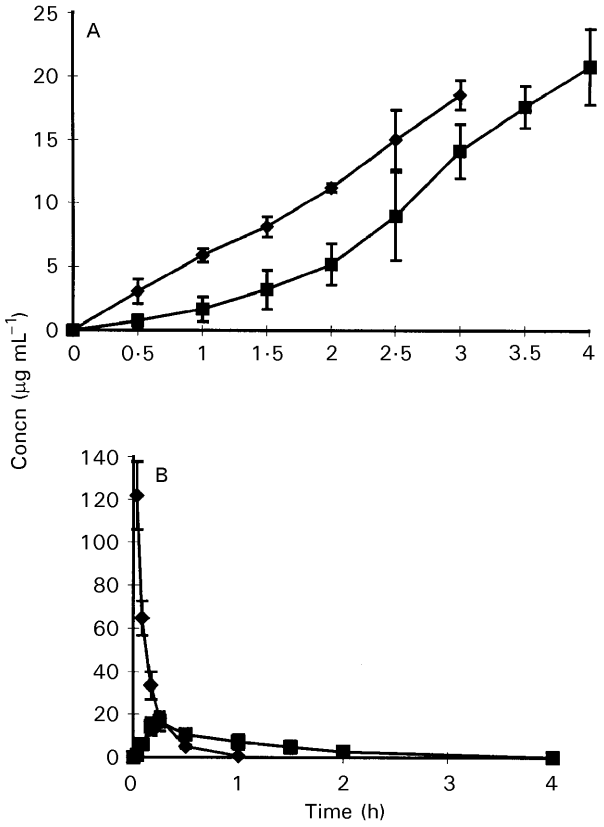

Figure 6. Plasma concentrations of penicillin V during intravenous (◆) and intraduodenal (■) accelerated infusion (A) and bolus dosing (30 mg kg^{-1}) (B).

The mobile phase, 40 : 40 : 20 (v/v) water–acetonitrile–0·1% phosphoric acid, was delivered at 1·0 mL min^{-1}. UV absorbance was monitored at 210 nm. The total run time was 20 min and the retention times of penicillin G and penicillin V were 11·5 and 15·5 min, respectively. The limit of quantitation for both drugs was 0·1 μg mL^{-1}.

Data analysis

After intraduodenal or intravenous bolus dosing AUCs were calculated, by non-compartmental methods, by use of WinNonlin (version 1·5; Pharsight). After intraduodenal or intravenous accelerated infusion, data points for the straight portion of the plasma concentration–infusion time curve were determined by linear regression, i.e. data points on both ends were included or excluded depending on their impact on the correlation coefficient (r^2). The slopes of the linear portion were calculated by linear regression analysis (Microsoft Excel; version 5.0).

Results and Discussion

The plasma drug concentrations of all five test compounds during accelerated infusion and after bolus dosing are presented in Figures 3–7. Intrinsic oral bioavailability values were calculated from the dose-normalized slope or AUC ratios and are summarized in Table 1.

Estimates of the bioavailability of paracetamol, a highly absorbed and highly metabolized drug, were very similar by both methods. Both values were higher than the reported oral bioavailability of 55% for paracetamol in rats (Belanger et al 1987). This difference might be because of the direct delivery of paracetamol into the duodenum, thus bypassing gastric emptying, or because a different dose of paracetamol (40 mg kg^{-1}) was used.

The bioavailability of cephalexin, a highly absorbed and poorly metabolized drug, was estimated to be 70% by accelerated infusion and 88·3% by bolus dosing. These values are comparable with the oral bioavailability of 85% (approx.) reported for cephalexin in rats (Sullivan et al 1969).

The bioavailability of aminopyrine was estimated to be 85% by accelerated infusion and 95·2% by bolus dosing. The values are qualitatively similar.

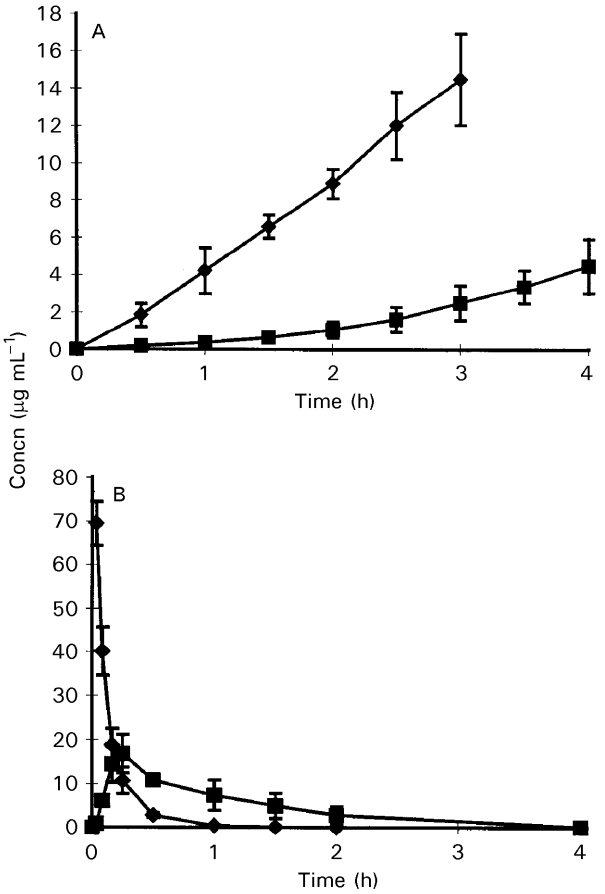

Figure 7. Plasma concentrations of penicillin G during intravenous (◆) and intraduodenal (■) accelerated infusion (A) and bolus dosing (30 mg kg^{-1}) (B).

Table 1. Comparison of bioavailability estimated by accelerated infusion and bolus dosing.

Drug	Ramping slope ratio (intraduodenal/ intravenous)	Bolus AUC ratio (intraduodenal/ intravenous)
Paracetamol	3·94/4·63 = 85·1%	24·47/27·94 = 89·3%
Cephalexin	3·93/5·62 = 70%	27·79/31·48 = 88·3%
Aminopyrine	8·42/9·90 = 85%	23·02/24·17 = 95·2%
Penicillin G	0·37/5·19 = 7·1%	0·83/11·89 = 7·0%

No reported literature value was available for the oral bioavailability of aminopyrine in rats. The values reported in this study might be higher, because absorption of aminopyrine has been shown to be dependent on gastric emptying (Tsuzuki et al 1974).

The bioavailabilities of penicillin V and penicillin G estimated by both methods were very similar. Both penicillins are mainly excreted into the urine, but are poorly absorbed because of inherently poor permeation (Kwan & Rogers 1983).

Conclusions

The intrinsic oral bioavailability of five drugs in rats estimated by accelerated infusion were similar or qualitatively comparable with those estimated by the conventional bolus dosing–AUC method. For the five drugs studied there is a good agreement between the qualitative rankings of intrinsic oral bioavailability values obtained by both methods. If good experimental designs are used it might be possible to obtain not only linear kinetic range and organ extraction ratio, but also intrinsic oral bioavailability in a single study with a relatively small number of animals.

References

Belanger, P. M., Lalande, M., Dore, F., Labrecque, G. (1987) Time-dependent variations in the organ extraction ratios of acetaminophen in rat. J. Pharmacokin. Biopharm. 15: 133–143

Charles, B. G., Ravenscroft, P. J. (1984) Rapid HPLC analysis of cefoxitin in plasma and urine. J. Antimicrob. Chemother. 13: 291–294

Colin, P., Sirois, G. (1987) Rapid high-performance liquid chromatographic assay of acetaminophen in serum and tissue homogenates. J. Chromatogr. 413: 151–160

Gaynall, W. E., Keller, J., Walker, B. E., Losornsky, M. S. (1979) The gastrointestinal absorption of paracetamol in the rat. J. Pharm. Pharmacol. 31: 157–160

Gembory, R. E., Mudge, G. H. (1981) Formation and disposition of the minor metabolites of acetaminophen in the hamster. Drug. Metab. Dispos. 9: 340–351

Kwan, K. C., Rogers, J. D. (1983) Pharmacokinetics of β-lactam antibiotics. In: Demain, A. L., Solomon, N. A. (eds), Antibiotics Containing the Beta-lactam Structure, Part II, Springer, pp 247–370

Li, J., Dobson, G. L., Marietta, M. P., Rhodes, G. R., Hidalgo, I. J. (1997) In vivo determination of drug kinetic linearity and individual organ elimination by the accelerated infusion technique. J. Pharmacol. Toxicol. Methods 37: 47–53

Sullivan, H. R., Billings, R. E., McMahon, R. E. (1969) Metabolism of cephalexin-^{14}C in mice and rats. J. Antibiot. 22: 195–200

Tsuzuki, O., Noda, A., Iguchi, S. (1974) Effect of gastric emptying on absorption of aminopyrine in rat. Chem. Pharm. Bull. 22: 2459–2462