J. Pharm. Pharmac., 1974, **26**, 937–944 *Received April* 1, 1974

Biliary excretion kinetics of phenolphthalein glucuronide after intravenous and retrograde biliary administration

J. H. GUSTAFSON AND L. Z. BENET

School of Pharmacy, University of California, San Francisco, California, U.S.A.

Several organic compounds of large molecular weight have previously been shown to be rapidly and primarily excreted via the biliary system of the rat after intravenous or retrograde biliary infusion. Poor biliary reabsorption has been suggested to explain these findings. The kinetics of the biliary excretion of phenolphthalein glucuronide have been examined with concurrent plasma data. A spectrophotometric method, capable of measuring phenolphthalein glucuronide in amounts as small as 4 nmol per 100 μl of plasma, was developed. The glucuronide (20–40 μmol kg^{-1}) was administered to 14 rats intravenously and to 12 rats by retrograde biliary infusion. There was a significant concentration of phenolphthalein glucuronide in the systemic blood after glucuronide administration by either route and the kinetics of elimination of the glucuronide were similar. The plasma availability of biliary infused doses was over 45% of the availability from the intravenous doses based on area under the curve calculations for the average plasma level-time curves. The results demonstrate the need to sample the plasma as well as the bile before any conclusion can be made about reabsorption of a compound from the biliary ducts.

In studies of the biliary uptake of organic compounds of large molecular weight in the rat, the possibility of some, albeit poor, absorption of representative compounds from the biliary tract was suggested by Clark, Hirom & others (1971). Czok & Dammann (1972) supported this suggestion by demonstrating that bromsulphthalein was absorbed from the bile duct into the hepatocytes of the rat. Nelson, Benet & Goldberg (1972) studied retrograde biliary infusion of iodipamide in the rabbit and found it to be rapidly reabsorbed (or leaked) into the systemic circulation.

We have examined biliary reabsorption phenomena for phenolphthalein glucuronide in the rat by measuring biliary and plasma concentrations. Phenolphthalein glucuronide, with a molecular weight of 495 for the anhydrous compound, falls within the category of substances of probable poor biliary reabsorption described by Clark & others (1971) who used this metabolite as a member of the group of large molecular weight organic anions (at physiologic pH) which are found in large amounts in the bile, and, thus, which may be relatively poorly reabsorbed from the primary bile. They found large amounts in the bile and small amounts, if any, in the urine. However, they presented no blood data to confirm the extent of reabsorption (or leakage) of the glucuronide into the systemic circulation from the primary bile. In this paper, a comparison of intravenous and retrograde biliary administration of phenolphthalein glucuronide, is presented with bile and concurrent plasma data.

METHODS

Animals

Male Sprague-Dawley rats 300–500 g were anaesthetized with pentobarbitone and occasionally with ether for short periods. Each bile duct was cannulated with PE 10 tubing with the cannula placed in the proximal third of the common duct preventing contamination of bile with pancreatic juices. The right jugular vein was cannulated with PE 50 or PE 60 tubing fitted to a luerlock with a syringe of heparinized saline in the lock.

For the intravenous studies, blood was drawn to the luerlock at zero time and a 10 or 20 mg kg^{-1} dose of phenolphthalein glucuronide (Sigma Chemical Co., St. Louis, MO) in saline (0·3–0·4 ml) was injected within 10 s, followed by a 0·4 ml saline wash.

In both the intravenous and retrograde biliary infusions, 8 to 10 blood samples (0·4 ml) were withdrawn at selected times up to 20 min after administration of the drug, these were immediately centrifuged and aliquots of plasma were withdrawn from the supernatant for assay.

For retrograde biliary infusion, a luerlock was placed on the PE 10 tubing. At zero time, a syringe with a 10 or 20 mg kg^{-1} dose of drug dissolved in 0·1 ml distilled water made isotonic by the addition of sodium chloride was placed in the lock and was introduced into the bile duct over 10 to 30 s followed by a 0·1 ml wash of normal saline. Thirty to 60 s later the catheter was cut, and the bile was allowed to flow freely.

Total bile was collected at selected times for up to 3 h after administration of the drug by either route.

One slow retrograde infusion of a 20 mg kg^{-1} dose of drug in a 0·1 ml of solution was made. A constant gravity pressure was the only infusing force. Before introduction of any drug, the PE 10 bile cannula was held vertically above the duct and bile allowed to flow until it was stopped when the bile reached a height of 11·8 cm in the cannula. The cannula was then placed horizontally until bile filled it and a zero time bile sample was collected. The drug solution (0·1 ml) was then placed beneath 0·2 ml of bile in the luerlock at a height of 18 cm above the rat. This retrograde gravity-assisted infusion lasted for 34 min, at which time the cannula was cut to 6·1 cm, and the bile allowed to flow freely. During the 34 min the drug solution was washed into the tree with 0·1 ml of bile. The visual line between the phenolphthalein glucuronide solution and the bile wash following it was sharp, suggesting a relatively complete dose administration.

Analytical

A modification of the spectrophotometric assay of Milburn, Smith & Williams (1967) was used. 8 N HCl (0·1 ml) was added to plasma or bile samples of known volume (generally 0·1 to 0·15 ml) and the mixture was hydrolysed for 1 h at 100°, partially neutralized with 5 N NaOH (0·1 ml) and made alkaline with 0·4 M glycine buffer, pH 10·4 (5·0 ml). Alkaline plasma and bile samples were stored in the dark for 24 h, and the absorbances of the samples were then read at 550 nm.

Control studies made after administration of saline solutions showed insignificant changes for plasma or bile baseline absorbances over the duration of the studies.

On some occasions the bladder was cannulated, and the urine was assayed for glucuronide content. Other control studies demonstrated insignificant changes in baseline absorbances for samples before acid hydrolysis of the glucuronide.

Plasma samples followed Beer's Law over a glucuronide concentration range of 4 to 200 nmol per 100 μl plasma. Bile samples followed Beer's Law over a glucuronide concentration range of 15 to 300 nmol per 100 μl bile.

RESULTS

Doses of 10 to 20 mg kg^{-1} (20 to 40 nmol kg^{-1}) of phenolphthalein glucuronide were administered to 14 rats intravenously and to 12 rats via the biliary route. Figure 1 shows representative biliary excretion rate curves for phenolphthalein glucuronide at 10 mg kg^{-1} for both routes of administration. Disposition rate constants for these

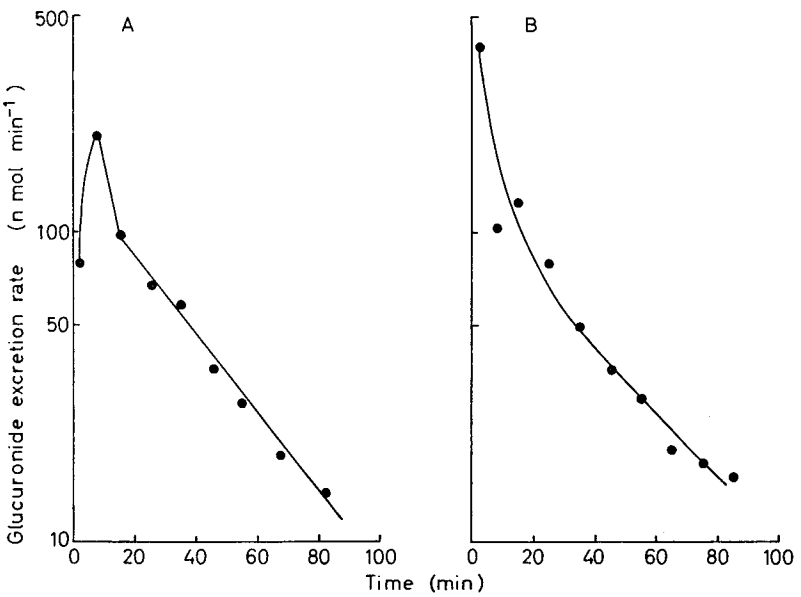

FIG. 1. Log biliary excretion rate of phenolphthalein glucuronide versus midpoint time curves: A—following a 10 mg kg^{-1} intravenous dose (Study 1, Table 1); B—following retrograde biliary administration of a 10 mg kg^{-1} dose (Study 1, Table 1). Lines drawn through the data are only to give the shape of the curves.

curves were calculated using a least-squares fit computer program for the log of the excretion rate versus the midpoint of the excretion interval. Excretion rate values for the time intervals preceding the terminal linear phase of excretion were excluded from the least-squares fitting of the data. Table 1 gives the rate constants and half-life data for 13 intravenous studies and for 12 biliary infusion studies. Average elimination rate constants and half-lives for the dosage subgroups are given in Table 2. Two sets of values are given for each dosage subgroup. The first line of values gives results for all the studies listed in Table 1 for each subgroup. The second line of values gives results for the studies in Table 1 excluding those marked *.

Table 3 summarizes the percentages of dose excreted via the biliary tract as a function of time after drug administration. Experiments in which erratic rates of bile

flow were observed (*Table 1) were not included in these calculations. Generally erratic bile flow rates resulted in over a 50% decrease in drug excretion for the times studied.

Within each of the four subgroups of phenolphthalein glucuronide administration, the glucuronide was evident in the plasma of every rat studied. Plasma concentra-

Table 1. *Terminal rate constants of elimination and corresponding half-lives for phenolphthalein glucuronide administered to rats by intravenous injection or retrograde biliary infusion at two dose levels.*

Intravenous administration			Biliary administration		
Study No.	k elim.	$t_{\frac{1}{2}}$	Study No.	k elim.	$t_{\frac{1}{2}}$
10 mg kg^{-1}					
1	0·035	19·8	1	0·034	20·4
2	0·015	46·2	2	0·029	23·9
3	0·014	49·5	3	0·016	43·3
4	0·018	38·5	4	0·029	23·9
5	0·027	25·7	5	0·032	21·7
6*	0·023	30·1	6*	0·035	19·8
			7*	0·035	19·8
20 mg kg^{-1}					
1	0·019	36·5	1	0·014	49·5
2	0·022	31·5	2	0·016	43·3
3	0·018	38·5	3	0·013	53·3
4*	0·026	26·7	4*	0·014	49·5
5*	0·033	21·0	5*	0·020	34·6
6*	0·025	27·7			
7*	0·015	46·2			

* Studies with erratic bile flow.
One intravenous result is not included because it gave values 10 times the s.d. for the group.

Table 2. *Average elimination rate constants and half-lives for the four subgroups of phenolphthalein glucuronide administration in the rat.* The first line of values includes data for all studies in Table 1; the second line of values excludes all studies marked * in Table 1.

Intravenous administration	Number of studies	k_{elim} (min^{-1})	$t_{\frac{1}{2}}$(min)	(Range of values)
10 mg kg^{-1}	6	0·022 ± 0·007	31·5	(19·8–49·5)
	5	0·022 ± 0·009	31·5	(19·8–49·5)
20 mg kg^{-1}	7	0·022 ± 0·006	31·5	(21·0–46·2)
	3	0·020 ± 0·002	35·5	(31·5–38·5)
Total	13	0·022 ± 0·007		
	8	0·021 ± 0·007		
Biliary administration				
10 mg kg^{-1}	7	0·030 ± 0·007	23·1	(19·8–43·3)
	5	0·028 ± 0·007	26·6	(20·4–43·3)
20 mg kg^{-1}	5	0·015 ± 0·003	46·2	(34·6–53·3)
	3	0·014 ± 0·002	49·5	(43·3–53·3)
Total	12	0·024 ± 0·009		
	8	0·023 ± 0·009		

tions decreased rapidly within the first few minutes after administration of the glucuronide, and the rate of decrease was similar within and between all subgroups.

Estimates of the half-life of elimination from the plasma compartment for each of the four subsets of glucuronide administration are presented in Table 4. Half-lives for both intravenous and biliary administration were also estimated from the log linear lines resulting from plots of the residuals of log biliary excretion rate versus time. These half-lives ranged from 5 to 9 min.

Table 3. *Average and range of values of the percentages of dose of phenolphthalein glucuronide excreted via the biliary tract as a function of time after drug administration for the four subgroups of phenolphthalein glucuronide administration in the rat.*

Intravenous administration	Number of studies	% of dose recovered (range of values) in bile during first: 5 min	15 min	30 min	60 min	180 min
10 mg kg^{-1}	5	4 (3–6)	26 (23–34)	43 (36–51)	57 (48–64)	73 (72–74)[a]
20 mg kg^{-1}	3	4 (1–6)	20 (14–27)	34 (27–42)	48 (40–56)	62 (54–66)
Biliary administration						
10 mg kg^{-1}	5	23 (19–29)	36 (30–40)	45 (36–53)	54 (46–67)	60 (51–67)
20 mg kg^{-1}	3	9·9 (6·6–12)	19 (13–23)	25 (18–30)	40 (36–46)	60 (50–63)

[a] Data from two studies.

Table 4. *Ranges of half-lives estimated from log plasma concentration-time plots of the individual studies within the four subgroups of phenolphthalein glucuronide administration in the rat.*

Dose	$t_{\frac{1}{2}}$ i.v.	$t_{\frac{1}{2}}$ biliary
10 mg kg^{-1}	3–8 min	7 min
20 mg kg^{-1}	3–6 min	5–10 min

Fig. 2 presents a log plot of the geometric averages of the plasma concentration versus time for the 10 mg kg^{-1} dose of phenolphthalein glucuronide after intravenous and biliary administration. The least-squares fit of the log-linear phase of these plots gives a rate of elimination of 0·083 min^{-1} after intravenous administration and 0·076 min^{-1} after biliary administration. These rates correspond to an elimination half-life of 8·4 min for the intravenous route and 9·1 min for the biliary route.

Controls demonstrated that the plasma and biliary concentrations measured were not functions of changing baseline absorbances. The measurements made before acid hydrolysis indicated that negligible quantities of unmetabolized phenolphthalein were present in the bile and blood samples. Insignificant amounts of glucuronide were present in the urine which is consistent with the findings of Clark & others (1971) that less than 2% of the glucuronide was present in urine 60 min after glucuronide administration.

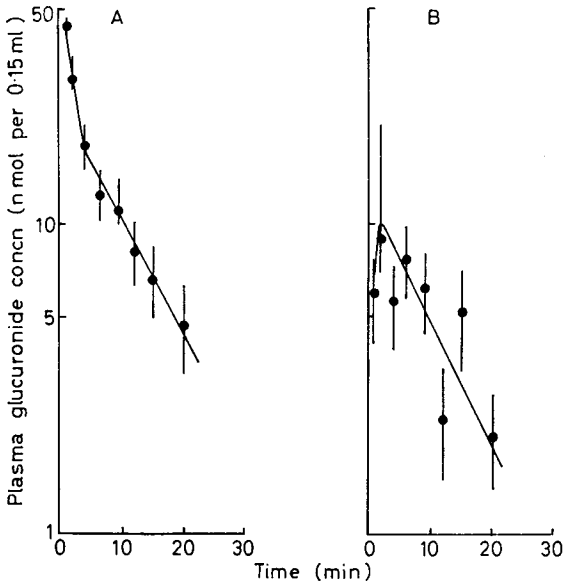

FIG. 2. Log plasma concentration of phenolphthalein glucuronide versus time curves: A—following intravenous administration of a 10 mg kg^{-1} dose; B—following retrograde biliary administration of a 10 mg kg^{-1} dose. Data are the geometric averages of combined subgroup values. Vertical lines represent standard deviations around each point. Lines drawn through the data are only to give the shape of the curves.

DISCUSSION

As may be seen from Fig. 2 there is a significant concentration of phenolphthalein glucuronide in the systemic blood after glucuronide is administered intravenously or by biliary infusion according to Clark & others (1971).

Plasma availability of a drug administered by different routes may be compared using the areas under the plasma level-time curves, summed from time zero to time infinity. Area under the curve from zero time to the last data point was calculated by the trapezoidal rule. A plasma concentration at that final time was calculated from the computed line of best fit to the log concentration versus time data, and the area under the curve from that time to infinity was calculated according to the following equation: $AUC_{t\to\infty} = \frac{Cp_t\, t_{\frac{1}{2}}}{0{\cdot}693}$. The $t_{\frac{1}{2}}$ in this equation is calculated for each grouped subset. For the lower dose (10 mg kg^{-1}) series of experiments, areas of 163 and 340 nmol-min per 0·15 ml were calculated for the retrograde biliary and intravenous administrations, respectively, using the geometric averages of the experimental plasma data at each time. Thus, the average plasma availability of the 10 mg kg^{-1} biliary-infused dose was about 46% of that of the intravenous dose.

For the 20 mg kg^{-1} dose, the areas under the curves were within 50% of each other with the area under the curve for the biliary route reaching the higher value. However, since these areas were calculated excluding the studies marked * in Table 1, these data are for two groups of three studies each. Since it was not possible to run a cross-over study in these animals, little significance can be placed on the relative areas from two different doses administered by two different routes in two different

routes in two different populations, beyond pointing out that there is significant plasma availability for the doses administered via the biliary duct, probably greater than 40%.

The large values for AUC measurements following retrograde biliary infusion suggest that the glucuronide is absorbed well enough by this route to require kinetic treatment in the same manner as data obtained after intravenous administration. When the glucuronide is administered by either route, the half-lives of plasma elimination are approximately equal (See Table 4) and the average terminal half-lives (for both doses) calculated using biliary excretion data are also similar (Table 2) but much greater than those values seen in the plasma. However, when the residuals of the biliary excretion plots are taken (see Results), the half-lives for the feathered lines yield approximately equivalent values to those seen for the plasma elimination curves. Thus, it would appear that the disappearance of drug from the plasma is not the rate limiting step in biliary excretion of this metabolite.

The percentages of glucuronide excreted in the bile as a function of time (Table 3) are lower than those reported by Clark & others (1971), yet they appear consistent with the 67 to 87% range reported by Milburn & others (1967) for drug excreted via the bile within 24 h of intraperitoneal administration of the glucuronide to three animals.

Peterson & Fujumoto (1973) point out that Clark's method of retrograde injection must distend the biliary tree, since the volume of the solutions injected exceeds the maximum capacity of the biliary tree. Peterson and Fujumoto claim that it is necessary to keep the injection volume below this maximum capacity to "accurately represent the biliary tree's absorption potential." The volumes Peterson & Fujumoto (1973) used for retrograde injection ranged from 18 to 46 μl. Their data indicated greater percentages of administered compounds recovered in the bile upon injection of the smaller volumes. They suggest that with the smaller volumes, which should distend the biliary tree less, the absorption process is most truly represented. However, it is possible that the decrease in percentage compound recovered with increasing retrograde volume administered may be a function of more extensive presentation of injected compound to the "absorption" sites. The smaller volumes could more easily be washed out of the distal portion of the biliary duct without ever being exposed to the absorption processes within the biliary tree.

Support for the capacity of the biliary system to handle the distension created by larger volumes of injected solutions and increased back pressure is presented by Barber-Riley (1963) in his studies on obstruction of the rat common bile duct. He used the normal bile flow found at the end of experiments with back pressures up to 150% of the maximum secretory pressure as an indication of no apparent damage to the secretory system. Although the absorption potential of Clark's system may well depend on the volume injected, there is no apparent indication of damage to the secretory system using Barber-Riley's criteria of bile flow for the studies in Table 3. When only those studies in Table 1 not marked * are used to calculate elimination rate constants, there is no significant change in average values for all four dosage subgroups (i.e., compare line 1 with line 2 in each study in Table 2). Nor is there any significant change in plasma rate constants and half-lives. This indicates that even with slower or more erratic bile flow rates, the rate constants of elimination are not significantly altered. That is, the rate constants of biliary elimination of glucuronide appear independent of the biliary flow rate for the two glucuronide dosages and for

the range of biliary flow rates observed in these experiments. For example, in comparing studies 1 and 6 for the 10 mg kg^{-1} biliary administration (Table 1), almost identical rate constants of elimination were obtained (0·034 and 0·035). However, the 1 h % dose excreted for study 1 is 67% while the corresponding value for study 6 is 35%. The concentrations of bile samples for study 1 were approximately twice those seen for study 6.

In addition to the criteria of bile flow, there is no apparent indication of damage to the secretory system due to rapid distension of the biliary tree. This was investigated by comparing the rapid retrograde infusion studies with the slow gravity-assisted infusion study. The plasma half-life for the slow infusion study (estimated from the apparent line of best fit to the log concentration versus time data after infusion) was 9·5 min. The biliary excretion rate constant, calculated by least squares fitting of log excretion rate versus midpoint of the excretion interval, for excretion intervals occurring after the infusion ended was 0·012 min^{-1}. Although the biliary rate constant is slightly lower than the other three rate constants for 20 mg kg^{-1} biliary administration, it is very close to the range of values presented. The plasma half-life estimate for the gravity-assisted infusion correlates very well with the plasma half-lives calculated from the data in Table 4.

CONCLUSION

A significant concentration of phenolphthalein glucuronide was found in the plasma after administration of this metabolite by both the intravenous and retrograde biliary routes. The kinetics of elimination of this glucuronide are similar by both routes. Area under the curve calculations demonstrated plasma availabilities of metabolite administered by retrograde biliary administration to be at least 45% of the plasma availabilities for the same doses administered intravenously. The results of these studies demonstrate the need to sample the plasma as well as the bile before one can conclude whether a compound is reabsorbed from the biliary ducts.

Acknowledgements

The authors wish to acknowledge the technical assistance of Mr. Peter Matsumoto and Mr. Richard Nishimoto with selected studies presented here.

This study was supported in part by Project Grant GM 16496 in the Institute of General Medical Sciences, National Institute of Health.

REFERENCES

BARBER-RILEY, G. (1963). *Am. J. Physiol.*, **205**, 1127–1131.

CLARK, A. G., HIROM, P. C., MILLBURN, P., & SMITH, R. L. (1971). *J. Pharm. Pharmac.*, **23** 150–152.

CZOK, G. & DAMMANN, H. G. (1972). *Ibid.*, **24**, 820–821.

MILLBURN, P., SMITH, R. L. & WILLIAMS, R. T. (1967). *Biochem. J.*, **105**, 1275–1281.

NELSON, J. A., BENET, L. Z., & GOLDBERG, H. I. (1972). *J. Pharm. Pharmac.*, **24**, 993–995.

PETERSON, R. E. & FUJUMOTO, J. M. (1973). *J. Pharmac. exp. Ther.*, **185**, 150–162.