Lumen Perfusion of the Human Rectum *In Situ*: A Method to Study Mechanisms for Rectal Drug Transport in Humans

W. M. BOTTGER ˣ, A. J. M. SCHOONEN, J. VISSER, and D. K. F. MEIJER

Received September 25, 1981, from the *Department of Pharmacology and Pharmacotherapeutics, Subfaculty of Pharmacy, State University of Groningen, 9713 AW Groningen, The Netherlands.* Accepted for publication November 12, 1982.

Abstract □ A lumen perfusion system was developed to study rectal transport mechanisms in humans. With this technique it is possible to perfuse a well-defined area of the rectum wall under single-pass and recirculation conditions. Sodium benzoate was used as a test drug. After absorption, sodium benzoate is conjugated with glycine to give hippuric acid which is rapidly eliminated ($t_{1/2}$ = 0.5 h). Due to the short half-life it is possible to reach a steady-state concentration within 2.5 h of perfusion. Plasma concentrations of hippuric acid were determined by HPLC. The absorption influx of sodium benzoate per unit area (Φ_{in}) could be calculated using the steady-state concentration of hippuric acid during rectal perfusion, the separately measured total body clearance after intravenous injection, and a designated absorption surface of the rectum. It was shown that with this technique reproducible Φ_{in} values within one subject could be obtained. Four volunteers were perfused with four different solutions of sodium benzoate, and it was found that Φ_{in} was proportional to the four concentrations used. In the case of recirculation perfusion (two volunteers), it was found that the amount of the perfusate lost equalled the amount absorbed into the general circulation. Therefore, the possibility of major drug accumulation in the rectal lumen or mucosa could be excluded. The perfusion technique elaborated in the present study can be used to investigate the mechanism of rectal absorption in humans as well as the factors that may influence this process.

Keyphrases □ Perfusion—lumen of the rectum, *in situ* technique in humans, sodium benzoate □ Drug transport, rectal—lumen perfusion, *in situ* technique in humans, sodium benzoate □ Sodium benzoate—*in situ* lumen perfusion of the rectum, humans

Drug absorption from the rectum is assumed to occur by mechanisms similar to those operating in other parts of the GI tract (1). Numerous studies have been done on drug absorption mechanisms *in vitro* as well as *in vivo*. Probably the most important mechanism of absorption is passive diffusion (2–5), but other mechanisms like convective, active, and ion-paired transport cannot be excluded (6, 7). Results from *in vitro* or *in vivo* rectal absorption studies in rats or rabbits are not always in agreement with the results from rectal absorption studies in humans (1, 8). Apart from anatomical species differences, an important factor in this respect may be the rectal formulations used, *e.g.*, suppositories and enemas.

Moolenaar and Schoonen (9) have investigated the rectal administration of various drugs with attention to the influence of physicochemical properties of drugs on the release from dosage forms *in vitro* and the absorption process *in vivo*. From these studies it was concluded that factors which markedly influence the delivery *in vitro* (*e.g.*, composition of suppository bases) have relatively little influence on the absorption *in vivo*. It was also shown that in case of poorly water-soluble drugs, the rectal absorption rate is determined by the release surface of the dosage form rather than the concentration of the drug. Finally, employing liquefied dosage forms they found a varying influence of pH of the administered solution on absorption rate.

When introducing a suppository or a rectal enema, however, neither the covered surface in the rectum nor changing conditions at the absorption site (*i.e.*, the rectal lumen) can be defined. Therefore, it was necessary to develop a technique that enabled these kinds of measurements. Devroede and Phillips (10) studied rectal absorption rates by perfusing a limited part of the rectal lumen, which involved separating the rectal lumen from the lumen of the colon by means of a distendable balloon fixed to the end of the perfusion apparatus. Bechaard (11) used an open perfusion technique with inner and outer tubes at a fixed distance from each other, and then instilled the perfusion solution until an equilibrium in the rate of inflow and outflow was reached and at this time measured the absorption. The rate and extent of absorption was determined either by the disappearance of the substance from the perfusion medium (10) or the appearance of the substance in the systemic circulation (11). A major problem encountered in these experiments was that a significant loss of perfusate through the colon could not be excluded, and therefore, the rectal absorption *per se* could not be accurately measured.

Other studies on rectal absorption in the human have been done with a dialyzing tube attached to a solid catheter, which was installed for 1 h in the rectum (12, 13). The initial and final drug concentrations of the rectally applied solution were measured to ascertain the amount absorbed. Although with these methods it was, in principle, possible to study rectal absorption as a function of the concentration in the perfusate, the technique did not allow quantitative measurement of the absorption process in a well-defined part of the rectum.

Therefore, a human rectal perfusion apparatus was developed enabling perfusion of a well-defined area of the rectal mucosa under single-pass and recirculation conditions. In the case of single-pass experiments, the apparatus delivers a solution with a constant rate creating a constant concentration of the substance under study within the lumen of the rectum. The absorption process can be measured as an influx per unit area of the rectal wall. In the case of the recirculation experiments, concentration in the perfusion fluid decreases. Since the total circulating volume can be estimated, the amount eliminated from the perfusate can be determined and compared with the total amount absorbed by the rectal wall.

Sodium benozate was used as the test drug because of its low toxicity, good water solubility (1:1.8), and convenient pharmacokinetic properties. It has been shown that after rectal administration, absorption of sodium benzoate is fast and complete (14). Benzoic acid is very rapidly conjugated with glycine in the liver to give hippuric acid.

0022-3549/84/0100-0095$01.00/0

Table I—Recovery of Hippuric Acid from Human Plasma

Concentration Added, μg/mL	Concentration Determined[a], μg/mL	CV, %	Recovery, %
0.75	0.74 ± 0.10	13.5	98.7
1.50	1.39 ± 0.17	12.2	92.6
3.00	2.99 ± 0.33	11.0	99.6
6.00	6.01 ± 0.30	5.0	100.2
12.00	11.76 ± 0.46	3.9	98.0
18.00	17.68 ± 0.77	4.4	98.2
24.00	23.62 ± 0.85	3.6	98.4

[a] Mean ± *SD*, $n = 7$.

Hippuric acid is excreted in urine almost quantitatively with a biological half-life of ~30 min. It was also shown that benzoic acid absorption can be estimated by measuring plasma concentrations of hippuric acid, provided that the benzoic acid level does not exceed ~0.4 mg/mL (15). When this concentration is exceeded, the hepatic glycine pool may become exhausted and, in addition to hippuric acid, unchanged benzoic acid will accumulate in the plasma. In the present study benzoic acid levels in plasma remained below detectable amounts.

EXPERIMENTAL

Solution Preparation—The perfusion solutions employed contained 116, 155, 232, and 310 mmol of sodium benzoate[1], respectively. A sodium benzoate solution of 155 mmol is isotonic with blood plasma. The other solutions were not corrected for osmolarity to exclude possible effects of additives.

A 0.02 M buffer solution (pH 7.2) was prepared by dissolving 5.024 g of disodium hydrogen phosphate 12-hydrate[2] and 0.824 g of sodium dihydrogen phosphate 1-hydrate[3] in 1000 mL of distilled water. An internal standard solution was prepared by dissolving 0.50 g of 4-methoxyphenylacetic acid[4] in 1000 mL of methanol[5]. A standard solution of benzoic acid was prepared by dissolving 0.177 g of sodium benzoate in 100 mL of distilled water. A standard solution of hippuric acid was prepared by dissolving 0.168 g of sodium hippurate[6] in 100 mL of distilled water.

Chromatographic Conditions—A high-performance liquid chromatograph[7] was equipped with an automated sampling injector[8] and a spectrophotometric variable-wavelength detector set at 254 nm. Samples were analyzed on a 4.6-mm × 25-cm reverse-phase column[9] fitted with a 2.1-mm × 10-cm precolumn[10]. The mobile phase was a solvent system of methanol in 0.02 M Na_2HPO_4/NaH_2PO_4 buffer (15:18, pH 7.2), and the flow rate was 2 mL/min at a pressure of 1700 psi.

Assay of Plasma Samples—To 1.0 mL of plasma in a 10-mL glass-stoppered tube, 3.0 mL of the internal standard solution was added. After shaking for some minutes, the tube was centrifuged for 5 min at 4000 rpm, and 25 μL of the supernatant liquid was injected into the HPLC. The retention times were 3.0, 3.8, and 5.5 min for benzoic, hippuric, and 4-methoxyphenylacetic acids, respectively.

The ratio of the peak height of the sample component to that of the internal standard was used to calculate concentrations of benzoic acid and hippuric acid, based on calibration curves prepared from spiked plasma samples (Table I). With 1.0-mL plasma samples this method is accurate to concentrations as low as 1.5 μg/mL for benzoic acid and 0.75 μg/mL for hippuric acid. The recovery from plasma was determined for seven different concentrations of hippuric acid and was found to be essentially complete.

Kinetic Studies—Because of the short half-life of benzoic acid it was possible to reach a steady-state blood concentration within the perfusion period of 2.5 h. At steady-state concentration (C_{ss}) the amount of hippuric acid eliminated per unit of time should be equal to the amount absorbed. Since the perfused surface of the rectum can be estimated with the method described, it was possible to calculate the absorption rate per unit area (Φ_{in}) as follows:

$$\Phi_{in} = \frac{C_{ss} \times CL}{\text{Area}} \times 0.68\ \mu\text{g of benzoic acid/min} \cdot \text{cm}^2 \quad \text{(Eq. 1)}$$

in which the factor 0.68 accounts for the molar ratio of benzoic acid to hippuric acid, C_{ss} is the mean steady-state plasma concentration calculated from the last four data points of the plasma curve (μg/mL), *CL* is the total body plasma clearance (mL/min), and the perfused rectal area is calculated from the length and the volume of the filled dialysis membrane (cm^2).

The total body clearance of hippuric acid was determined for each volunteer participating in the perfusion experiments. Sodium hippurate (660 mg in 10 mL of distilled water) was injected in the cubiti medialis vein. Blood samples (8.0 mL) were taken from the other arm 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, 120, and 150 min postinjection. The blood samples were heparinized and centrifuged. The plasma thus obtained was immediately frozen until analyzed.

From the hippuric acid plasma disappearance curve, the pharmacokinetic parameters were calculated by a nonlinear curve-fitting program (16). Control experiments, in which hippuric acid clearance was determined several times within a few months, showed a good reproducibility with variations not deviating more than 5% from the mean value. To investigate whether the total body clearance of hippuric acid was dose dependent, a lower dose (250 mg of sodium hippurate in 10 mL of distilled water) was administered in addition to the high dose to three volunteers.

Perfusion Technique and Procedure—The apparatus consisted of an outer tube with a funnel-shaped end and an inner tube ending in a sphere with holes. Both tubes could be adjusted with respect to each other, so that the distance between sphere and funnel could be changed from 1 to 12 cm. A dialysis bag[11] (diameter 2.9 cm) was tightened over the funnel and sphere (Fig. 1).

The perfusion solution, warmed in a water bath to 37°C, was pumped by a peristaltic pump[12] through the outer tube into the dialysis bag and drained away through the holes of the inner tube (Fig. 2). In the case of single-pass studies, the perfusate was collected *via* an overflow vessel and a calibrated tube with a tap. By closing the tap, the flow rate of the effluent fluid can be estimated and compared with the infusion rate (10 mL/min). The difference in height between the outlet of the perfusion apparatus and the outlet of the overflow vessel was maintained at 110

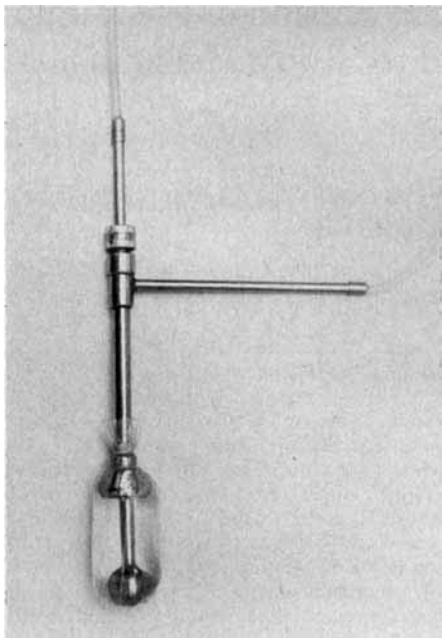

Figure 1—*Perfusion apparatus with filled dialysis bag.*

[1] Brocacef E 211, Maarsen, Netherlands.
[2] Merck 6579, Darmstadt, West Germany.
[3] Merck 6346, Darmstadt, West Germany.
[4] Aldrich M 1920-1, Beerse, Belgium.
[5] Merck 6009, Darmstadt, West Germany.
[6] Sigma, Chemical Co., St. Louis, Mo.
[7] Waters Associates model 440, Milford, Mass.
[8] Wisp; Waters Associates, Milford, Mass.
[9] Lichrosorb 10 RP 18; Chrompack, Middelburg, Netherlands.
[10] Vydac 201 SC; Chrompack, Middelburg, Netherlands.
[11] Thomas technological service, 3787 D50, Mw, Cutoff 12,000; Philadelphia, Pa.
[12] Gilson minipuls 2; Meyvis and Co., Bergen op Zoom, Netherlands.

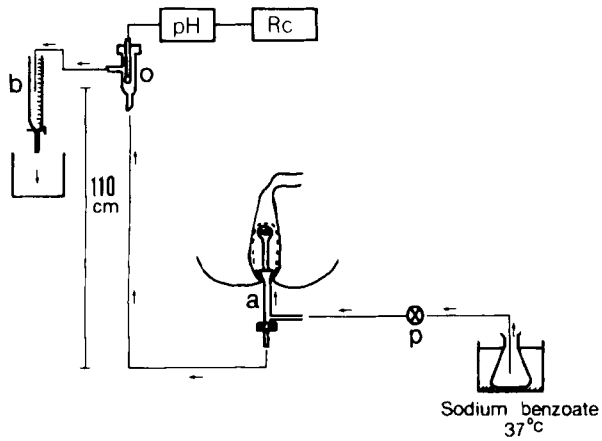

Figure 2—*Experimental setup for rectal absorption measurement* in vivo. *Key: (a) perfusion apparatus, (p) peristaltic pump, (o) overflow vessel, (b) buret, (pH) pH-electrode, (Rc) recorder. Arrows indicate the direction of flow.*

cm such that the inner pressure of the dialysis bag was constant. Due to the positive pressure in the system, the dialysis bag regained its maximal volume. A pH electrode was placed in the overflow vessel to record the pH of the perfusate during perfusion.

In the case of recirculation, a constant volume of perfusate was circulated by connecting the tube of the overflow vessel with the inlet tube of the perfusion apparatus. Before starting an experiment all the air was expelled by filling the whole system with perfusion solution. After emptying the dialysis bag, the apparatus was introduced into the rectum by placing the funnel on the inner side of the anus (Fig. 2). The perfused area of the rectum wall was in close contact with the surface of the dialysis bag.

The volunteers fasted overnight and throughout the perfusion experiment. Consequently, the endogenous hippuric acid level was almost zero before starting the infusion of sodium benzoate, confirming the results of Moolenaar *et al.* (14). One hour before the actual start of a perfusion, the rectum was cleaned by a phosphate buffer enema. The results of the experiment were rejected if the dialysis bag appeared to be contaminated with feces at the end of the perfusion.

The subject was seated on a specially designed chair so that the perfusion tube with connections did not cause inconvenience. After introducing the apparatus and filling it with the warm solution of sodium benzoate, the rectum was constantly perfused for 2.0–2.5 h under recirculation or single-pass conditions. Blood samples of 8.0 mL were taken every 15 min from a forearm vein and the plasma analyzed for hippuric acid and benzoic acid. Four volunteers were perfused for 2.5 h under single-pass conditions with four different concentrations of sodium benzoate on four different days. The time between the experiments was at least 14 d. From the established steady-state concentration, the Φ_{in} was calculated as described.

Two volunteers were perfused under recirculation conditions with a

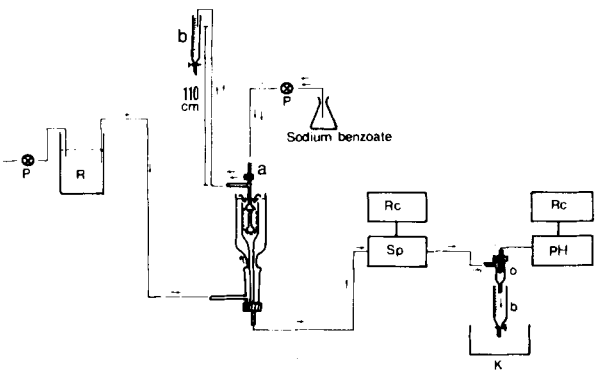

Figure 3—*Experimental setup for membrane-release measurement* in vitro. *Key: (a) perfusion apparatus; (R) reservoir; (p) peristaltic pump; (b) buret; (o) overflow vessel; (Sp) spectrophotometer; (Rc) recorder; (pH) pH-electrode; (k) collection vessel. Arrows indicate the direction of flow along the dialysis membrane (→) and through the dialysis membrane (⇉).*

Table II—Pharmacokinetic Parameters of Hippuric Acid after Intravenous Administration of Two Different Doses of Sodium Hippurate to Three Healthy Volunteers [a]

Parameter	Subject A	A	B	B	C	C
Dose (free acid), mg	588	222	588	222	588	222
AUC, μg·min/mL	1701	734	1828	657	1700	626
CL, mL/min	346	302	322	338	346	355

[a] Pharmacokinetic analysis was performed according to a two-compartment system with elimination from the central compartment.

155-mmol sodium benzoate solution. After 2 h the apparatus was taken out of the rectum, but blood sampling was continued for at least 1 h to determine the postperfusion elimination of hippuric acid. From the plasma concentration–time curve the area under the curve (AUC) was calculated using the trapezoidal rule. Using the AUC and clearance value, the total amount of absorbed sodium benzoate could be determined and compared with the amount that disappeared from the perfusate.

Determination of the Release from the Dialysis Bag *In Vitro*—To exclude the possibility that passage of sodium benzoate through the dialysis membrane would be rate limiting, the release of sodium benzoate from the dialysis bag was studied *in vitro* with the apparatus shown in Fig. 3. From a reservoir, nonbuffered water (pH 7.2) warmed to 37°C flowed into the outer tube and, *via* the inner tube, along the membrane, through a flow cell in the spectrophotometer, and was finally collected *via* overflow vessel and buret into a collection vessel. A pH-electrode was placed in the overflow vessel to detect any change in the pH of the solution coming out of the inner tube. The difference in height between the reservoir and the overflow vessel determined the water flow rate, which was measured by the buret.

The perfusion apparatus, with dialysis bag was hung inversely in the inner tube. The distance between the membrane and tube wall was such that a laminar water flow along the dialysis membrane occurred. The flow rate along the membrane (150–175 mL/min) ensured that measurements were performed under sink conditions, *i.e.*, that the concentration (C) in the water layer was almost zero compared with the concentration inside the membrane, C_0. The pressure inside the dialysis membrane was similar to that in the *in vivo* studies, since the distance between the inlet and outlet tubes of the perfusion apparatus was also maintained at 110 cm. In the case of *in vitro* recirculation experiments a pH electrode was also placed into the circulation system to detect any change of pH in the perfusate analogous to the *in vivo* situation.

The dialysis bag was filled with a solution of sodium benzoate and perfused until there was a steady-state release. Release per unit area was calculated as follows:

$$\Phi_{out} = \frac{C_{out} \times F}{\text{Area}} \ \mu\text{g/min} \cdot \text{cm}^2 \qquad \text{(Eq. 2)}$$

where C_{out} is the measured concentration of the solution coming out of the inner tube at steady state (μg/mL), F is the flow rate (mL/min), and Area = the membrane surface (cm^2).

Four different concentrations of sodium benzoate (72.5, 145, 218, and 290 mmol) were perfused as a single pass, and the release rate per unit of surface area was determined. To establish whether the membrane release characteristics change during perfusion *in situ*, control experiments were performed in which release from the dialysis bag was determined before and after use in a human rectal perfusion experiment. The presence of the bag in the human rectum for 2.5 h appeared not to change the *in vitro* release characteristics.

RESULTS AND DISCUSSION

Perfusion Under Single-Pass Conditions—Before starting the perfusion experiments it was determined whether dose-dependent kinetics in the elimination of hippuric acid could occur in the concentration range used for the perfusion studies. The clearance value can either be calculated according to the model-independent technique (CL = Dose/AUC, where AUC is the area under the plasma concentration *versus* time curve and dose is the amount of drug in the body) or by $CL = V_d \cdot \beta$, where V_d is the volume of distribution and β is the overall elimination rate constant. In the case of dose-dependent kinetics, a lower clearance value should be detected at the higher concentrations. The clearance will also change with any changes in V_d, when β remains constant. Table II shows the results of two different intravenous doses of sodium hippurate to three volunteers. No difference in CL was found, indicating that dose-dependent kinetics does not occur.

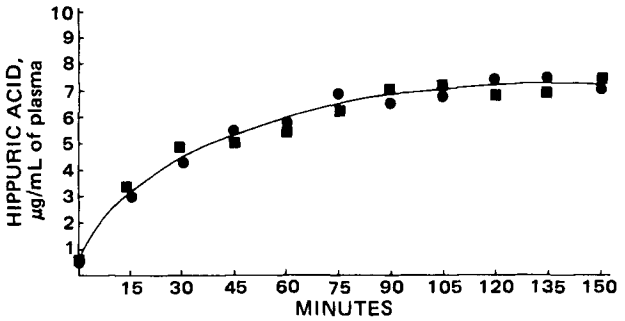

Figure 4—*Plasma concentration–time curves of perfusions with a 155-mmol sodium benzoate solution performed on two different occasions in one human subject.*

Figure 4 shows plasma concentration *versus* time curves following perfusions with 155 mmol on two different occasions in one subject. The calculated Φ_{in} values ($\mu g/min \cdot cm^2$) of the perfusion experiments, performed on two different occasions with four different concentrations of sodium benzoate, were, respectively, 19 and 16 for 116 mmol, 24 and 31 for 155 mmol, 58 and 59 for 232 mmol, 93 and 110 for 310 mmol of sodium benzoate. The identical curves and the established Φ_{in} values indicate a good reproducibility of the perfusion method.

Figure 5 shows the plots of the established Φ_{in} values of single-pass perfusion experiments with four different concentrations of sodium benzoate in four volunteers. The perfused length of the rectum was 7.0 cm. The plots show a linear relationship between Φ_{in} and sodium benzoate concentration. This pattern indicates a passive transport process, probably in the form of benzoic acid. However, paracellular or carrier transport in the form of sodium benzoate, operating under saturating conditions, can not be excluded at this stage. Further studies have been initiated to study the influence of pH on the absorption rate of this compound.

It is important that the perfused length of the rectum remains constant in these studies. Although Φ_{in} is determined per square centimeter, it is still unknown whether there is any difference in absorption between different parts of the rectum. Furthermore, there has to be a severe restriction in food intake the day before the experiment; otherwise, endogenous hippuric acid levels will be too high (2.0–8.0 μg/mL) and will influence steady-state concentration verifiability.

Figure 6 shows the *in vitro* transport across the membrane under sink conditions. For the sodium benzoate concentrations used in the present study, the transport across the membrane (per square centimeter) *in vitro* was found to be at least two times higher than the absorption rate (per square centimeter) observed *in vivo*. The amount transported was found to be proportional to the surface area of the membrane. Therefore, it is concluded that under the chosen conditions transport through the dialysis membrane is not rate limiting, so that the measured influx reflects the absorption capacity per unit area of the rectal membrane. This conclusion is supported by the high variation in Φ_{in} values between individuals, the good reproducibility of values within one individual, and the fact that membrane release does not change during the time of perfusion.

Perfusion Under Recirculation Conditions—Figure 7 shows a typical example of a perfusion curve following a recirculation experiment.

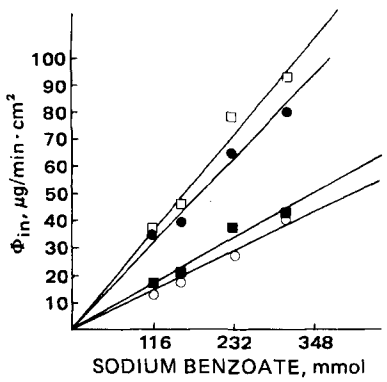

Figure 5—*Absorption rate (Φ_{in})* versus *sodium benzoate concentration after rectal perfusion with four different doses of sodium benzoate in four volunteers. Each symbol represents a single volunteer.*

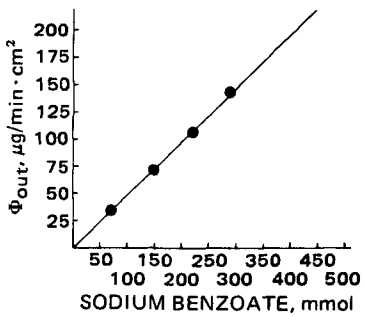

Figure 6—*Membrane release (Φ_{out})* versus *sodium benzoate concentration determined* in vitro.

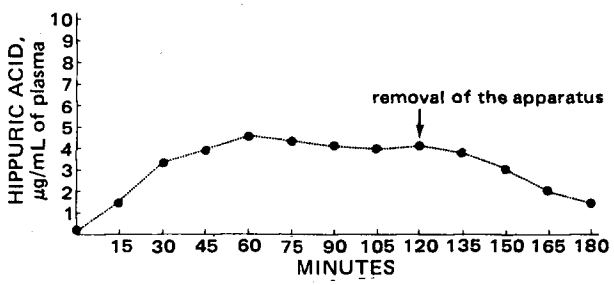

Figure 7—*Typical plasma concentration–time curve following a recirculation perfusion in one human subject.*

After the apparatus was taken out of the rectum, the plasma concentration of hippuric acid decreased very quickly. The slope of the terminal log concentration–time points (β-phase) was calculated and used to establish the $AUC_{t\to\infty}$. In the case of recirculation experiments, it is essential that the endogenous hippuric acid level is very low, because otherwise the calculated AUC is not in accordance with the amount really absorbed.

Table III represents the results of three recirculation experiments in two subjects (D and E). The total amount of absorbed benzoic acid equalled the measured amount of sodium benzoate eliminated from the perfusate. Thus, the loss of sodium benzoate within the lumen of the rectum or accumulation of sodium benzoate in the mucosa can be excluded.

In addition, it is interesting to note that in each experiment the pH of the perfusion solution rose during the time of perfusion at least one pH-unit, from 7.1 to 8.1. Assuming that sodium benzoate is absorbed in the uncharged form as benzoic acid, hydrogen ions have to be provided in the rectal wall or rectal lumen, because more benzoic acid disappeared from the perfusion solution than could be present at pH 7.1. With loss of benzoic acid, the sodium ion concentration increases, which combined with the hydroxyl ions from water cause the increase in pH. However, in our studies no relationship between the amount of sodium benzoate eliminated from the perfusion solution and the enhancement of the pH during perfusion was found. Therefore, it is concluded that the human rectum wall probably is able to secrete a fluid that adjusts the pH of the perfusion solution to a physiological pH. Crommelin *et al.* (17) found a similar phenomenon with perfusion experiments in rats. Future investigations should give a more quantitative picture of this process.

The rectal lumen perfusion technique described provides a reproducible method for quantitative absorption studies in humans. With sodium benzoate as the test drug, absorption could be measured as an influx per unit area of the rectal wall (Φ_{in} = $\mu g/min \cdot cm^2$). *In vitro* release studies indicate that the passage across the dialysis membrane of the perfusion apparatus is not rate limiting in the absorption process *in vivo*. With recirculation perfusion experiments it could be shown that the

Table III—Results of Recirculation Perfusion Experiments using 155-mmol Sodium Benzoate Solution in Two Subjects

Subject	Amount of Sodium Benzoate, mg Eliminated from the Perfusate	Absorbed	pH of the Perfusate Start	End
D	422	420	7.0	8.0
E	218	226	7.2	8.3
E	260	259	7.1	8.1

amount of sodium benzoate which disappeared from the perfusion solution equaled the amount of benzoic acid absorbed. With the single-pass perfusion experiments, a linear relationship between concentration of sodium benzoate and the rectal absorption was found, which may indicate passive diffusion possibly as benzoic acid.

REFERENCES

(1) N. Senior, "Advances in Pharmaceutical Sciences," vol. 4, Academic, London, 1974, p. 363.

(2) L. S. Schanker, D. J. Tocco, B. B. Brodie, and C. A. Hogben, *J. Pharmacol. Exp. Ther.*, **123**, 81 (1958).

(3) K. Kakemi, T. Arita, and S. Muranishi, *Chem. Pharm. Bull.*, **13**, 861 (1965).

(4) N. F. H. Ho and W. I. Higuchi, *J. Pharm. Sci.*, **60**, 537 (1971).

(5) S. Riegelman and W. J. Crowell, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 115, 123, 127 (1958).

(6) R. E. Beck and S. Schultz, *Science*, **170**, 1302 (1970).

(7) K. Kakemi, T. Arita, R. Hori, R. Komishi, and K. Nishimura, *Chem. Pharm. Bull.*, **17**, 248 (1969).

(8) K. Thoma, "Arzneiformen zur Rectalen und Vaginalen Applikation," Werbe- und Vertriebsgesellschaft Deutscher Apotheker mbH, Frankfurt, West Germany, 1980, p. 17.

(9) F. Moolenaar and A. J. M. Schoonen, *Pharm. Int.*, **1**, 144 (1980).

(10) C. J. Devroede and S. F. Phillips, *Gut*, **11**, 438 (1970).

(11) E. Bechaard, *Acta Pharmacol. Toxicol.*, **33**, 123 (1973).

(12) N. I. McNeil, *Gut*, **20**, 400 (1979).

(13) C. J. Edmonds, *Gut*, **12**, 356 (1971).

(14) F. Moolenaar, A. G. G. Stuurman-Bieze, J. Visser, and T. Huizinga *Int. J. Pharm.*, **1**, 323 (1978).

(15) A. J. Quick, *Am. J. Med. Sci.*, **185**, 630 (1933).

(16) N. H. Nie and C. H. Hull *et al.*, "SPSS-6000," 2nd ed., McGraw-Hill, New York, N.Y., 1975, p. 4.

(17) D. J. A. Crommelin, J. Modderkolk, and C. J. De Blaey, *Int. J. Pharm.*, **3**, 299 (1979).

Quantitation of Norfloxacin, a New Antibacterial Agent in Human Plasma and Urine by Ion-Pair Reverse-Phase Chromatography

L. T. PAULIUKONIS, D. G. MUSSON ˣ, and W. F. BAYNE

Received August 12, 1982, from *Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.* Accepted for publication November 22, 1982.

Abstract □ A specific and sensitive high-performance liquid chromatographic method for the analysis of norfloxacin in human plasma and urine is described. Norfloxacin was extracted from the sample matrix using dichloromethane under neutral conditions, followed by back extraction into dilute phosphoric acid for chromatographic analysis on a reverse-phase column with a mobile phase consisting of methanol, phosphate buffer, and ion-pairing reagent (pH 3.0) at a flow rate of 2.0 mL/min. The ability of this method to distinguish intact norfloxacin from its metabolites was demonstrated. The method is linear, quantitative, and reproducible for both plasma analysis (0.05–2.5 μg/mL) and urinalysis (1.0–500 μg/mL) using peak area ratios (norfloxacin–internal standard) for quantitation. The stability of norfloxacin and its metabolites in dilute phosphoric acid was studied. To assess the presence of norfloxacin conjugates in the urine of dosed individuals, the effects of urine hydrolysis on drug quantitation were examined. Urine and plasma levels of norfloxacin at selected time points following the administration of single drug doses are presented.

Keyphrases □ Norfloxacin—quantitation, antibacterial agent, human plasma and urine, ion-pair reverse-phase, high-performance liquid chromatography □ High-performance liquid chromatography—norfloxacin, human plasma and urine, ion pairing □ Antibacterial agents—quantitation of norfloxacin, human plasma and urine, ion-pair reverse-phase high-performance liquid chromatography

Norfloxacin (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid) (I) is a new quinolinecarboxylic acid antibiotic which exhibits a broad spectrum of antibacterial activity. *In vitro* studies have demonstrated its efficacy towards most Gram-positive and Gram-negative bacteria, including nalidixic acid-resistant pathogens (1, 2).

The development of a rapid, sensitive, and specific assay for norfloxacin quantitation was critical for the measurement of drug levels in clinical specimens. Although a high-performance liquid chromatographic (HPLC) method specific for norfloxacin has been described (3), this method proved unsuitable for use in clinical studies since it offers inadequate sensitivity and requires extensive sample preparation. The use of nonspecific microbiological methods[1] (4) in pharmacokinetic and bioavailability studies is also precluded due to the antibacterial nature of several norfloxacin metabolites (III–VIII) (3).

This report describes a reverse-phase ion-pair HPLC method developed for the quantitation of norfloxacin in plasma and urine, using pipemidic acid (II) as the internal standard. The assay was applied to the measurement of norfloxacin in the plasma and urine of human volunteers given increasing bolus doses of the drug.

EXPERIMENTAL

Reagents and Materials—All solvents[2] were distilled-in-glass and liquid chromatography grade, and purified water[3] was used throughout. All chemicals were ACS grade and used without further purification. Norfloxacin and its metabolites were used as received[4]. Pipemidic acid was extracted from 250-mg tablets[5].

Apparatus—The isocratic HPLC system consisted of a constant-flow pump[6], an autosampler[7], and variable-wavelength detectors; the assay for norfloxacin in plasma utilized a fluorescence detector[8] (excitation, λ = 280 nm; emission, λ = 445 nm; slits, 8 nm), while the urine assay used a UV absorbance detector[9] set at 280 nm. Data were recorded and reduced

[1] Dr. J. A. Bland, Merck Sharp & Dohme Research Laboratories, Rahway, N.J., personal communication.

[2] Burdick & Jackson Laboratories, Muskegon, Mich.

[3] Milli-Q System; Millipore Corp., Bedford, Mass.

[4] Kyorin Pharmaceutical Co., Ltd., Japan.

[5] Dolcol tabs from Dainippon Pharmaceutical Co., Japan.

[6] Model 6000A solvent delivery system; Waters Associates, Milford, Mass.

[7] WISP 710A autosampler; Waters Associates, Milford, Mass.

[8] 650S fluorescence detector equipped with 150B Xenon power supply; Perkin-Elmer, Norwalk, Conn.

[9] Schoeffel SF770 UV-spectroflow monitor; Kratos Analytical Instruments, Westwood, N.J.

0022-3549/84/0100-0099$01.00/0