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BIOPHARMACEUTICS OF RECTAL ADMINISTRATION OF DRUGS IN MAN. ABSORPTION RATE AND BIOAVAILABILITY OF GLAFENINE AFTER ORAL AND RECTAL ADMINISTRATION

F. MOOLENAAR, J. VISSER and T. HUIZINGA

Laboratorium voor Farmacotherapie and Recepteur, Ant. Densinglaan 2, Groningen (The Netherlands)

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SUMMARY

In this report it will be shown that rectal administration of glafenine and its salt can not be considered as a rational therapy. Plasma concentrations of glafenine and glafenic acid were measured, by means of HPLC analysis, after single oral and rectal doses of glafenine (400 mg), suspended in aqueous vehicles. After oral administration negligible plasma concentrations of free glafenine could be detected, probably due to a substantial first-pass effect. Peak concentrations of glafenic acid were reached after one hour. The decline in plasma concentrations was observed to be multiphasic. Rectal adsorption of glafenine (from micro-enemas) or glafenine HCl (from fatty suppositories) was extremely slow and incomplete, due to the slight water-solubility of glafenine at the prevailing pH in the rectum lumen.

INTRODUCTION

Glafenine (N-(7-chloro-4-quinolyl)-anthranilic acid 2,3-dihydroxypropyl ester) was developed from the synthetic anti-malarials and was introduced as a peripherally acting analgesic.

As an analgesic, glafenine was found to be more effective than acetylsalicylic acid (Boeijinga, 1977). However, in a double-blind study in patients suffering from post-operative dental pain, no significant difference in analgesic efficacy was observed between a dose of 400 mg of glafenine and a dose of 1000 mg of paracetamol in combination with 100 mg of caffeine (Phaf et al., 1973).

In animals anti-inflammatory and antipyretic properties were demonstrated but these effects were not as marked as the analgesic action (Jequier and Peterfalvi, 1966; Branceni et al., 1964).

Data on the pharmacokinetics of glafenine in man available at present are of limited

value, due to the rather insensitive and unspecific spectrophotometric method used, which determines glafenine including its metabolites (Pottier et al., 1974).

Reviewing the most relevant observations from literature it can be concluded that after oral administration of glafenine, absorption from the gastrointestinal tract occurs readily and quickly. Since there are no intravenous data available, it is uncertain if absorption is complete or not. Biotransformation to glafenic acid, which is the main metabolite, is extremely rapid (Rondelet et al., 1966).

It is uncertain whether hydrolysis occurs in the gut lumen, the gut wall or in the liver. However, first-pass metabolism seems to be rather complete, since only low concentrations of free glafenine could be detected in human plasma (Mallein et al., 1966).

The contribution of hepatic clearance to the total body elimination was about 65%, while 35% of the dose was excreted via the urine, probably mainly in the form of O-conjugates with glucuronic acid.

Chemically, glafenic acid is closely related to the peripheral analgesic, floctafenine. The analgesic activity of the main metabolite, floctafenic acid, was reported to be almost equal to that of floctafenine (Pottier et al., 1974).

Clinical studies after rectal administration of glafenine in man have been reported (Lepinard and Andrieu, 1969; Bonhomme and Lemaire, 1969; Fontaine and Maillard, 1969; Bouche and Bruker, 1969; Camus, 1969). In these studies the more water-soluble glafenine hydrochloride was used. The general conclusion from these investigations was that rectally administered glafenine had an important analgesic effect. However, apart from the lack of double-blind cross-over comparisons with a standard analgesic drug, in none of these studies were plasma concentrations of glafenine and its metabolites measured.

The authors therefore designed a study to investigate the nature of the rectal absorption process. For this purpose it was necessary to work out an adequate method for bioanalysis, in order to discriminate between the concentration of glafenine and its metabolites. To establish differences in relative bioavailability, a comparison was made with an orally administered suspension of glafenine.

MATERIALS AND METHODS

Dosage forms

Glafenine and glafenine HCl, obtained from Roussel Laboratoria, were used in this study. Glafenic acid was prepared from glafenine, as described by Moolenaar et al. (1977).

The oral dosage form consisted of 400 mg of glafenine, suspended in 200 ml of water. For rectal use a suspension was prepared containing 400 mg of glafenine, suspended in 10 ml of a medium, which consisted of 0.5% methylcellulose 400 cP (Ph. Ned. VI). The pH of the suspension was 5.8. A coarse powder of glafenine HCl was prepared by recrystallizing from a hot saturated solution of the HCl salt. A coarse fraction (125–250 μm) was separated by sieving. The powder was mixed with a molten base Witepsol H 15 (Interpharm), poured into brass moulds (3 ml) and stored in the refrigerator for at least one night before use. The weight of the suppositories was adjusted to 2.8 g and they contained 439 mg of glafenine HCl.

In vitro determinations

The release of glafenine HCl from suppositories was determined using the release apparatus described by Schoonen et al. (1976).

Human experiments

Six healthy human subjects, ranging in age from 23 to 32 years of age and in body weight from 65 to 82 kg, participated in the study. No drugs were taken for two weeks prior to and during the study. The experiments were initiated in the morning and the volunteers did not take any food during the experiments. They were asked to remain in a sitting position. No discomfort following application of any rectal dosage form was reported by the volunteers. The micro-enema was administered using a plastic disposable syringe to which a plastic application tube was connected. The tube was introduced into the rectal lumen enabling quantitative emptying of the syringe into the rectum. Blood samples of 10 ml were taken using Venoject tubes (Terumo) with 15 mg EDTA–sodium granules at 0, 30, 60, 120, 180, 240 and 300 min after administration. Blood samples were cooled in ice and plasma was obtained by centrifugation of blood samples. Plasma was pipetted off and stored in dry-ice until required for analysis.

The volunteers were asked to collect urine every hour. Every hour 200 ml water was ingested. The urine samples were frozen until the time of analysis.

Determination of glafenine and glafenic acid in plasma and urine

Glafenine and glafenic acid concentrations were estimated by reverse-phase high pressure liquid chromatography.

Standard solutions

A 0.007 M buffer solution (pH 6.6) was prepared by dissolving 716 mg disodium hydrogen phosphate 12-hydrate (Merck 6579) and 644 mg sodium dihydrogen phosphate 1-hydrate (Merck 6346) in 1000 ml of distilled water.

An internal standard solution was prepared by dissolving 150 µl nitrobenzene (Fluka A56190) in 1000 ml of methanol (Merck 6009).

A standard solution of glafenine was prepared by dissolving 10.00 mg glafenine in 250 ml of methanol.

A standard solution of glafenic acid was prepared by dissolving 10.00 mg glafenic acid in 250 ml 50% (v/v) 0.007 M buffer solution (pH 6.6) in methanol.

Apparatus and chromatographic conditions

A liquid chromatograph (Waters Associates) equipped with an UV-detector (model 440) set at 365 nm was employed. The detector was operated at 0.05 AUFS. The column (25 cm × 4.6 mm) was packed with Nucleosil 10 C18 and guarded with a pre-column Co pell ODS (10 cm × 2.1 mm). Both columns were made by Chrompack Nederland. The mobile phase was 40% (v/v) 0.007 M buffer solution (pH 6.6) in methanol. The flow rate was 2.0 ml/min at a pressure of 3500 psi.

Sample injections were made on-column through a Waters U6K septumless injector with a 100 µl syringe (PSC B-110).

Procedure in plasma

To 1.0 ml plasma in a 10 ml glass-stoppered tube, 3.0 ml of the internal standard solution was added. After shaking for 10 min the content of the tube was centrifugated during 5 min at 4000 rpm; 100 µl of the liquid was injected into the HPLC. For the determination of glafenine the detector was operated at 0.01 AUFS after reaching the internal standard peak.

The retention times were respectively: glafenic acid 2.0 min, nitrobenzene 3.0 min and glafenine 10.5 min.

The ratio of the peak area of the sample component to that of the internal standard was used to calculate concentrations of glafenine and glafenic acid, based on calibration curves prepared from spiked plasma samples (Table 1).

With 1.0 ml plasma samples this method is accurate to concentrations as low as 0.2 µg per ml plasma for glafenic acid and 0.5 µg per ml plasma for glafenine. The recovery from plasma (urine) was determined for 7 different concentrations of glafenine and glafenic acid and was found to be essentially complete (Table 1).

Procedure in urine

To determine the excreted amount of glafenic acid in urine, glafenic–glucuronide was transformed into glafenic acid.

To 0.1 ml urine in a 10 ml glass-stoppered tube 0.1 ml 4 M NaOH was added. The tube was securely capped and heated for 16 h at 95°C. After cooling 3.0 ml of the internal standard solution was added. From this point the same procedure was followed as in plasma.

Pharmacokinetic parameters

The absorption was characterized by two parameters: the peak concentration (C_{max}) and the peak concentration time (t_{max}). The area under the plasma concentration time curve (AUC) from t = 0 to t = 5 was determined by the trapezoidal rule. Relative bioavailability was determined using the equation:

$$F_{rel} = \frac{AUC_{rectal}}{AUC_{oral}} \times \frac{Dose_{oral}}{Dose_{rectal}}$$

RESULTS AND DISCUSSION

Bioanalysis

The calibration curves of glafenine and glafenic acid are rectilinear and the recovery from plasma is essentially complete (Table 1 and Fig. 1).

A typical chromatogram from a plasma extract is shown in Fig. 2.

As a result of the relatively high detection wavelength (365 nm) no interference with peaks in the same region from other plasma components has been encountered. The peaks of the compounds were found to be almost symmetrical and were well separated from each other. About 1.5 min after injection of the plasma extract a peak can be detected, possibly belonging to the glucuronide conjugate of glafenic acid.

TABLE 1

RECOVERY OF GLAFENINE (Gf) AND GLAFENIC ACID (Gfa) FROM PLASMA

Concentration added (μg/ml)		Number of determinations		Calculated concentrations ± S.D. (μg/ml)		Coefficient of variation (%)		Recovery (%)	
				Gf	Gfa	Gf	Gfa	Gf	Gfa
Gf	Gfa	Gf	Gfa						
0.52	0.21	7	7	0.48 ± 0.03	0.22 ± 0.01	6.25	4.55	92.3	104.5
0.99	0.99	7	7	0.97 ± 0.03	0.98 ± 0.02	3.09	2.06	97.9	99.0
2.46	2.46	7	7	2.34 ± 0.06	2.40 ± 0.05	2.57	2.50	95.0	97.5
4.85	4.85	7	7	4.65 ± 0.11	4.68 ± 0.09	2.37	1.92	95.8	96.5
9.41	9.41	7	7	9.31 ± 0.17	9.36 ± 0.15	1.83	1.60	98.9	99.4
13.71	13.71	7	7	13.48 ± 0.25	13.74 ± 0.21	1.85	1.53	98.3	100.2
17.78	17.78	7	7	17.62 ± 0.25	17.69 ± 0.21	1.42	1.19	99.1	99.5

After an oral dose of 400 mg glafenine only in the plasma sample, obtained 15 min after administration, a trace of unmetabolized glafenine (much less than 0.5 μg/ml) could be detected. In urine mainly glafenic–glucuronide was found; less than 2% was detected as glafenic acid. No free glafenine was found in the urine samples.

The present HPLC method is more specific and sensitive than the spectrophotometric method used in earlier studies (Pottier et al., 1974). Also the latter method is more laborious, since the extinction in plasma and urine must be corrected for extinction at 365 nm of naturally occurring plasma and urine constituents. In addition it does not differentiate between glafenine, glafenic acid or its glucuronide.

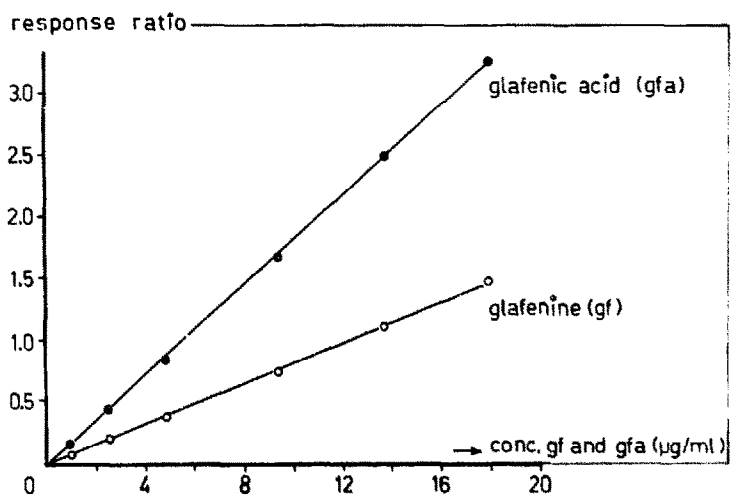

Fig. 1. Calibration graphs for the determination of glafenine (Gf) and glafenic acid (Gfa) using nitrobenzene as internal standard. Mean values of 7 determinations. Coefficinet of variation at the lowest concentration: 1.95%.

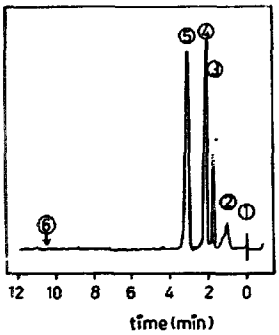

Fig. 2. Liquid chromatogram of a plasma extract. The plasma sample was obtained 30 min after oral administration of 400 mg glafenine. 1 = injection (100 μl); 2 = solvent front; 3 = glafenic–glucuronide; 4 = glafenic acid; 5 = nitrobenzene; 6 = glafenine.

Oral versus rectal administration

The mean plasma concentrations of glafenic acid in 7 subjects after oral and rectal administration of a dose of 400 mg glafenine, suspended in water, are given in Table 2 and Fig. 3.

TABLE II

ABSORPTION CHARACTERISTICS, RELATIVE BIOAVAILABILITY AND URINE EXCRETION PATTERN OF GLAFENIC ACID (MEAN ± S.D.) AFTER ORAL AND RECTAL ADMINISTRATION OF 400 mg (439 mg) GLAFENINE (HCl).

	Oral in 200 ml (400 mg)	Rectal	
		Micro-enema (10 ml) (400 mg)	Suppository (4 ml) (439 mg)
Plasma concentration (μg/ml) at t:			
30 (min)	9.2 ± 3.4	0.20 ± 0.02	
60	12.8 ± 2.7	0.36 ± 0.05	
120	4.6 ± 0.7	0.40 ± 0.07	
180	1.6 ± 0.5	0.37 ± 0.05	
240	1.1 ± 0.2	0.34 ± 0.06	
300	0.6 ± 0.1	0.30 ± 0.02	
Number	7	7	7
C_{max} *(μg/ml)*	12.6 ± 3.1	0.44 ± 0.09	
t_{max} *(min)*	50 ± 27	125 ± 28	
AUC_{0-5} *(μg/min/ml)*	1308 ± 68	98 ± 9	
F_{rel}	1.00	0.075	
Urine concentration (mg) *at t:*			
60 (min)	31.1 ± 6.7	3.8 ± 0.4	0.8 ± 0.07
120	44.2 ± 8.2	5.1 ± 0.3	2.1 ± 0.2
180	41.0 ± 6.7	4.8 ± 0.4	1.7 ± 0.1
240	12.1 ± 3.1	4.7 ± 0.5	1.5 ± 0.2
300	9.2 ± 2.7	3.5 ± 0.2	0.2 ± 0.04

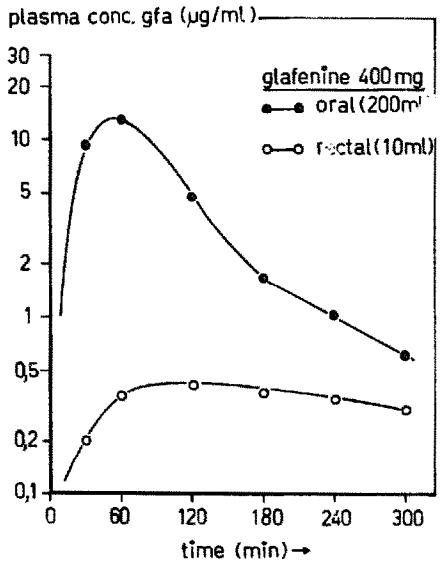

Fig. 3. Mean plasma concentrations of glafenic acid (Gfa) on logarithmic scale after oral and rectal administration to 7 subjects of a dose of 400 mg glafenine, suspended in water.

Glafenine from the orally administered suspension was rapidly absorbed. The mean peak plasma level of glafenic acid was reached after about 1.0 h. The plasma profile indicates that glafenic acid kinetics are more complicated than previously supposed (Mallein et al., 1976; Boeijinga, 1977; Moolenaar et al., 1977). The absorption kinetics and the elimination half-lives from either of these studies do not provide meaningful information, since these values are based on the combined plasma concentrations of glafenic acid and its glucuronide.

Fig. 3 shows that the decline in plasma concentrations of glafenic acid is multiphasic and incompatible with a one compartment model. More detailed studies following both intravenous and oral administration of glafenine are required to establish quantitative distribution and elimination kinetics of glafenic acid. The concentration of unmetabolized glafenine in plasma after oral administration was negligibly small. A substantial first-pass elimination by liver or gut is therefore implied. Thus the absorption process of glafenine can only be estimated by measuring glafenic acid levels. After rectal administration only small concentrations (less than 0.5 µg/ml) of glafenic acid could be detected in plasma (Table 2).

A likely explanation for this marked difference in absorption conditions is that the water-solubility of glafenine – which is a prerequisite for passing biological membranes – is strongly dependent on the pH. Glafenine is very slightly soluble in water at neutral pH range (1 in 60,000) but at pH 1.0 solubility increases remarkably (1 in 25). Therefore it is reasonable to suppose that after oral administration glafenine is protonated in the acid medium of the stomach, resulting in a fair solubility. However, the pH in the rectum is about 7.5, which is extremely unfavourable for dissolution.

If it is assumed that the driving force in absorption depends on the rate of dissolution

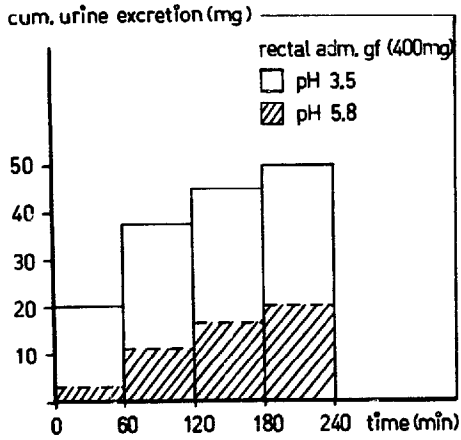

Fig. 4. Cumulative urine concentrations (mg) of glafenic acid (Gfa) after rectal administration of a dose of 400 mg glafenine, suspended in 10 ml of medium with a difference in pH, to subject F.M.

it can be rationalized that rectal absorption of glafenine will occur extremely slowly. In this respect is it interesting to notice that after oral administration of glafenine to patients with a so-called pentagastrin refractory anacidity, absorption of glafenine was found to be poor (Preiss, 1971).

In our study no trace of glafenine could be detected in plasma after rectal administration. It is obvious that the supposed advantage of the rectal route to by-pass enzymatic activity in the liver or gut wall is not relevant since rectal absorption conditions for the drug are very unfavourable, compared with the oral route of administration. Even a reduction of the pH of the micro-enema to a value of 3.5 did not result in appreciable plasma concentrations of glafenic acid. Yet, in view of the amount of glafenic glucuronide excreted in the urine during 4 h, it can be observed that absorption conditions at this lower pH range were improved (Fig. 4).

Suppositories containing glafenine HCl

The release characteristics in vitro of glafenine HCl from Witepsol H 15 suppositories, containing 439 mg of the drug under study, have been determined by the method of Schoonen et al. (1976). To maintain sink conditions, the pH of the fluid in the release apparatus was kept on pH 4.0 (solubility is 1 in 2000 at this pH). Coarse particles (125–250 μm) were used to achieve an optimal release (Schoonen et al., 1979). About 30% of the amount glafenine HCl was released in two hours. This low amount may be explained by the poor water-solubility of the drug used. In this respect it is remarkable that from commercial suppositories, containing 500 mg of the HCl salt in 2 ml suppositories (Roussel), only 4% was released, probably due to the use of micronized particles in the fatty vehicle.

From Table 2 it can be observed, however, that practically no rectal absorption occurs. As discussed earlier, it seems likely that in view of the pH of 7–8 in the rectal lumen, the released amount of glafenine HCl will be precipitated as unsoluble glafenine, resulting in extremely unfavourable absorption conditions.

CONCLUSIONS

For the determination of glafenine and its metabolite concentrations in plasma or urine the HPLC method used in the present study is more specific and more sensitive than the spectrophotometric method used by Pottier et al. (1974) and others.

After oral administration of glafenine, peak concentrations of the main metabolite, glafenic acid, are reached after about one hour. The decline in plasma concentrations is multiphasic and incompatible with a one compartment model. In view of the lack of free glafenine in the central compartment, a substantial first-pass elimination by liver or gut wall can be assumed.

Rectal absorption of glafenine is very incomplete, probably since the water-solubility at the prevailing pH in the rectum lumen is extremely poor. The HCl salt is also very slightly soluble at this pH range and therefore it can be concluded that rectal administration of micro-enemas or suppositories containing glafenine or its HCl salt can not be considered as a rational therapy.

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