

Quantitative measurement of biliary excretion and of gall bladder concentration of drugs under physiological conditions in man

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SUMMARY Gall bladder storage of hepatic bile prevents complete recovery of biliary excretion of drugs to be obtained under physiological conditions in man. The aim of this study was to develop and validate a method for simultaneous measurement of gall bladder storage of a cholephilic drug, and of its duodenal excretion and $t_{1/2}$ in bile. Duodenal perfusion using polyethylene glycol as intestinal recovery marker for measurement of drug duodenal excretion, with an iv bolus of ^{99m}Tc HIDA for measurement of drug mass within the gall bladder was used. Gall bladder volume was measured by ultrasonography. $T_{1/2}$ in bile was measured by relating drug duodenal excretion to that of bile acid used as an endogenous bile marker. The use of bile acid as biliary marker was validated in two subjects receiving simultaneous iv infusion of indocyanine green. Seven healthy subjects were studied using a beta-lactam antibiotic, Cefotetan 1 g iv, as test drug. Median values during the study period (seven hours) were 51.1 mg for Cefotetan duodenal excretion, 45.2 mg for gall bladder mass and 2.8 mg/ml for concentration within the gall bladder. $T_{1/2}$ of the drug in bile was 100 minutes. This technique enables measurement of mass and concentration of drugs within the gall bladder to be carried out, in addition to measurements of $t_{1/2}$ of drugs in bile. These measurements may have specific application for assessment of potential efficacy of antibiotics in biliary tract infections, as well as general application for assessment of biliary excretory kinetics of drugs.

Current methodology for measurement of biliary excretion of drugs and biliary pharmacokinetics is unsatisfactory.¹ Methods based on biliary drainage through a T tube after cholecystectomy² may not ensure quantitative recovery of biliary secretions. Furthermore, results may be affected by the underlying disease. Methods based on duodenal perfusion techniques can, on the one hand, be applied to healthy subjects but, on the other hand, are of limited value because the mass of drug stored in the gall bladder is neglected. This gall bladder mass is likely to account for a major proportion of drug biliary

excretion, because about 50% to 80% of hepatic bile is stored in the gall bladder during fasting.^{3,5} Attempts to abolish gall bladder storage function by using a continuous intraduodenal infusion of nutrients⁶⁻⁹ has been recently shown by Everson *et al*¹⁰ to be ineffective because of failure to maintain the gall bladder tonically contracted.

A method combining cholescintigraphy with nasoduodenal intubation has recently been developed for measurement of gall bladder storage function in healthy subjects¹¹ and has been applied to measurement of biliary lipid mass stored in the fasting gall bladder. It is of potential value for measuring the mass of drug in the gall bladder.

The aim of this study was to apply this method to quantitative measurement of gall bladder mass of a cholephilic test drug; and to combine this technique

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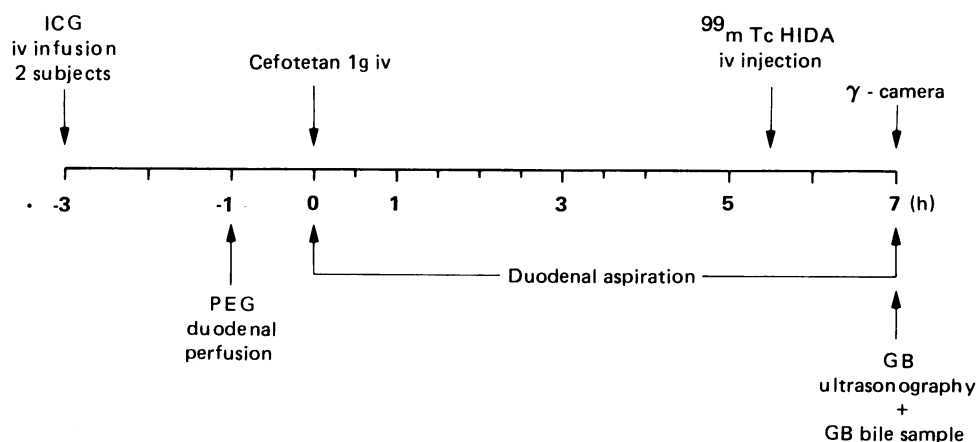


Fig. 1 Time table of the study (ICG=indocyanine green, PEG=polyethylene glycol and GB=gall bladder).

with measurements of duodenal excretion rates in order to obtain quantitative measurement of total biliary excretion of drugs under physiological conditions in man. Further aims were to measure the drug concentration within the fasting gall bladder, and its half-life in bile. A duodenal perfusion of polyethylene glycol (non-absorbable intestinal recovery marker) was used for measurement of duodenal excretion⁵ of a test drug. A cholescintigraphic technique involving ^{99m}Tc HIDA (gall bladder bile marker) given as an iv bolus was used for measurement of drug mass within the gall bladder.^{5,11} This latter measurement was related to gall bladder volume as measured by ultrasonography¹² in order to measure drug concentration within the gall bladder. The half life ($t_{1/2}$) of the test drug in bile was measured by relating its duodenal excretion rate to that of an endogenous biliary marker, bile acid. We applied this combination technique to five healthy volunteers. Two further subjects were studied during a continuous iv infusion of indocyanine green (ICG, exogenous biliary marker) in order to validate bile acid as an endogenous biliary marker. As test drug we used a beta lactam antibiotic, Cefotetan.

Methods

SUBJECTS

We studied seven healthy volunteers (five women, two men; age range 28–51 years, mean (SE) 48.3 (4.5) years) with normal physical examination, blood count, and liver function tests. Creatinine clearance was >125 ml/minute in each subject. Informed consent was obtained from all subjects. Radiation exposure was calculated to be <0.5 rad for the whole gut.

CLINICAL PROCEDURE (Fig. 1)

On the day before the study, the subjects were admitted to a clinical investigation ward. On the following morning, a double lumen nasoduodenal tube (Anpro-20, Andersen, USA) was advanced under fluoroscopic control in the fasting state to position the proximal outlet (duodenal perfusion tube, id 1 mm) opposite the ampulla of Vater. The distal tube (aspiration tube, id 2.5 mm) was positioned at the ligament of Treitz, allowing 20 cm mixing segment. This distal tube was radiopaque, and radiopaque marks were also attached at the distal end of the perfusion tube to assist correct fluoroscopic positioning.

Two and a half grams per litre polyethylene glycol solution (PEG, mw 4000) was prepared in normal saline and continuously perfused into the duodenum (Fig. 1) at the rate of 5 ml/min by a peristaltic pump (model 502S, Watson Marlow, UK). After allowing 60 minutes to achieve a steady state, 1 g Cefotetan (ICI, UK) was injected as a single iv bolus, and duodenal aspirate was obtained by syphonage at 30 minute intervals for four hours and at hourly intervals thereafter for a total seven hours. As part of a validation study, two of the seven subjects were studied during continuous iv infusion of ICG. The ICG solution (Indocyanine green, HW&D, USA) was prepared in normal saline containing 5% human albumin immediately before the study. Intravenous infusion started three hours before Cefotetan administration (Fig. 1), to allow equilibration of hepatic output with its iv input,^{3,13} and continued until the end of the study at a constant rate of 0.2 mg/kg/h (model 975, Harvard Infusion Pump, USA). The ICG solution was kept in the dark during preparation and iv administration.

Two millicuries ^{99m}Tc HIDA (^{99m}Tc sodium n-[n(2,6-diethylphenyl-carbamylmethyl)] iminodiacetate, Amersham, UK) was injected as an iv bolus in five subjects 90 minutes before the end of the duodenal perfusion of polyethylene glycol. Two hours later, anterior and posterior abdominal gamma camera counting (one minute interval) was carried out with a gamma camera/computer system (model KR7, SELO, Italy). Data were stored in the computer and later reanalysed using an area of interest facility to outline the gall bladder area. Interference for liver background activity was corrected by subtracting from gall bladder counts the activity detected in an equivalent area of the liver adjacent to the gall bladder. Differences in gall bladder depth from the gamma camera crystal were compensated for by using the formula:¹¹ GB counts = (anterior GB counts² + posterior GB counts²)^{1/2} (where GB = gall bladder).

After gall bladder gamma camera scanning, gall bladder volume was measured by real time ultrasonography (model MK 300C, sector probe 3.0 MHz, ATL, USA) as described by Everson *et al.*¹¹ A sample of gall bladder bile was later obtained by stimulating gall bladder contraction with iv infusion of caerulein solution (20 μg in 100 ml normal saline, Farmitalia, Italy).

LABORATORY METHODS

Polyethylene glycol concentration in duodenal aspirate was measured by the turbidometric method of Hyden.¹⁴ Cefotetan concentration was measured by HPLC (model 590, Waters, USA) as described by Adam *et al.*¹⁵ Bile acid concentration was measured enzymatically,¹⁶ and ICG was measured spectrophotometrically within 24 hours from collection as previously described.⁴ ^{99m}Tc HIDA activity in 1 ml

gall bladder bile samples was measured for 10 seconds with a gamma counter (model ST5, SELO, Italy). In order to relate measurements obtained with gamma counter and gamma camera [see (ii) in calculations], a correction factor was applied to take account of their different sensitivities for ^{99m}Tc HIDA detection (1/2054). Time dependent decay of ^{99m}Tc HIDA activity and differences in counting time were also taken into account.

CALCULATIONS AND EXPRESSION OF RESULTS

Duodenal excretion of Cefotetan, bile acid and ICG was calculated according to the following formula:

$$(i) C_{de} \text{ (or Ba or ICG)} = [C \text{ or Ba or ICG}] \times \frac{[\text{PEG}]_p \times v \times t}{[\text{PEG}]_a}$$

Where de = duodenal excretion, C = Cefotetan, Ba = bile acid, ICG = indocyanine green, PEG = polyethylene glycol, [] = concentration, a = duodenal aspirate, p = duodenal perfusate, v = volume of duodenal perfusate, t = time of duodenal perfusion.

Gall bladder mass of Cefotetan was calculated according to the following formula:

$$(ii) C_m \text{ in GB} = \frac{C_m \text{ (1 ml GB bile)} \times ^{99m}\text{Tc HIDA activity over the Gb area}}{^{99m}\text{Tc HIDA activity (1 ml GB bile)}}$$

where m = mass and GB = gall bladder

Total biliary excretion of Cefotetan was calculated according to the following formula:

$$(iii) C_b = (i) + (ii)$$

where b = biliary excretion

Concentration in the gall bladder of Cefotetan was calculated according to the following formula:

$$(iv) C_c = C_m / \text{GB volume}$$

where c = concentration

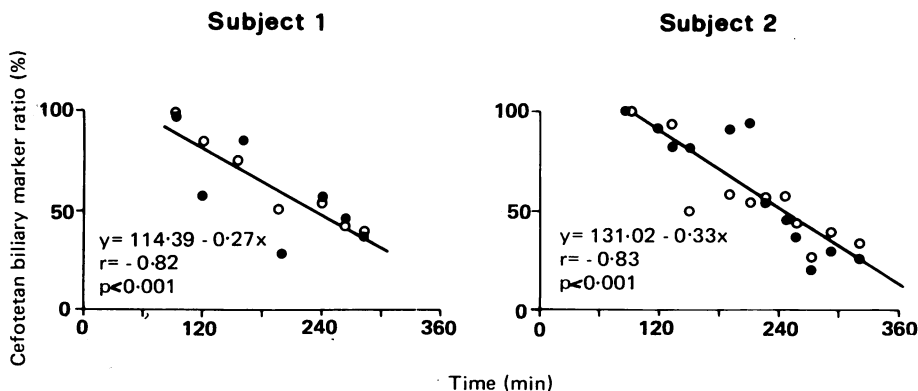


Fig. 2 Reproducibility of Cefotetan/indocyanine green ratio (open circles) and of Cefotetan/bile acid ratio (closed circles) in two subjects studied during continuous iv infusion of ICG.

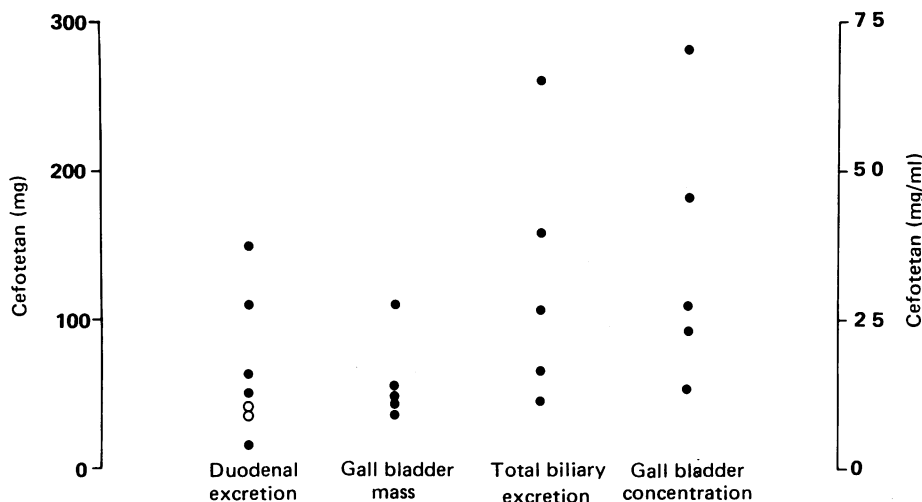


Fig. 3 Duodenal excretion, gall bladder mass, total biliary excretion and concentration of Cefotetan in the gall bladder. Subjects marked (O) were studied during indocyanine green iv infusion.

Cefotetan/bile acid and Cefotetan/ICG ratios were calculated from duodenal outputs (i). Absolute values for both measurements were expressed as a percent of the peak values, which were considered to be 100%.

$$\begin{aligned} (v) \quad C/Ba &= C_{de}/Ba_{de} \\ C/ICG &= C_{de}/ICG_{de} \end{aligned}$$

T_{1/2} in bile of Cefotetan was defined as the time taken for both C/Ba and C/ICG ratios to reach 50% of the peak value.

STATISTICAL ANALYSIS

Results were expressed as median values. Regression lines were calculated using the least square method. The linearity was tested by fitting a power curve to log transformed variables, a power less than 1 indicating a curvilinear relationship.

Results

VALIDATION STUDIES (Fig. 2)

Cefotetan/bile acid ratio and Cefotetan/ICG ratio declined with time in both subjects studied during ICG intravenous infusion. A single regression line was derived for the combined data from the two measurements in both subjects, and the data from both individual measurements were equally distributed around this regression line.

GALL BLADDER MASS AND CONCENTRATION (Table, Fig. 3)

The mass of Cefotetan stored in the gall bladder in

the five subjects studied ranged from 17.9 to 111.9 mg (45.2 mg median value). Gall bladder volume in individual subjects ranged from 10.6 to 21.1 ml (16.4 ml median value). Cefotetan concentration in the gall bladder ranged from 1.4 to 7.1 mg/ml (2.8 mg/ml median value).

BILIARY EXCRETION (Table, Fig. 3)

Cumulative duodenal excretion of Cefotetan in the seven subjects studied ranged from 18.4 to 151.9 mg (51.1 mg median value) during the seven hours study. Total biliary excretion (duodenal excretion plus gall bladder mass) ranged in individual subjects from 47.2 to 263.8 mg (108.6 median value).

T_{1/2} IN BILE (Figs 4 and 5)

The profile of Cefotetan duodenal excretion in

Table Individual values for duodenal excretion, gall bladder (GB) mass and total biliary excretion of Cefotetan in the seven subjects studied. Gall bladder volume and Cefotetan concentration in the gall bladder are also shown

n	Duodenal excretion (mg)	GB mass (mg)	Total biliary excretion (mg)	GB volume (ml)	GB concentration (mg/ml)
1	51.1	17.9	69.0	21.1	2.4
2	18.4	28.8	47.2	21.1	1.4
3	151.9	111.9	263.8	15.8	7.1
4	63.4	45.2	108.6	16.4	2.8
5	111.6	48.9	160.5	10.6	4.6
6	44.4	-	-	-	-
7	39.0	-	-	-	-

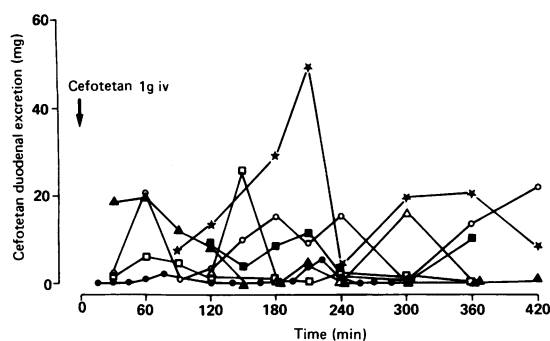


Fig. 4 Duodenal excretion rate of Cefotetan (mg/h) in individual subjects. Each subject is identified by a different symbol.

individual subjects is shown in Figure 4. Hourly Cefotetan duodenal excretion fluctuated during the whole study period in all subjects, and there was no relationship between Cefotetan duodenal excretion and time ($r=0.11$, NS). By contrast, there was a decline of Cefotetan/bile acid ratio with time in pooled data from all subjects. This relationship was better described by a curve ($y=979.5 \times X^{-0.684}$, $r=0.315$, $p<0.05$) than by a straight line. $T_{1/2}$ of Cefotetan in bile calculated on this curvilinear relationship was 100 minutes.

Discussion

Our validation study has shown that $t_{1/2}$ of a cholephilic substance in bile can be measured under

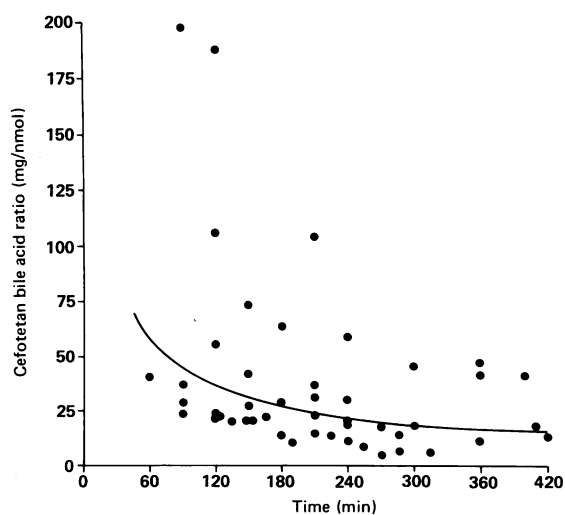


Fig. 5 Relationship between Cefotetan/bile acid ratio (mg/nmol) and time in all subjects studied.

physiological conditions in man by using bile acid as an endogenous biliary marker. The validity of this measurement was confirmed by comparison with results based on ICG as an exogenous biliary marker. The reason for choosing ICG as control bile marker was that ICG acts as an ideal biliary marker, as indicated by its complete duodenal recovery in cholecystectomised subjects.^{3,5,13}

We have previously validated the duodenal perfusion technique by showing 102% duodenal recovery of a gall bladder bile marker, ^{99m}Tc HIDA.⁵ Measurement of the mass of cholephilic substances in the gall bladder using ^{99m}Tc HIDA cholescintigraphy has also been validated by Jazrawi *et al*¹⁷ by direct comparison with measurements obtained in surgically removed gall bladders.

The combination of a cholescintigraphy technique with a duodenal perfusion technique has enabled quantitative measurement of gall bladder mass and concentration, and of total biliary excretion of drugs to be carried out under physiological conditions in man. These measurements have several potential applications. In relation to safety of drugs like parenteral antibiotics, dose dependent changes in colonic flora caused by biliary excretion may occur,¹⁸ thus underlining the importance of quantitative measurement of the amount of drug entering the intestine. In relation to drug efficacy, although therapeutic serum concentrations of an appropriate antibiotic may often prevent complications of biliary tract infections,¹ eradication of bacteria from bile may be difficult as indicated by the high incidence of septic complications in biliary surgery.¹⁹ Eradication of bacteria from bile is only guaranteed by a therapeutic biliary concentration of an antibiotic.¹ Our technique for measuring drug concentration in gall bladder bile provides a simple and cheap method of obtaining this information. In the case of Cefotetan, drug concentration in the gall bladder bile was 21- to 71-fold greater in individual subjects than the minimal inhibitory concentration measured *in vitro* for sensitive bacteria.²⁰

A further advantage of our technique is that it also enables measurement of drug $t_{1/2}$ in bile to be carried out by using bile acids as an endogenous biliary marker. The advantage of calculating the ratio of Cefotetan to bile acid is born out by visual comparison of Figure 4 with Figure 5. Duodenal excretion of the antibiotic fluctuated during the whole study in all subjects (Fig. 4), probably as a result of sphincter of Oddi activity²¹ and/or changes in gall bladder storage and emptying function.⁵ There was no relationship between Cefotetan duodenal output and time (Fig. 4), and biliary $t_{1/2}$ could not be measured on these results. By contrast, results for Cefotetan/bile acid ratio were inversely related with time, thus enabling

calculation of $t_{1/2}$ of the drug in bile to be carried out (100 minutes). The high value for $t_{1/2}$ of Cefotetan is consistent with the strong binding of this antibiotic to serum proteins, and with its high serum $t_{1/2}$.²²

In conclusion, our results indicate that we have a valid and simple technique for the quantitative measurement of biliary excretion and gall bladder concentration of drugs under physiological conditions in man. This technique should be of specific value in the case of cholephilic antibiotics.

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