

LIVER

Intestinal absorption of the bile acid analogue ^{75}Se -homocholic acid-taurine is increased in primary biliary cirrhosis, and reverts to normal during ursodeoxycholic acid administration

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Background: Whether ileal absorption of bile acid is up or downregulated in chronic cholestasis is still debated, and most evidence has come from animal studies.

Aims: To compare ileal bile acid absorption in patients with primary biliary cirrhosis (PBC) and in healthy control subjects, and to assess the effect of ursodeoxycholic acid (UDCA).

Patients: We studied 14 PBC patients before and during (n=11) UDCA administration, 14 healthy control subjects, and 14 Crohn's disease patients (as disease controls).

Methods: We used cholescintigraphy to measure retention in the enterohepatic circulation over five successive days of the bile acid analogue ^{75}Se -homocholic acid-taurine ($^{75}\text{SeHCAT}$) as an index of ileal bile acid absorption. Results were expressed as $^{75}\text{SeHCAT}$ fractional turnover rate (FTR) and $t_{1/2}$.

Results: $^{75}\text{SeHCAT}$ FTR was 0.19 (0.11)/day, 0.34 (0.11)/day ($p<0.001$), and 0.83 (0.32)/day in PBC patients, healthy controls ($p<0.0001$), and Crohn's patients ($p<0.001$), respectively, which increased to 0.36 (0.16)/day in PBC patients during UDCA treatment ($p<0.005$). $^{75}\text{SeHCAT}$ $t_{1/2}$ was 4.8 (2.1) days in PBC patients, 2.2 (0.5) days ($p<0.001$) in healthy controls, and 1.0 (0.5) days ($p<0.001$) in Crohn's disease patients. $^{75}\text{SeHCAT}$ $t_{1/2}$ decreased to 2.2 (0.93) days ($p<0.001$) in PBC patients during UDCA treatment.

Conclusions: Our results support the concept that ileal bile acid absorption is upregulated in PBC patients, and that this effect may contribute towards damaging the cholestatic liver. This upregulation of bile acid absorption is abolished by UDCA.

Primary biliary cirrhosis (PBC) is a chronic autoimmune liver disease characterised by progressive cholestasis.¹ As a consequence of chronic cholestasis, bile acid concentration may increase in serum and decrease in bile and in the small intestine, and these latter effects are likely to cause adaptive changes in expression and function of hepatic² and intestinal transporters³ for bile acid. Intestinal adaptive changes are thought to be important in PBC⁴ because upregulation of intestinal bile acid transport due to reduced substrate availability may favour bile acid retention in the enterohepatic circulation to a point that the capacity of the canalicular bile acid export pump is exceeded. This effect may lead to accumulation of bile acids in the hepatocyte and to hepatocyte necrosis or apoptosis.⁵

The adaptive regulation of intestinal bile acid transport during cholestasis has been extensively studied but available information is based mainly on animal or ex vivo models of acute and severe cholestasis,^{6–12} experimental conditions unlikely to represent the pathophysiological situation of chronic cholestasis at steady state in humans. Furthermore, these animal studies have provided conflicting results as downregulation,^{6,8,9,11,12} upregulation,⁷ and lack of regulation¹⁰ of intestinal bile acid transporters or transport have been reported.

Very little information is available on ileal absorption of bile acid in humans with chronic cholestasis studied under physiological conditions. This objective can be achieved using a scintigraphic technique involving oral administration of ^{75}Se -homocholic acid-taurine ($^{75}\text{SeHCAT}$), a radiolabelled bile acid analogue of taurocholic acid.¹³ Previous studies have shown that $^{75}\text{SeHCAT}$ behaves as the natural taurocholic acid

in the overall turnover of the enterohepatic circulation^{14,15} with the only difference that $^{75}\text{SeHCAT}$ undergoes negligible deconjugation by colonic bacteria¹⁴ and cannot be absorbed by passive non-ionic diffusion in the colon as for deconjugated bile acids. For this latter characteristic, $^{75}\text{SeHCAT}$ fulfils the prerequisites for an ideal marker of ileal function¹⁶ in that it can only be absorbed by active bile acid uptake in the terminal ileum. Using $^{75}\text{SeHCAT}$ scintigraphy it has been reported that intestinal absorption of bile acid in patients with chronic cholestatic liver diseases is normal¹⁷ but these results must be interpreted cautiously because the methodology used in these studies is inaccurate. By measuring $^{75}\text{SeHCAT}$ retention over the whole intestinal area, this methodology cannot distinguish between $^{75}\text{SeHCAT}$ activity within the enterohepatic circulation from that of $^{75}\text{SeHCAT}$ retained within the colon. This colonic retention of the isotope has been reported by Ferraris and colleagues¹⁸ to cause overestimation of $^{75}\text{SeHCAT}$ absorption to a variable extent in individual subjects and for different diseases by comparison with results obtained using the faecal isotope ratio, the gold standard for measurement of intestinal bile acid absorption.^{16,19}

The aim of our study was to assess ileal absorption of bile acids in patients with chronic cholestasis due to PBC by using a validated cholescintigraphic technique.^{14,18} This technique involves sequential measurement of $^{75}\text{SeHCAT}$ activity over the

Abbreviations: PBC, primary biliary cirrhosis; $^{75}\text{SeHCAT}$, ^{75}Se -homocholic acid-taurine; UDCA, ursodeoxycholic acid; FTR, fractional turnover rate.

Table 1 Characteristics of the primary biliary cirrhosis (PBC) patients studied

Patient No	Age (y)	Body weight (kg)	Pretreatment				Histology (stage)	During UDCA			
			Bilirubin (mg/dl) (0.3–1.2)	AP (mU/ml) (30–85)	γ GT (mU/ml) (5–50)	ALT (mU/ml) (5–50)		Bilirubin (mg/dl)	AP (mU/ml)	γ GT (mU/ml)	ALT (mU/ml)
1†	64	75	0.7	133	73	60	II	0.6	92	25	30
2†	55	47	0.6	103	92	32	II	0.6	83	46	30
3†	63	47	0.6	236	419	60	II–III	0.6	110	152	29
4†	23	82	0.4	591	400	360	I–II	0.4	414	303	250
5†	52	58	0.4	320	120	104	I–II	0.8	240	80	80
6†	50	55	0.4	500	202	80	I	1.0	340	82	55
7†	46	61	1.0	448	180	92	I–II	0.8	208	102	48
8†	70	56	0.5	182	311	50	II–III	0.6	95	105	36
9†	49	77	1.4	750	836	182	III–IV	1.4	180	190	42
10†	40	60	0.6	512	260	48	I	0.8	138	88	40
11†	50	50	0.6	694	193	123	III	0.5	272	82	86
12	57	58	1.0	652	297	113	I–II	1.0	657	317	50
13	48	43	0.5	748	226	81	II	0.6	236	309	57
14	65	50	1.0	282	310	125	IV	0.8	133	124	50
Mean	52	59	0.7	439	280	108		0.8	228***	143***	63***
SD	12	12	0.3	230	191	83		0.3	158	98	56

†Patients were studied twice with the $^{75}\text{SeHCAT}$ test: pretreatment and during ursodeoxycholic acid (UDCA) administration.

Normal ranges for alkaline phosphatase (AP), γ -glutamyl transpeptidase (γ GT), and alanine aminotransferase (ALT) are shown in parentheses.

*** $p < 0.001$ compared with pretreatment.

gall bladder area on five successive days following oral administration of the isotope. This technique is based on the observation in healthy subjects that a constant fraction of the bile acid pool is stored in the gall bladder in the fasting state,²⁰ and has been shown to be independent of the phenomenon of colonic retention and to provide measurements similar to those obtained using the faecal isotope ratio.¹⁸ We have also tested the effect of ursodeoxycholic acid (UDCA), a bile acid currently used for the treatment of PBC,²¹ on intestinal retention of $^{75}\text{SeHCAT}$. We studied 14 patients with PBC and 14 healthy control subjects. We also studied, as a disease control group, 14 patients with ileal Crohn's disease, a condition known to cause intestinal bile acid malabsorption. Eleven of the 14 PBC patients were studied twice, before and during UDCA administration.

PATIENTS AND METHODS

Patients

Fourteen female PBC patients entered the study. Patients characteristics are shown in table 1. Diagnosis of PBC was based on histological, serological, and immunological criteria, and all patients tested positive for antimithochondrial antibodies. None of the patient was overtly icteric on admission to the study, and serum bilirubin was slightly above the normal limit in only one patient.

All 14 PBC patients underwent a $^{75}\text{SeHCAT}$ study while off bile acid treatment and 11 agreed to have a repeat study during UDCA treatment. In order to avoid a sequence effect, four patients were first studied during UDCA and underwent a repeat study three months after stopping UDCA. Seven patients were studied during the opposite sequence: off treatment first and then three months after starting UDCA treatment. UDCA was administered in divided doses at meal-time, and the dose ranged from 13 to 15 mg/kg/day in individual patients.

Fourteen healthy control subjects were also studied (13 postmenopausal females and one male), of comparable age and body weight to patients with PBC (54 (4) v 52 (3) years and 61 (3) v 59 (3) kg, respectively). None of the control subjects had diarrhoea at the time of investigation. Fourteen patients with chronic or recurrent diarrhoea due to Crohn's disease were also studied (seven males, seven females, aged 42–70 years). Crohn's disease was diagnosed on the basis of characteristic clinical, radiological, and endoscopic findings.

All 14 patients had involvement of the terminal ileum, and seven had colonic involvement. Eight of these patients had undergone previous surgical treatment with resection of the terminal ileum that was longer than 100 cm in only one patient. Written informed consent was obtained from each subject, and the study was approved by the local ethics committee.

$^{75}\text{SeHCAT}$ test

The $^{75}\text{SeHCAT}$ test was performed as follows. On the day of the study, patients and healthy control subjects were admitted to a day case unit after fasting, and blood samples were taken for measurement of serum enzymes and serum bile acids. A capsule containing 370 kBq (10 μCi) $^{75}\text{SeHCAT}$ (Amersham International, Saluggia (VC), Italy)²² was administered orally and anterior and posterior abdominal γ -camera (model SP6; Elscint, GE Medical System, Milan, Italy) counting was carried out for 300 seconds on successive 4–5 early mornings and in a fasting state. ^{75}Se activity was calculated by selecting an area of interest over the gall bladder and following correction for background.

Laboratory methods

Serum concentrations of bilirubin, alkaline phosphatase, γ -glutamyltranspeptidase, and alanine aminotransferase were measured by standard automated laboratory techniques. Serum bile acid composition was measured by gas chromatography-mass spectrometry, as described in details elsewhere.²³

Calculation and statistical methods

In order to correct for different depths of ^{75}Se activity within the abdominal cavity, total counts over the gall bladder area of interest were obtained by relating anterior (ant) to posterior (post) counts according to the formula:

$$\text{total counts} = \text{sqr}(\text{ant}^2 + \text{post}^2)$$

Assuming first order kinetics, the slope K of the decrease in ^{75}Se activity over the gall bladder area was obtained by relating the ln of ^{75}Se activity measured on successive days (y) versus time in days (x). The $t_{1/2}$ of $^{75}\text{SeHCAT}$ was calculated according to the formula:

$$t_{1/2} \text{ (days)} = \ln 2 / K \text{ (per day)}$$

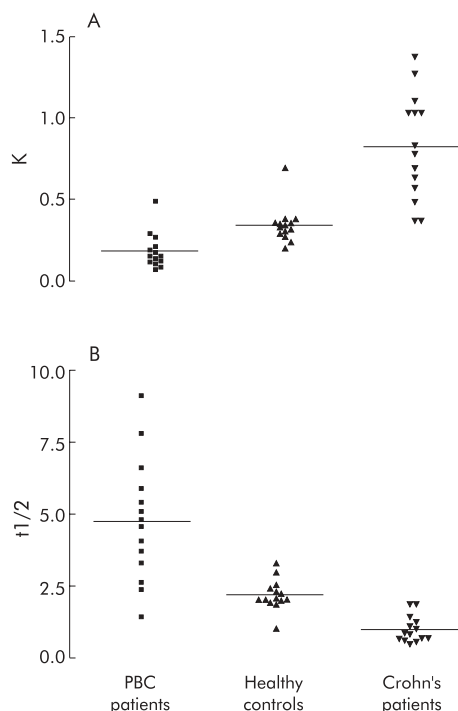


Figure 1 Daily fractional turnover rate (K) (A) and $t_{1/2}$ (B) of ⁷⁵Se-homocholeic acid-taurine (⁷⁵SeHCAT) in healthy controls, Crohn's disease patients, and in patients with primary biliary cirrhosis (PBC). Bars represent the mean value for each group.

where K represents the fractional turnover rate (FTR) of ⁷⁵SeHCAT.¹⁸

Results are expressed as means (SD). Differences between groups were tested for statistical significance using the Student's *t* test for paired and unpaired observations, as appropriate. A *p* value <0.05 was used to indicate statistical significance of differences. Parameters of linear regression were calculated using the least squares method.

RESULTS

PBC patients versus healthy control subjects and Crohn's disease patients

⁷⁵SeHCAT FTR ranged from 0.041 to 0.487/day in PBC patients, from 0.207 to 0.690/day in healthy controls, and from 0.368 to 1.380/day in Crohn's disease patients (fig 1A). The mean value for ⁷⁵SeHCAT FTR was significantly lower in PBC patients (0.182 (0.107)/day) compared with healthy controls (0.341 (0.112)/day) (*p*<0.0001) and Crohn's disease patients 0.829 (0.325)/day) (*p*<0.0001).

⁷⁵SeHCAT retention in the enterohepatic circulation expressed as $t_{1/2}$ ranged from 1.4 to 9.1 days in PBC patients, from 1.0 to 3.3 days in normal controls, and from 0.5 to 1.9 days in Crohn's disease patients (fig 1B). The mean value for ⁷⁵SeHCAT $t_{1/2}$ was significantly higher in PBC patients (4.8 (2.1) days) compared with healthy control subjects (2.2 (0.5) days) (*p*<0.001) and Crohn's disease patients (1.0 (0.5) days) (*p*<0.0001).

Values of ⁷⁵SeHCAT FTR and $t_{1/2}$ in PBC patients did not correlate with the histological stage of the disease or with biochemical parameters of cholestasis or cytolysis.

Effect of UDCA treatment

During treatment, the bile acid pool enriched with UDCA and the proportion of this bile acid in serum bile acids increased from 6 (5)% before treatment to 26 (5)% during UDCA (*p*<0.01). Corresponding values for the primary bile acids cholic acid and chenodeoxycholic acid decreased from 30 (15)% to 21 (12)% and from 33 (11)% to 25 (11)%,

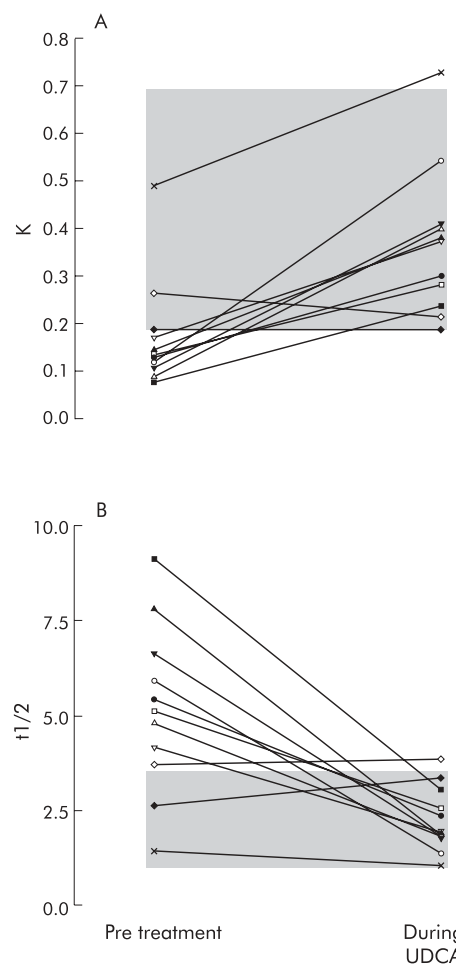


Figure 2 Effect of ursodeoxycholic acid (UDCA) treatment on daily fractional turnover rate (K) (A) and on $t_{1/2}$ (B) of ⁷⁵Se-homocholeic acid-taurine (⁷⁵SeHCAT) in patients with primary biliary cirrhosis. The shaded area represents the range of values for healthy controls.

respectively, but these differences were not statistically significant. The proportion of lithocholic acid increased from 8 (10)% to 13 (12)% (NS) and deoxycholic acid remained virtually unchanged (17 (9)% *v* 16 (11)% for pretreatment and during UDCA, respectively).

During UDCA, ⁷⁵SeHCAT FTR increased in nine individual PBC patients and slightly decreased in two of 11 patients studied before and during UDCA (fig 2A). These two latter patients were among the four patients with an FTR within the normal range before treatment. The mean value for ⁷⁵SeHCAT FTR increased from 0.173 (0.116)/day pretreatment to 0.363 (0.157)/day (*p*<0.005) during UDCA.

During UDCA, ⁷⁵SeHCAT $t_{1/2}$ decreased in nine patients and slightly increased in two patients (fig 2B). The mean value for $t_{1/2}$ decreased significantly from 5.1 (2.2) days pretreatment to 2.2 (0.9) days (*p*<0.001) during UDCA, and visual inspection of fig 1 clearly indicates that the size of this effect of UDCA was greater for patients with higher pretreatment $t_{1/2}$ values. The $t_{1/2}$ values measured during treatment fell within the range observed in healthy controls in all patients except one. The mean value for ⁷⁵SeHCAT $t_{1/2}$ during UDCA was the same as that observed in control subjects (2.2. (0.9) days *v* 2.2 (0.5) days, respectively).

Serum concentrations of alkaline phosphatase, γ -glutamyltranspeptidase, and alanine aminotransferase decreased in each individual patient during UDCA (table 1). Treatment was well tolerated by all patients. No side effects were reported.

DISCUSSION

Our study indicates that retention in the enterohepatic circulation of the bile acid analogue $^{75}\text{SeHCAT}$ is increased in patients with PBC compared with healthy controls, a finding in keeping with the low FTR of primary bile acid reported by other authors^{24,25} in these patients. Differences between PBC patients and control subjects were striking, as indicated by visual inspection of fig 1. Values for PBC patients varied widely for both $^{75}\text{SeHCAT}$ FTR and $t_{1/2}$, probably as a result of the variable degrees of perturbation of the enterohepatic circulation in individual patients. This phenomenon may also explain the slight overlap between the two populations. As expected, $^{75}\text{SeHCAT}$ FTR was increased and $t_{1/2}$ was reduced in patients with ileal disease or resection compared with healthy controls and PBC patients (fig 1), a finding confirming the intrinsic validity of $^{75}\text{SeHCAT}$ as a test of ileal absorption.

In contrast with our results, Chazouilleres and colleagues¹⁷ reported no difference in $^{75}\text{SeHCAT}$ retention in 12 PBC patients compared with control subjects but the validity of their results is questionable for two reasons. Firstly, they measured whole abdominal retention of $^{75}\text{SeHCAT}$, a measurement influenced by colonic retention of isotope, thus introducing a large error in their measurements,¹⁸ as admitted by Chazouilleres and colleagues,¹⁷ in relation to their own studies in healthy volunteers.²⁶ Secondly, Chazouilleres and colleagues¹⁷ included five cholecystectomised patients among a total of 12 PBC patients studied. Abolition of gall bladder storage function in cholecystectomised patients has been reported to result in a decreased bile acid pool size^{27,28} and in an increased FTR of primary bile acids,^{29,30} and this latter phenomenon may have contributed to the increase in FTR of $^{75}\text{SeHCAT}$ in the subgroup of cholecystectomised PBC patients studied by Chazouilleres and colleagues.¹⁷ In contrast with these authors, our study was carried out using a validated cholescintigraphic technique for measurement of ileal bile acid absorption,¹⁸ and all 14 PBC patients studied had functioning gall bladders, as ascertained by $^{75}\text{SeHCAT}$ accumulation in the gall bladder area during cholescintigraphy.

We are well aware that increased retention of $^{75}\text{SeHCAT}$ in the enterohepatic circulation does not prove that all natural bile acid species are retained in the same way as $^{75}\text{SeHCAT}$. $^{75}\text{SeHCAT}$ handling within the enterohepatic circulation has been shown to be similar to that of taurocholic acid,¹⁴ but no information is available on comparisons between $^{75}\text{SeHCAT}$ and chenodeoxycholic or deoxycholic acid. The observation that biliary bile acid composition shows little difference between healthy subjects and patients with early cholestatic disease^{31,32} indirectly suggests that in our study there was retention of all endogenous bile acids.

Our study strongly supports the view that chronic cholestasis in humans is accompanied by upregulation of ileal bile acid absorption. Cholestasis and/or sluggish gall bladder contraction may have theoretically contributed to the reduced $^{75}\text{SeHCAT}$ FTR in our study. We believe that the effect of cholestasis was marginal because serum bile acid composition was normal in our patients, suggesting very mild cholestasis. Furthermore, gall bladder motility has been reported by others³³ as normal in PBC patients.

In vitro and in vivo animal studies on the adaptive changes in the expression and function of intestinal transporters for bile acids have provided conflicting results. Downregulation of ileal bile acid uptake has been reported in rats with decreased intestinal bile acid concentrations, resulting from acute extrahepatic cholestasis, and in studies involving measurement of bile acid uptake by ileal brush border membranes.^{3,6,8,11} In contrast with these findings, upregulation of ileal bile acid uptake was reported by other authors in anaesthetised bile fistula guinea pigs⁷ during administration of cholestyramine, a bile acid sequestrant resin. The effect on ileal absorption of increasing the bile acid load to the intestine by means of bile

acid feeding is also controversial,^{10,12} adding further uncertainty to the adaptive changes in intestinal bile acid uptake to different bile acid loads. This controversy has been explained by several factors, including species specific differences^{3,7} or differences between measuring transport function of the whole intestine or transporter activity in a specific intestinal segment that may not detect changes in zonal distribution of transporters.⁴ Furthermore, all of these studies were carried out under conditions of acute or short term changes in intestinal bile acid load, a condition extremely different from the chronic and slowly progressive cholestatic condition that characterises PBC.¹ To overcome these limitations, the importance of measuring intestinal transport function in humans has been authoritatively emphasised,⁴ and the method used in the present study is consistent with this recommendation.

The second important observation in our study was that $^{75}\text{SeHCAT}$ FTR increased and retention within the enterohepatic circulation was reduced to normal in PBC patients during UDCA treatment. These findings are consistent with the current view that UDCA administration may cause endogenous bile acid malabsorption.³⁴⁻³⁶ This effect of UDCA on endogenous bile acid absorption is not the only mechanism advocated to explain the beneficial effect of UDCA in PBC and other cholestatic liver diseases. A cytoprotective effect^{37,38} and an improvement in hepatobiliary excretory function have also been reported for UDCA.^{39,40} The scintigraphic finding of Jazrawi and colleagues⁴⁰ that the reduced hepatic excretion of intravenous $^{75}\text{SeHCAT}$ observed in patients with PBC improved but was not corrected by UDCA in the majority of their patients indirectly suggests that normalisation of $^{75}\text{SeHCAT}$ retention during UDCA observed in our study is partly independent of the choleric effect of this bile acid and emphasises the effect of UDCA on intestinal absorption of the isotope. The observation of Invernizzi and colleagues⁴¹ that faecal bile acid excretion is increased in PBC patients during UDCA treatment, and that this increased excretion is mainly due to secondary bile acid (lithocholic and deoxycholic acid) excretion is consistent with the hypothesis of primary bile acid ileal malabsorption during UDCA treatment with consequent colonic biotransformation to secondary bile acid.

In conclusion, our findings indicate that intestinal bile acid absorption is upregulated in PBC patients, and that this upregulation is reversed by UDCA treatment. This ability of UDCA to compete with other bile acids for ileal absorption may prevent accumulation in the hepatocyte of toxic bile acids, thus representing a mechanism to explain the beneficial effect of UDCA in PBC.

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