INVESTIGATIVE NUCLEAR MEDICINE

Enterohepatic Circulation in Man of a Gamma-Emitting Bile-Acid Conjugate, 23-Selena-25-Homotaurocholic Acid (SeHCAT)

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A conjugated bile acid, 23-selena-25-homotaurocholic acid (SeHCAT), labeled with the gamma emitter Se-75, has been evaluated in man. Absorption and excretion were compared with that of simultaneously administered [23-^{14}C]cholic acid. SeHCAT is absorbed quantitatively following oral administration, secreted into the bile at the same rate as cholic acid, reabsorbed from the small intestine, and resecreted. It is not absorbed when the terminal ileum has been excised or bypassed. SeHCAT is therefore the first of a new class of radiopharmaceuticals, namely, gamma-emitting tracers of the complete cycle of the enterohepatic circulation. Its use will simplify investigation of the functional state of the terminal ileum by eliminating the need to collect and process feces.

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Clinical investigation of the enterohepatic circulation—in particular the absorptive capacity of the ileum—would be greatly facilitated by the availability of a gamma-emitting bile acid that can be detected by external counting, thus eliminating the need for fecal collection or duodenal aspiration (*1*,*2*).

A suitable compound would be absorbed from the lumen of the gut by the same mechanism as the natural bile acids, and at the same rate. It must be extracted from the portal blood by the liver and excreted into the bile by the same processes and at the same rate as the natural compound, and must be reabsorbed and recirculated by the same mechanisms. The radioactive label should not be detached or transferred to any significant extent in vivo and it should be stable in vitro.

In the rat, the taurine conjugate of 23-[^{75}Se]selena-25-homocholic acid (SeHCAT) (Fig. 1) has been shown to be absorbed from the gut and excreted into the bile at the same rate as cholic acid (*3–5*). We report here the initial studies in man with this compound.*

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METHODS AND RESULTS

Bile collections. A mixture of an aqueous solution containing 24–28 μCi (0.9–1 MBq) of SeHCAT† at a specific activity of 68.5 mCi/mmol was administered orally to four subjects on the third day after surgical insertion of a T tube into the common bile duct. At the time of the SeHCAT dose, the same activity of [23-^{14}C]cholic acid (52 mCi/mmol) was given. At this time oral feeding of fluids had been reestablished, and nasogastric suction and intravenous fluids discontinued. The T tube was draining freely and was not clamped. Patients received dihydrocodeine tablets, 60 mg every 6 hr if necessary for pain, but no other medication.

The indication for exploration of the common bile duct was cholelithiasis with choledocholithiasis in three subjects and carcinoma of the ampulla of Vater in the fourth. The first three had normal hepatic function and were otherwise healthy. The fourth was jaundiced, with a serum bilirubin of 250 μmol/l preoperatively. This had fallen to 100 μmol/l by the end of the bile collection. Drainage of the common bile duct was preliminary to definitive surgery.

The total drainage from the T tubes was collected over 3-hr periods for the first 24 hr, and further collections

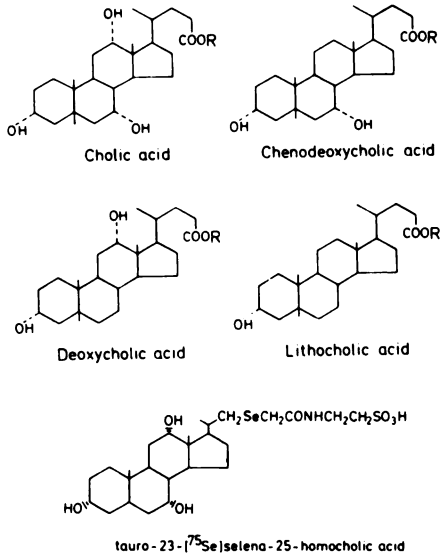

FIG. 1. Structural formulas of the principal human primary bile acids (top line), secondary bile acids (middle line), and SeHCAT (bottom line).

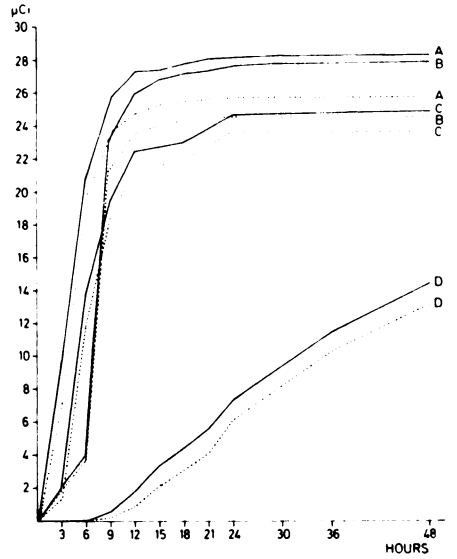

FIG. 2. Cumulative recovery from bile of C-14 (solid lines) and SeHCAT(Se-75) (dotted lines) in three normal subjects (A, B, C) and one jaundiced patient (D).

were made at 30, 36, and 48 hr. The volume of each sample was measured and a 1-ml aliquot transferred to a counting vial and decolorized with hydrogen peroxide. Three milliliters Instage‡ scintillation mixture was then added and, after stabilization, C-14 radioactivity was counted under preset conditions. The vials were then transferred to an automatic gamma counter and counted again for Se-75 activity. A computer program corrected for the detection of gamma photons in the beta counter and for radioactive decay in the interval between beta and gamma counting.

Of the administered C-14 activity, (98 ± 2)% was recovered from the bile within 24 hr of oral administration in the three subjects with normal hepatic function. Over the same period (90.9 ± 2.8)% of the administered Se-75 activity was recovered. The time course of appearance of both tracers in the bile was similar in each individual, despite differences from one patient to another (Fig. 2). The amounts of both tracers recovered from the bile was much lower in the jaundiced patient, but the rates of recovery were similar. Whole-body counting (of the subject and the collected bile) confirmed that there was no loss of Se-75 by any other route over this period.

Intact normal subjects. Whole-body retention (WBR) of Se-75 was measured after oral administration of SeHCAT, using a shadow-shield whole-body counter equipped with four NaI(Tl) detectors 150 mm in diameter by 100 mm thick. The pulse-height window covered from 10–510 keV to include all of the primary peaks and the Compton scatter (7) but to exclude base-line noise. The counter was calibrated daily against two phantoms containing 0.5 μCi (19 kBq) and 0.1 μCi (4 kBq) Se-75 in 1 l of water.

Nine members of the medical or scientific staff were counted for 70 days after administration of 10 μCi (0.4 MBq) of SeHCAT orally, or until the radioactivity became undetectable. Nine volunteers among patients attending the out-patient clinic, without evidence of gastrointestinal disease, were counted for up to 40 days after 1 μCi (40 kBq) orally.

Excretion can be described in man, as in the rat, by an equation of the form $x = Ae^{-at} + Be^{-bt} + C$. The constant A is the intercept of the faster-clearing component on the activity axis. (Fig. 3) It has a mean value of 155%, with a range from 100% to 376%. A value greater than 100% retention clearly has no physical meaning, and is a mathematical artifact, due to slow mixing in the colon. Compartmental analysis is valid only when there is complete mixing within the compartments. Errors arise when measurements are made before mixing is complete. In this case it is more meaningful (but mathematically unconventional) to regard the displacement of the 100% value along the time axis as an indicator of the mixing or transit time in the colon (range 0–4.5 days).

The value of the index a is the rate constant of excretion of the fast component. This has a half-time of 2.6 ± 0.7 days (range 1.4–4.7 days).

The slower-clearing component, comprising (3.8 ± 2.8)% of the administered activity, has a half-life of 62 ± 17 days. Residual nonexcreted activity (equivalent to constant C) was too small to be measured. If present it comprises less than 1% of the administered activity and probably much less.

In four of the subjects, blood clearance and whole-body retention (WBR) were measured on a second occasion, this time following intravenous administration, approximately 5 mo after the initial oral dose. The initial

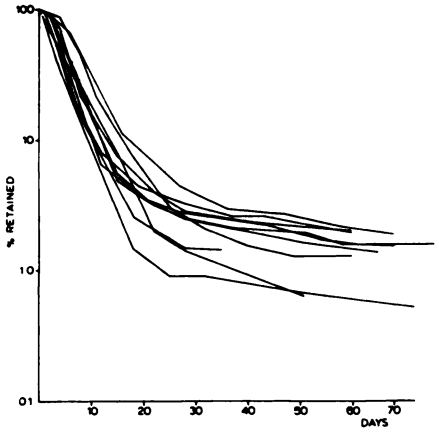

FIG. 3. Whole-body retention in nine normal subjects. Lines connect measured points and are not fitted or extrapolated.

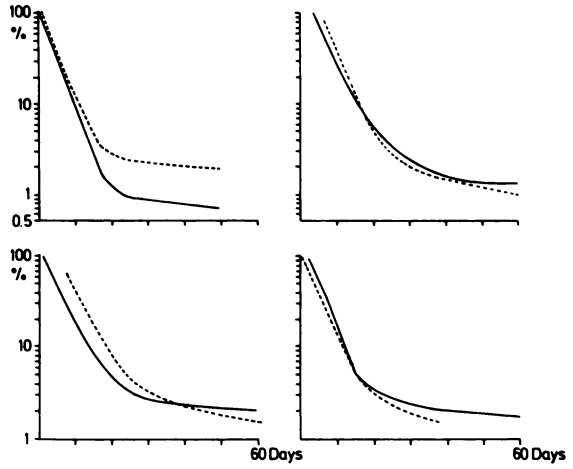

FIG. 4. Whole-body retention of SeHCAT in four subjects after oral (solid lines) and intravenous administration (dotted lines).

clearance from the blood has a half-time of 5.1 ± 0.8 min. Random blood samples taken during the week after i.v. administration (or after oral administration in some other subjects) gave plasma levels that varied between 0.007 and 0.0001% of the administered activity per milliliter. Values did not follow any simple pattern but resembled that of circulating plasma bile-acid levels, the highest values being found between 1 and 3 hr after meals, and the lowest in fasting subjects. There was little difference in WBR curves between those made following oral and those following intravenous administration (Fig. 4).

Ileal exclusion. WBR was measured under conditions identical to the above after oral administration of 1 μCi of SeHCAT to four subjects who had previously undergone resection of more than 300 cm of ileum for Crohn's disease or mesenteric thrombosis, and four who had undergone 14-4 jejuno-ileal bypass operations for refractory obesity (*8*). These latter four, and two of the former, were each examined on two separate occasions. Excretion was much more rapid in these subjects, with a mean WBR of (0.7 ± 0.7)% at 7 days (Table 1). In contrast, the WBR of normal subjects at one week was (29.9 ± 8.9)%. The rapid excretion precluded long-term measurements in the group with ileal resection.

DISCUSSION

Bile acids follow a complex enterohepatic circulation. The primary human bile acids (cholic and chenodeoxycholic) are synthesized in the liver and conjugated with glycine or taurine before excretion into the bile. Normal bile contains no free bile acids or salts. The conjugates are reabsorbed principally by active ionic transport. This occurs only in the distal ileum. In the presence of Crohn's disease or other pathological conditions affecting the ileum, absorption of conjugates is reduced and more bile acids enter the colon (*2*).

Free bile acids, both primary and secondary, are passively absorbed to some extent in the jejunum and colon. Under normal circumstances free acids are not present in the jejunum. However, in the presence of bacterial overgrowth, conjugates may be hydrolyzed to form free acids. Bacterial hydrolysis occurs to a much greater extent in the colon, where there is in addition bacterial dehydroxylation to form a number of secondary bile acids. Under normal circumstances, passive diffusion is quantitatively small compared with active transport. There is little passive absorption of glycine conjugates at any site, and virtually none of taurine conjugates (*9*). Secondary bile acids are reabsorbed along with the primary, and recirculate similarly. On passing through the liver, all are conjugated before reappearing in the bile. Thus, absorption of taurine conjugates is confined to the ileum, and a stable taurine conjugate, resistant to de-

TABLE 1. PERCENTAGE WHOLE-BODY RETENTION OF SeHCAT

	Number of		4 days			7 days		
	subjects	examinations	Mean % retained	s.d.	Range	Mean % retained	s.d	Range
Normal	18	21	68.6	18.9	43–100	37.7	17.3	19–79
Resection of ileum	4	6	2.3	2.0	0–4.5	0.56	0.75	0–2
Ileal bypass	4	8	8.3	8.6	1–28	0.83	0.87	0–2.7

TABLE 2. ABSORBED DOSE FROM SeHCAT IN NORMAL SUBJECTS (SUMMARIZED FROM REF. 6)

	mrad/μCi	μGy/kBq
Liver	1.3	0.35
Small-intestinal wall	10.2	2.8
Large-intestinal wall	8.0	2.2
Gallbladder	4.9	1.3
Total body	0.9	0.24

conjugation, should be a specific indicator of the functional state of the distal ileum.

Selenium is not a normal constituent of bile acids, and it is therefore necessary to prove that the biological behavior of the synthetic analog resembles that of the natural compound. We have previously compared the absorption and excretion of selena bile acids with that of cholic acid in the rat (*3*), and have shown that replacement of the C-23 methylene group by selenium in the extended side chain of homocholic acid does not affect the rate of absorption or of excretion. If a longer side chain is present, the compound no longer behaves as a bile acid.

The active transport of cholic acid is supplemented by some passive diffusion. In contrast, there is little or no passive diffusion of taurine conjugates. The difference in recovery of C-14 and Se-75 in subjects with bile-duct drainage is almost certainly due to this difference in properties between cholic acid and the taurine conjugate. Such a difference would be present only on the first recirculation. The chemical form of the C-14 cholic acid excreted was not assayed but was almost certainly present principally as a glycine conjugate. The actual ratio of glycine to taurine conjugate varies from one individual to another, but in most subjects glycine conjugates predominate. Rapid conversion between the free acid and the various conjugates makes study of the properties of the separate conjugates difficult in intact subjects.

In the rat (*3*) both Se-75 and C-14 appear in the bile at the same rate following the intravenous administration of a mixture of C-14 cholic acid and SeHCAT(Se-75). As both also appear in the bile at the same rate after oral administration (in man as well as in the rat), it is evident that both must be absorbed at the same rate. Exclusion of the ileum, by either excision or bypass, eliminates absorption of SeHCAT in man, confirming that this must be the site of absorption.

The biexponential excretion of SeHCAT requires some consideration. The fast component, comprising approximately 96% of the administered activity, is excreted with a half-life of 2.6 ± 0.7 days. This is not significantly different from the figures obtained previously using C-14 cholic acid (*10*), despite important differences between whole-body counting and techniques using beta emitters. Whole-body counting measures the total amount of radioactivity remaining, but gives no information about its chemical form or its distribution within the body. Standard methods require fecal collection and analysis. Recovery of C-14 is always incomplete and the fate of the "lost" activity is unknown. The possibility of a long-half-life component has been postulated but is unproven. The similarity of the results obtained by the two techniques, despite the different sources of error, supports the accuracy of both. Further experiments are necessary to confirm that the slow pool of selenium detected with SeHCAT is still bile acid and not a breakdown product yet to be isolated in vitro.

The biological interpretation of the >100% intercept of the fast component (constant A) can be explained by the finite transit time between ingestion and fecal excretion. In the majority of subjects this is small, and the constant is only a little over 100. In some, however, the value is much greater and the entire curve is shifted to the right. This delay may be regarded as an index of the duration of the process of mixing in the colon.

Thus SeHCAT fulfills many, possibly all, of the requirements of the ideal tracer for investigating the integrity of the enterohepatic circulation of bile acids. Following oral administration it is absorbed from the gut and excreted into the bile at the same rate as 24-[^{14}C]-cholic acid. Excision or bypass of the terminal ileum—the only part of the intestine capable of active transport of bile acids—almost totally abolishes absorption of SeHCAT. The only other substance known to have an active transport process confined to this part of the intestine is vitamin B_{12}, and it is inconceivable that SeHCAT should follow this pathway.

The discrepancy between the rapidity with which SeHCAT appears in the bile and its biological half-life indicates that it must be reabsorbed and recirculated in a manner closely analogous to that of natural bile acids. The identity of WBR following both oral and intravenous administration is further evidence of the completeness of absorption.

Preliminary experiments suggest that it is very stable both in vivo and in vitro. These will be reported in detail later. The gamma energy of Se-75 and the absence of beta emissions are favorable for whole-body counting; this eliminates the need to collect and process feces, while the rapid biological turnover compensates for the long physical half-life of Se-75 (Table 2). On the other hand, the long half-life and chemical stability at ambient temperature permit an extended shelf life, a useful attribute in a clinical diagnostic agent.

In normal subjects excretion might be accelerated after termination of measurements by the administration of a bile-acid-binding agent such as cholestyramine. Even without this, however, the radiation dose is so low

that the expedient is probably unnecessary. Thus the properties of SeHCAT justify a trial of its clinical efficacy.

FOOTNOTES

* These studies were performed with the approval of the appropriate committees on safety and informed consent was obtained from all patients.

† Amersham International Ltd.

‡ Packard Corporation.

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