Effects of a meal on plasma clearance of \([^{14}\text{C}]\)glycocholic acid and indocyanine green in man

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Summary

1. The fasting plasma disappearance curve of \([^{14}\text{C}]\)glycocholic acid after intravenous injection was compared in nine normal subjects with that obtained 100 min after a standard liquid test meal.

2. Plasma disappearance curves of indocyanine green were determined in 13 normal subjects under the same conditions.

3. Plasma clearances were significantly increased after the meal for both \([^{14}\text{C}]\)glycocholic acid (median 455 ml min\(^{-1}\) m\(^{-2}\), range 376–672 increased to 704, 528–1968; \(P < 0.01\)) and indocyanine green (359, 227–473 increased to 435, 358–985; \(P < 0.01\)).

4. Median initial volume of distribution was unaltered, but in four subjects it was greatly increased after the meal, although no alteration in plasma volume, measured with Evans blue dye, was observed.

5. The increased postprandial plasma clearance of glycocholic acid is probably due to an increase in liver blood flow, and suggests that in health this part of the enterohepatic circulation of bile acids also varies with meals.

Key words: bile acids, glycocholic acid, indocyanine green, liver.

Introduction

The determinants of serum bile acid levels in health and disease have been the subject of many recent investigations. In normal subjects total serum bile acid levels rise after a meal [1, 2], though less than in patients with liver disease [3, 4]. It has therefore been suggested that the 2-h postprandial level is a more sensitive test of liver function than the fasting level and other commonly used tests [3, 5], although this has not been confirmed by others [6, 7]. The postprandial rise in total serum bile acids in health could be the result of one or more of the following mechanisms: the same fraction of an increased mass of bile acids extracted from the portal blood, a reduced hepatic extraction ratio, the opening of extra- and intra-hepatic portal-systemic shunts and, less likely, saturation of the hepatic uptake mechanism.

LaRusso, Hoffman, Korman, Hoffmann & Cowen [8] gave \([^{14}\text{C}]\)glycocholic acid and bromsulphthalein intravenously before and after a standard liquid test meal to six normal subjects. Although there was a trend towards a faster plasma disappearance of \([^{14}\text{C}]\)glycocholic acid postprandially, the increase in the distribution and elimination half-life times \((t_1\alpha\) and \(t_1\beta\) did not reach significance; it was therefore suggested that the fractional extractions of bile acids and bromsulphthalein remain constant after a meal. However, on theoretical grounds the postprandial disappearance should be faster than the preprandial. This is because glycocholic acid is highly extracted by the normal liver, with an extraction ratio of 0.85 in man [9]; its clearance after intravenous administration should therefore...
be closely related to changes in hepatic blood flow [10]. Since liver blood flow is increased postprandially in man [11, 12] and animals [13, 14], an increase in bile acid clearance, and therefore plasma disappearance, would be expected.

To establish the validity of these physiological principles applied to bile acid clearance, we have given \([^{14}\text{C}]\text{glycocholic acid} \) intravenously to normal subjects, and compared the pre- and post-prandial plasma disappearance curves, clearances and initial volumes of distribution. In addition, the dye indocyanine green, which has a high extraction ratio of about 0.70 and is therefore suitable for measuring liver blood flow in normal subjects when given in low doses [15, 16], was given simultaneously. Because four subjects were found to have large increases in volumes of distribution of \([^{14}\text{C}]\text{glycocholic acid} \) or indocyanine green postprandially, we also investigated the effect of a meal on plasma volume using Evans blue dye [17].

Materials and methods

Subjects

\([^{14}\text{C}]\text{Glycocholic acid} \). Nine healthy medical students and doctors were studied; six males and three females, aged 21–34 years. None had evidence of liver disease and all had normal routine liver function tests except for one male who was found to have mild Gilbert's syndrome. Two of the women were taking the contraceptive pill.

Indocyanine green and Evans blue. Indocyanine green was given simultaneously with \([^{14}\text{C}]\text{glycocholic acid} \) to eight of the above nine subjects, while two further normal female subjects (aged 22 and 33 years) received indocyanine green alone and three male subjects (aged 30–34 years) received indocyanine green and Evans blue simultaneously. Four of the nine subjects were found to have large increases in volumes of distribution for either \([^{14}\text{C}]\text{glycocholic acid} \) or indocyanine green postprandially; two of these were given Evans blue and indocyanine green simultaneously on another occasion. Thus a total of 13 subjects received indocyanine green and five subjects received Evans blue.

The study was approved by the Ethical Committee of the hospital and all subjects gave informed written consent.

Materials

\([1^{\text{14}}\text{C}-\text{Glycine}]\text{Glycocholic acid}, \) specific radioactivity 51 mCi/mmol (The Radiochemical Centre, Amersham, Bucks., U.K.) was dissolved in sodium chloride solution (150 mmol/l: saline) to give a concentration of 1 μCi/ml (4 × 10⁴ Bq/ml), passed through a 0.22 μm pore membrane filter and stored in sealed ampoules. Thin-layer chromatography on 0.25 mm silica-gel plates (E. Merck, Darmstadt), with butanol-acetic acid/water (12:3:5, by vol.) as solvent, confirmed radiopurity of greater than 98%.

On the day of study, after addition of 20 ml of aqueous solvent to 25 mg ampoules of indocyanine green (Hyson, Westcott and Dunn, Baltimore, U.S.A.), 0.25 mg (0.32 μmol/kg body wt.) was administered/kg.

The standard liquid test meal was of the same composition as that used by LaRusso et al. [8]: 20% protein, 40% carbohydrate, 40% fat; 10 cal/kg body weight. The homogenate was prepared from milk, eggs, double cream, skimmed milk powder and Maxijul (Scientific Hospital Supplies, Liverpool, U.K.).

Techniques

Subjects were studied after an overnight fast and, at least 1 week later, 100 min after the liquid test meal. Although the two studies were not carried out in random order, two randomly selected subjects underwent the postprandial study before the fasting one. After lying semirecumbent for at least 15 min, an initial blood sample was taken and 5 μCi (2 × 10⁵ Bq) of \([^{14}\text{C}]\text{glycocholic acid} \) and 0.25 mg of indocyanine green were rapidly injected intravenously/kg in less than 15 s. Those who had plasma volume estimations received 5 ml of a 5 mg/ml (5·2 μmol/ml) solution of Evans blue. Heparinized blood (4 ml) was taken from an indwelling 19G cannula in the opposite antecubital fossa at 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 25, 30, 40, 50, 60 and 90 min.

Samples were centrifuged at 1800g for 20 min and 1 ml of plasma was added to 10 ml of liquid scintillator (NE260, New England Nuclear, Edinburgh, Scotland, U.K.) and counted for 1000 s thrice in a Nuclear Enterprises 8312 liquid-scintillation counter. The mean value was corrected for quenching by the external-standard channels-ratio method, and results were expressed as d.p.m. per ml of plasma.

Indocyanine green and Evans blue concentrations were determined by using an absorption spectrophotometer (CE272, Cecil Instruments, Cambridge, U.K.) at wavelengths of 800 and 610 nm respectively. Repeated centrifugation was sometimes necessary to ensure that all samples
Meals and bile acid clearance

gave an absorbance reading at 900 nm within 0.015 of the plasma blank sample; this made correction for variable blank density [18] unnecessary.

Analysis of results

The plasma clearance of $[^{14}\text{C}]$glycocholic acid was calculated from eqn. (1),

\[
\text{Clearance} = \frac{\text{dose}}{\text{Auc}^{00}}
\]

(1)

where \text{Auc}^{00} (calculated by the trapezoidal rule) is the area under the graph of concentration (d.p.m. per ml) against time (min), with the initial part of the curve extrapolated to time zero and the terminal part to infinity. The initial volume of distribution ($V_D$) was calculated from eqn. (2)

\[
V_D = \frac{\text{dose}}{\text{concn. at } t = 0}
\]

(2)

The concentration-time curve for $[^{14}\text{C}]$glycocholic acid was also analysed by an unweighted iterative non-linear least-squares fit programme to fit an equation with two exponential components, of the form shown by eqn. (3),

\[
Ct = Ae^{-\alpha t} + Be^{-\beta t}
\]

(3)

where $Ct$ is the concentration at time $t$, $A$ and $B$ are the intercepts and $\alpha$ and $\beta$ are the slopes of the distribution and elimination exponentials. The distribution and elimination half-life times ($t_{1/2a}$ and $t_{1/2b}$) were then calculated by dividing 0.693 by $\alpha$ and $\beta$ respectively.

The plasma concentration of indocyanine green fell mono-exponentially with time and therefore plasma clearance was calculated from eqn. (4),

\[
\text{Clearance} = k \cdot V_D
\]

(4)

where $k$ is the fractional disappearance rate (min$^{-1}$); $k$ and $V_D$ were calculated by a least-squares fit programme.

Plasma volume (ml) was calculated from eqn. (5).

\[
\text{Plasma volume} = \frac{\text{dose}}{\text{concn. of Evans blue at } t = 0}
\]

(5)

All data were expressed per square metre body surface area, calculated from height and weight [19]. Since the derived parameters (clearance, volume of distribution and plasma volume) were not normally distributed, the median and range were calculated, and Wilcoxon’s signed-rank test for paired observations was used to test the significance of any difference between pre- and post-prandial measurements.

Results

The mean plasma disappearance curves for $[^{14}\text{C}]$glycocholic acid and indocyanine green are shown in Figs. 1 and 2 respectively. The postprandial mean values for both bile acid and indocyanine green were lower at all times after injection. For $[^{14}\text{C}]$glycocholic acid, neither the distribution ($t_{1/2a}$) nor the elimination half-life ($t_{1/2b}$)
were significantly altered by the meal ($t_1$: median 3.0 min, range 1.4–4.1 and 1.5, 0.6–3.0; sum of positive ranks 8, negative 37; $P > 0.05$. $t_2$: median 14.7 min, range 7.4–33.0 and 6.5, 5.0–15.1; sum of positive ranks 9, negative 36; $P > 0.05$).

The increase in the postprandial fractional disappearance rate ($k$) of indocyanine green also did not reach significance (0.24, 0.20–0.46 and 0.32, 0.24–0.44; rank 20.5, 70.5; $P > 0.05$). Plasma clearance (Fig. 3a, b) was significantly increased after the meal for both $[^14]C$glycocholic acid (455 ml min$^{-1}$m$^{-2}$, 376–672 increased to 704, 528–1968; rank 0, 45; $P < 0.01$) and indocyanine green (359, 227–473 increased to 435, 358–85; rank 0, 91; $P < 0.01$). Plasma clearance was increased postprandially in all subjects studied. $[^14]C$Glycocholic acid clearance was significantly correlated with that of indocyanine green ($r = 0.54; P < 0.025$).

The initial $V_D$ was not altered postprandially for either $[^14]C$glycocholic acid (3679 ml/m$^2$, 2148–5401 and 3667, 2362–9391; rank 27, 18; $P > 0.05$) or indocyanine green (1404, 810–2632 and 1510, 1001–2058, rank 26, 55; $P > 0.05$). However, four subjects had large increases in $V_D$ of bile acid or indocyanine green postprandially. To determine whether these increases in $V_D$ were due to changes in plasma volume, measurements of plasma volume with Evans blue were made in two of these and in a further three subjects. No difference in plasma volume before and after the meal was detected (1884 ml/m$^2$, 1303–2114 and 1683, 1446–1863; rank 4, 11; $P > 0.05$).

**Discussion**

The results confirm that, as predicted from physiological principles, the pre- and postprandial plasma disappearance curves of $[^14]C$glycocholic acid and indocyanine green are not identical, and this is reflected in the significant increases in clearance of both anions after the meal. The bile acid clearance is correlated with that of indocyanine green, suggesting that there is a common mechanism underlying the effect of the meal. The most likely explanation is the
known postprandial rise in liver blood flow. This would also explain why $^{14}$Cglycocholic acid clearance is increased more than that of indocyanine green after the meal, because, being more highly extracted by the liver, its clearance will be the more dependent on changes of liver blood flow. Indocyanine green has a different hepatic uptake mechanism from bile acids in the isolated perfused rat liver [20] and in man [21]. Likewise no interference of uptake of Evans blue by the liver has been shown when indocyanine green is given concurrently [21].

Liver blood flow has been shown to increase by 30–40% after a meal in man [11, 12] and animals [13, 14]. Protein given orally or intravenously increases liver blood flow more than carbohydrate or fat, and hypertranscytosis of portal blood after amino acid absorption has been suggested as the cause of this effect [13]. Whatever the mechanism, the postprandial increase of liver blood flow should increase the clearance of highly extracted substances such as bile acids and indocyanine green.

An alternative explanation for our results is an increase in extrahepatic clearance. Bile acids [22] and indocyanine green [23, 24] are almost exclusively removed by the liver; less than 5% of an intravenous dose of a bile acid appears in the urine [25]. There is no reason to suggest that extrahepatic clearance should be greatly increased after a meal, but there is no evidence to refute it.

A third possible explanation is an alteration in plasma protein binding after a meal. However, for compounds such as indocyanine green and bile acids with high hepatic extraction ratios, alterations in plasma protein binding have little effect on clearance. A decrease of even 50% in protein binding would only increase clearance of glycocholic acid by about 3%.

Although LaRusso et al. [8] reported that the plasma disappearance curves of $^{14}$Cglycocholic acid were identical before and after a meal, small reductions in $t_{1/2\alpha}$ and $t_{1/2\beta}$ were found. However, clearance rates, which have the advantage over half-life times of being unaltered by changes in volume of distribution [10, 26], were not calculated. We did not find a significant postprandial change in volume of distribution, but there was considerable intersubject variation. This may have influenced estimations of half-life times, but not those of clearance rates. This might explain why we and LaRusso et al. [8] have found only small changes in fractional disappearance rates (or half-life times), although clearances were increased for both anions postprandially.

Another difference from our study was that all six of their subjects were men, while three of our subjects were women. It has been suggested that the fractional disappearance rate of indocyanine green is faster in women compared with men when low doses are used [27], although there was no sex difference in our small number of subjects.

Luey & Heaton [28] have reported that clearance of glycocholic acid did not change postprandially in seven normal patients and 20 with liver disease. Their study differs from ours in that the normal patients were older, the postprandial study was started 120 min after the meal and, perhaps most importantly, their meal contained an amino acid mixture (Casilan), corn oil and a glucose solution. In cats intraduodenal Casilan or a glucose solution did not increase superior mesenteric artery blood flow, whereas milk increased it by up to 60% [14]. Also, after oral ingestion of Casilan, peak blood levels of all the amino acids occur at 30 min, returning to normal by 4 h [29]; since the $^{14}$Cglycocholic acid was not given until 120 min after the meal, the maximum effect of the food on liver blood flow may have been missed. Our postprandial study was started 100 min after the meal, at which time choly conjugates are at their maximum serum level in healthy subjects [30], and therefore presumably the hepatic uptake capacity for this bile acid is under most stress.

Although studying only four patients with liver disease, Thjodleifsson et al. [5] found a decreased plasma retention of $^{14}$Cglycocholic acid 10 min after an intravenous injection given 90 min after a fatty breakfast. This suggests that $^{14}$Cglycocholic acid disappearance is increased postprandially in liver disease, as well as in health. In six normal ponies mean plasma clearance of $^{14}$Ccholic acid was increased by 36% after feeding compared with the fasting state [31], further supporting our finding in normal human subjects.

No difference in plasma volume, estimated by the Evans blue dye method, was detected postprandially in our five subjects, including two of the subjects in whom large increases in volume of distribution of $^{14}$Cglycocholic acid or indocyanine green were observed. These variable increases in volume of distribution may be due to a change in plasma protein binding of the anions after a meal, but remain unexplained.

Plasma clearance of conjugated cholic acid has been shown to be unimpaired in the postprandial state [5, 8, 28]. The present results further suggest that, as theory predicts, plasma clearance of this bile acid is actually increased after a meal, probably as a result of the increase in liver blood flow.
flow. Thus, like other parts, this part of the enterohepatic circulation of bile acids also varies with meals.

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References


