Galactose Clearance Measurements and Liver Blood Flow

SUSANNE KEIDING
Medical Department A, Division of Hepatology, Rigshospitalet, Copenhagen, Denmark

Galactose clearance, measured during low galactose infusion and calculated as infusion rate divided by peripheral galactose concentration (systemic clearance), has been proposed as a measure of liver blood flow. This requires nearly complete hepatic extraction as well as negligible extrahepatic elimination. The purpose of the study was to examine if these assumptions are fulfilled in subjects with no liver disease, and to compare the galactose clearance measurement with an independent measurement of liver blood flow. Liver blood flow was measured in 6 subjects by means of a constant indocyanine green infusion, indocyanine green concentration measurements in a peripheral artery and a hepatic vein, and calculation according to Fick's principle. The mean (± SEM) blood flow rate was 1.2 ± 0.1 L/min. Galactose was given at a constant infusion rate of 142 ± 10 μmol/min, and steady-state concentrations were measured in the peripheral artery (A) and the hepatic vein (V). The hepatic extraction fraction [$(A - V)/A$] was 0.91 ± 0.03. The hepatic galactose elimination rate [$(A - V) \times$ flow] was 101 ± 12 μmol/min; this is about two-thirds of the total elimination rate (viz., infusion rate). Urinary excretion was negligible. This indicates an extrahepatic galactose elimination of ~41 μmol/min. Systemic galactose clearance, calculated as mentioned above, was 1.5 ± 0.1 L blood/min. It was significantly higher than the liver blood flow in each subject (paired t-test, each $p < 0.02$), on average 133% of the flow. Thus the systemic galactose clearance value overestimates liver blood flow, probably due to a small, but in this context quantitatively important, extrahepatic galactose elimination.

Liver blood flow measurements based on intravenous infusion of a dye taken up by the liver, concentration measurements in arterial and hepatic venous blood, and calculation according to Fick's principle have been used extensively since the method was introduced by Bradley et al. in 1945 (1). Several attempts have been made since then to develop a clearance measurement of liver blood flow without the need for a concentration measurement in hepatic venous blood.

Tygstrup and Winkler in 1958 (2) examined the use of galactose clearance as a measure of hepatic blood flow. The idea was recently taken up by Henderson et al. (3), who used a constant low galactose infusion rate to achieve constant low galactose concentrations; galactose clearance was calculated as the infusion rate divided by the mean galactose concentration in the peripheral venous blood during the measurement period. The method has also been used in dogs (4), and has recently been extended to a procedure using a single-injection technique and higher blood galactose concentrations in humans (5).

It has been argued (6) that the hepatic elimination capacity of galactose is not high enough to ensure satisfactory approximation of clearance to hepatic blood flow in subjects with liver impairment (7). On the other hand, the hepatic extraction fraction found by Henderson et al. (3) in 2 subjects with no liver disease was 93% and 95%, respectively. Thus the "hepatic clearance," i.e., hepatic elimination rate divided by the arterial concentration, approximates liver blood flow by this factor.

Hepatic clearance, however, only equals the "systemic clearance" measurement used by Henderson et al. (3) if galactose elimination outside the liver (viz., splanchnic area) is negligible. Henderson et al. (3) measured urinary excretion and erythrocyte metabolism of galactose; both were only ~2% of the total elimination. At steady-state galactose concentrations from 6 to 9 mmol/L, Lindskov et al. (8) did not find statistically significant extrahepatic metabolic conversion of galactose in patients with cirrhosis examined by means of hepatic vein and renal vein catheterization. It is not known, however, whether a possible extrahepatic galactose metabo-

Abbreviation used in this paper: ICG, indocyanine green.

0016-5085/88/$3.50

lism could be of quantitative importance at the low concentrations used for the clearance measurements. Furthermore, none of the above-mentioned clearance studies compared the galactose clearance measurement with an independent and valid quantitative measurement of liver flow.

The present study was undertaken in subjects with no liver disease to compare total elimination rate and hepatic elimination rate of galactose at low steady-state galactose concentrations, and to compare the galactose clearance measurement with the liver blood flow as assessed by a constant indocyanine green infusion and Fick's principle.

Materials and Methods

Subjects

Six subjects with no sign of liver disease were studied. Their alcohol intake ranged from 0 to 20 g/day; they had no regular medicine intake. All subjects had normal values of alanine aminotransferase, alkaline phosphatase, albumin, and prothrombin time. Splanchnic hemodynamics were evaluated because of various abdominal complaints. Body weights are given in Table 1.

Informed written consent was obtained from all subjects and the protocol was approved by the local ethics committee of the hospitals in Copenhagen. No complications caused by the procedures were observed.

Catheterization

The subjects fasted for 12 h and were given 10 mg of diazepam if wanted. The hepatic vein catheterization was performed through a cubital vein with a Cournand catheter 7 (Surgimed, Copenhagen, Denmark). The position of the catheter was verified by fluoroscopy and measurements of blood oxygen content. Hepatic venous blood samples were drawn from this catheter.

An indwelling catheter was placed in a femoral artery for blood sampling.

Experimental Design

Galactose (Kabi, Sweden, with measured purity in the present study of 96%–100%) was given as a constant intravenous infusion of a solution containing 51–100 mmol of galactose per liter (mean 71 mmol/L) at an infusion flow rate of 1.8–2.1 ml/min (mean 2.0 ml/min) by means of a roller pump (Dick, Copenhagen, Denmark) that was calibrated before and after each experiment. No priming dose was used. Infusion rates are given in Table 1.

Indocyanine green (ICG; Hynson, Westcott, and Dunning, Baltimore, Md.) (0.20 μmol/min) was given simultaneously with the galactose infusion; no priming dose was used (9).

Blood samples were taken simultaneously from the peripheral artery and the hepatic vein every seventh minute in a 35-min measurement period preceded by a 90-min equilibration period. For galactose concentration measurements, ~1 ml of blood was sampled in a tube with 50 i.e. dried heparin and kept on ice at 4°C until analysis the following day. For concentration measurements of ICG, ~3.5 ml of blood was kept on ice until analysis immediately after the infusion period. Urine was collected during the whole infusion period (125 min). Urine specimens were handled in the same manner as the blood samples.

Analyses

Galactose concentration was measured by the galactose dehydrogenase method (10) in duplicate precipitations of 300 μl of blood or urine in 900 μl of perchloric acid (0.3 mol/L). The analytic accuracy, as assessed by the mean difference between these double determinations, was 1.1 ± 2.3 μmol/L (mean ± SEM, n = 24) for the arterial concentrations (~113 μmol/L, see Table 1), and 5.2 ± 2.3 μmol/L for the hepatic vein concentrations (~9 μmol/L).

Table 1. Hepatic Galactose Elimination at Low Constant Galactose Infusion in Subjects With No Liver Disease

Case No.	Body wt (kg)	Hepatic blood flow[a] (ml/min)	Galactose infusion rate (μmol/min)	Measurements: Arterial concentration[b] (μmol/L)	Measurements: Hepatic venous concentration[b] (μmol/L)	Calculated values: Hepatic extraction fraction[c]	Calculated values: Hepatic elimination[d] (μmol/min)	Calculated values: Systemic clearance[e] (ml/min)	Calculated values: Hepatic clearance[f] (ml/min)
1	46	995 ± 60	106	78.2 ± 3.9	10.2 ± 3.0	0.87 ± 0.08	67.7 ± 3.3	1355 ± 67	866 ± 78
2	54	844 ± 23	132	101.3 ± 3.6	1.4 ± 2.4	0.99 ± 0.06	84.3 ± 2.0	1303 ± 46	832 ± 43
3	55	986 ± 23	143	127.1 ± 3.0	18.9 ± 3.8	0.85 ± 0.04	106.6 ± 4.8	1125 ± 26	839 ± 43
4	65	1212 ± 35	152	77.5 ± 2.8	6.7 ± 1.7	0.91 ± 0.04	85.8 ± 3.2	1961 ± 70	1107 ± 58
5	75	1216 ± 20	138	89.2 ± 3.8	−1.7 ± 2.8	1.02 ± 0.06	110.6 ± 4.0	1547 ± 66	1240 ± 69
6	87	1665 ± 68	183	106.7 ± 3.3	16.3 ± 5.4	0.85 ± 0.06	150.6 ± 10.9	1715 ± 125	1411 ± 111
Mean ± SEM		1153 ± 118	142 ± 10	113 ± 14	8.6 ± 3.3	0.91 ± 0.03	101 ± 12	1501 ± 124	1049 ± 99

Calculated values are given ± standard error of the estimate. [a] Hepatic blood flow (Q) is calculated as a mean value in each study by Fick's principle: Indocyanine green infusion rate (corrected for possible accumulation due to non-steady state) divided by the arteriovenous indocyanine green concentration difference, and corrected for hematocrit values. [b] Arterial (A) and hepatic venous (V) galactose blood concentrations are given as mean values of six measurements ± SEM; concentrations may be negative due to measurement error at low concentrations. [c] Hepatic galactose extraction fraction is calculated as (A−V)/A. [d] Hepatic galactose elimination rate is calculated as $Q \cdot (A-V)$. [e] Systemic blood galactose clearance is calculated as galactose infustion rate/A. [f] Hepatic blood galactose clearance is calculated as $Q \cdot (A-V)/A$.

The recovery of galactose added to blood samples taken from the subjects before the start of the infusions was on average 83% (range 77%–89%, n = 28) in the present concentration interval; the concentration measurements were corrected by this mean value. Recovery of galactose added to urine (≥0.2 mmol/L) was similar to that in blood.

Galactose concentration in the infusion solution was measured during each experiment and corrected according to the average recovery of 98%. Indocyanine green concentration was determined in undiluted plasma samples by spectrophotometry (11). The hematocrit was determined after centrifugation in microcapillary tubes. It was 0.31–0.45 L/L.

Calculations

The hepatic plasma flow rate Q, or rather the splanchnic plasma flow rate, was calculated from the plasma concentrations of ICG in the artery (C_i) and in the hepatic vein (C_o) by Fick's principle, assuming no extrahepatic elimination (9):

$$Q = \text{Hepatic ICG elimination}/(C_i - C_o). \quad (1)$$

The hepatic ICG elimination was calculated as the infusion rate corrected for possible accumulation of ICG in the course of the measurement period (9), being <3% in the present study. The blood flow rate was calculated by appropriate correction for the hematocrit value (12). The experimental error of Q was calculated from the concentration measurements as described in Reference 12.

Galactose blood concentration was calculated as a mean value in each period in the artery (A) and in the hepatic vein (V).

The hepatic extraction fraction of galactose (E) was calculated as

$$E = (A - V)/A. \quad (2)$$

The hepatic elimination rate of galactose (v, μmol/min) was calculated as

$$v = Q \cdot (A - V). \quad (3)$$

The hepatic galactose clearance (Cl_h, ml/min) was calculated as

$$Cl_h = v/A. \quad (4)$$

The systemic galactose clearance (Cl_s, ml/min) was calculated as

$$Cl_s = \text{Galactose infusion rate}/A. \quad (5)$$

Statistical Analysis

The statistical analysis included analysis of experimental errors at three levels. First, the analytic errors of the galactose concentration measurements were accounted for as discussed above.

Second, analysis of the variation of the concentration measurements during the course of the infusion period, due to variation in the physiologic and experimental conditions, was performed. Steady state is defined as measurements in which the slope of the linear regression line of the arterial concentration-time curve is not statistically significantly different from zero; this was examined by means of a t-test of the slope relative to the calculated error of the slope (13). (The arterial concentration is considered representative of the concentration in the volume of galactose distribution.) Steady state was achieved within the experimental error in each case (each $p > 0.3$).

The experimental errors of the individual mean concentrations A and V were calculated from the residual variation of the respective regression lines (13). The values are given in Table 1. It is seen that this variation is of the same order of magnitude as the analytic error (see above), which accordingly was not included in the calculation of the error of the mean concentrations and the following derived data.

The experimental errors of E, v, Cl_h, and Cl_s were calculated from the experimental errors of Q, A, and V (Table 1) by standard procedures (13). The calculated error of the infusion rate was <0.5%, and is not included in the calculations. An example will illustrate the calculation procedure. The experimental error of E, $s(E)$, is calculated from A, V, and their respective errors $s(A)$ and $s(V)$ as follows:

$$s(E) = \left[\frac{(A-V)^2 \cdot s^2(A)}{A^4} + \frac{s^2(A) + s^2(V)}{A^2}\right]^{1/2} \quad (6)$$

It should be noted that the mean hepatic venous concentration may be recorded to be <0 at very low concentrations, due to the analytic procedure of the enzymatic analysis, which includes subtraction of a (individually measured) blind value. It should also be noted that, although the low hepatic venous concentration is measured with a large relative error, this does not add to the calculated error of E, v, and Cl_h, because this is based on the absolute values of the errors, as illustrated for $s(E)$ in Equation (6). The error of Cl_s depends only on the values of the infusion rate, A, and the error of A [cf. Equation (5)].

Comparison between individual data was performed by a paired t-test.

The third level of the statistical analysis deals with the mean values for the 6 subjects regarded as a group, given at the bottom of Table 1. These values can be used to get an overview of the data and to compare the data with data from other studies.

Results

Figure 1 shows the galactose concentration measurements in each of the six experiments. It is seen that there is no systematic tendency for the concentration to increase or decrease with time. As mentioned in Materials and Methods, steady state was approximated within the experimental error in each case. Urine galactose was not detectable in any of the specimens.

Table 1 gives the individual values of the measured hepatic blood flow rate and of measured and derived galactose data. It is seen that the hepatic extraction fraction is ≥0.85. In agreement with this, the *hepatic* clearance value given in Table 1 is ≥85%

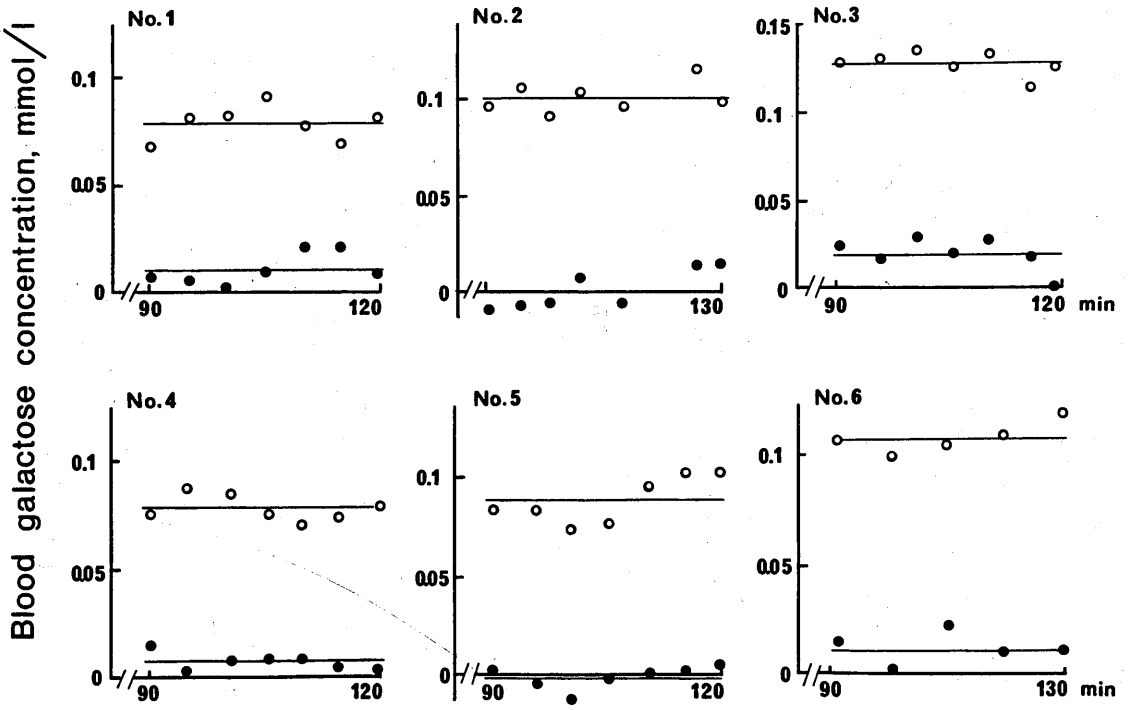

Figure 1. Galactose blood concentrations in a peripheral artery (○) and a hepatic vein (●) in 6 subjects during constant intravenous infusion of galactose (mean 142 μmol/min, see Table 1). Measurements were performed 90–120 (or 90–130) min after the start of the infusion, as indicated. The *lines* give mean concentrations.

of the hepatic blood flow rate [$Cl_h/Q = E$; Equations (2), (3), and (4)].

The calculation of the *systemic* clearance [Equation (5)] is based on the assumption that the infusion rate equals the total elimination rate. As the galactose blood concentration did not deviate significantly from steady state, this was obtained within the experimental accuracy. As seen in Table 1, the systemic clearance was higher than the hepatic blood flow in each subject (on average 133%; $p < 0.02$).

Comparison of the data in Table 1 shows that the hepatic elimination rate is systematically lower than the infusion rate (viz., total elimination rate) ($p < 0.001$), the difference being on average 41 μmol/min, with the 95% confidence limits being 14–69 μmol/min.

Discussion

Steady state was obtained in each subject, with arterial blood concentrations ranging from 78 to 127 μmol/L (see Table 1). The blood concentrations in the study by Henderson et al. (3) were 99 and 128 μmol/L, as recalculated from the measured plasma concentrations in peripheral venous blood in their 2 control subjects (0.021 and 0.027 mg/ml, respectively, mol wt 180). Assuming equal galactose concentrations in erythrocyte water and plasma water, the galactose concentration in blood is 0.85 times that in plasma (14); accordingly, the present concentration range is identical with that used by Henderson et al. (3).

In agreement with the similar concentrations, the hepatic extraction fraction of galactose in the present study, ranging from 0.85 to 1.02, is equal to the values in the 2 control subjects (0.95 and 0.93, respectively) in the study by Henderson et al. (Reference 3, Table 4). The variation of the extraction fraction values (Table 1) corresponds to what is often seen for substances used for clearance measurements (15). It may be noticed that our galactose infusion rate, 106–183 μmol/min (see Table 1), was a little lower than that used in 5 control subjects by Henderson et al. [140–300 μmol/min (26–54 mg/min); Reference 3, Table 3]. We aimed to give a low infusion rate to improve the approximation to first-order kinetics required by clearance measurements, as the experiments by Henderson et al. (3) showed a significant deviation from first-order kinetics (6). The data do not allow for evaluation of the present approximation, but extraction is high, and the experimental error of the extraction fraction of ~6% (Table 1) seems reasonable for this kind of study.

The value of the liver blood flow rate is similar to that found in other studies using ICG infusion and Fick's principle (see references cited in Reference 9).

The approximation of the *hepatic* galactose clear-

ance to the liver blood flow is equal to the hepatic extraction fraction, i.e., 0.85–1.02.

The *systemic* blood galactose clearance ranged from 1.1 to 2.0 L blood/min (mean 1.5 L/min). The values in the study by Henderson et al. (3) ranged from 1.3 to 2.2 L blood/min (mean 1.8 L/min) in 11 normal subjects, as recalculated from the published plasma clearance values (1.1–1.9 L plasma/min). This difference is so small that, in our opinion, it can be ascribed to the well-known variation in experimental data obtained from different laboratories.

The *systemic* galactose clearance was higher than the flow rate in each subject, being on average 133% of the flow rate. A possible reason for this could be that part of the galactose elimination takes place outside the splanchnic organs, amounting to as much as 66 μmol/min at the present concentrations. There was no detectable urinary excretion of galactose (indicating a renal threshold for galactose higher than the present arterial blood galactose concentrations of ~0.1 mmol/L). Metabolism in the erythrocytes (3,16) is of no quantitative importance in the present context. A possible site for extrahepatic galactose elimination could be renal metabolism. This is consistent with the 6–9 mmol/L steady-state studies in humans by Lindskov et al. (8), where there was a nonsignificant difference between renal elimination evaluated by means of renal vein catheterization and urinary excretion of the same order of magnitude as the present (statistically significant) possible extrahepatic galactose metabolism. It is not possible, however, to evaluate from the present data whether the observed extrahepatic elimination is due to extrahepatic metabolic conversion of galactose or to a continued distribution of galactose into a large volume of distribution in spite of the observed good approximation to steady state. The purpose of the present study was not, however, to study this question, which has to be addressed in future studies.

This study demonstrates that the systemic galactose clearance overestimates liver blood flow systematically and significantly, and it suggests that extrahepatic galactose elimination, although small, may contribute to this. The study also emphasizes that whenever a clearance measurement is being proposed as a new measure of liver blood flow, it should be compared with an independent and valid quantitative flow measurement.

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Received July 18, 1986. Accepted September 8, 1987.

Address requests for reprints to: S. Keiding, Medical Department A 2152, Rigshospitalet, 2100 Copenhagen Ø, Denmark.