Digestive Diseases and Sciences, Vol. 32, No. 7 (July 1987), pp. 753–758

Hepatic Clearance of D-Sorbitol

Noninvasive Test for Evaluating Functional Liver Plasma Flow

GIANPAOLO MOLINO, MD, ALBERTO CAVANNA, MD, PAOLO AVAGNINA, MD, MARCO BALLARÉ, MD, and MAURO TORCHIO, MD

The hepatic clearance of D-sorbitol, a natural polyol which is metabolized by the liver, was studied in normal and cirrhotic subjects after bolus intravenous injection (2 g) and during constant infusion (54 mg/min) with the aim of providing a noninvasive and simple measure of functional liver plasma flow. The high hepatic extraction of D-sorbitol and the dose-independence of its clearance pointed to a flow-dependent clearance regimen. The renal excretion was taken into account when computing the hepatic clearance. Day-to-day reproducibility of the test was good. No significant difference was found when the hepatic clearance was measured by bolus injection or constant infusion methods. As measured by the bolus injection method, the mean (± SD) hepatic clearance in the normal subjects (911 ± 137 ml/min) was significantly greater ($P < 0.001$) than that of the cirrhotics (456 ± 181 ml/min).

KEY WORDS: D-sorbitol; hepatic clearance; functional liver plasma flow.

Hepatic function depends on the interaction between liver cell activity and liver blood perfusion. Liver blood flow is thus a determinant of many metabolic processes and of the systemic bioavailability of enterally absorbed substances. According to the clearance principles (1–3), the functional liver plasma flow (FLPF), which is assumed as the fraction of liver plasma supply which perfuses functioning parenchyma, can be measured by any substance which is essentially metabolized by the hepatocytes and completely extracted at any passage through the liver. This is known to occur whenever the hepatic intrinsic clearance largely exceeds liver blood flow.

Among these compounds, D-sorbitol (S) was chosen owing to its biologic and kinetic features. S biotransformation depends on an enzymatic process involving sorbitol dehydrogenase, which was found in large amounts in hepatocytes, while very low concentrations were detectable in other body tissues (4–10). Minor amounts are excreted by glomerular filtration in the normal condition (4, 10). As extrapolated from *in vitro* data (6, 9), the intrinsic clearance of S is quite high in humans, largely exceeding the average value of liver plasma flow.

This study was designed to directly ascertain the indicated requirements of S clearance, to define the pathophysiologic meaning of the test and its limitations, and eventually to assess a standard protocol for routine application.

MATERIALS AND METHODS

Subjects. Seventy-five subjects entered the study and were submitted to the experiments included in the study

Manuscript received September 16, 1985; revised manuscript received October 7, 1986; accepted October 16, 1986.

From the Dipartimento di Biomedicina, Università di Torino, Italy.

This study was partially supported by grants from Consiglio Nazionale Ricerche (84.01760.04) and Ministero Pubblica Istruzione (2.12.2).

Address for reprint requests: Prof. G. Molino, Dipartimento di Biomedicina, Clinica Medica E, Università di Torino, via Genova 3–10126 Torino, Italy.

design, as outlined below. Preliminary experiments were carried out to validate the method in 18 normal subjects, 18 patients affected by liver cirrhosis, and six patients affected by nephrovascular hypertension without any detectable liver alteration.

A standard protocol for the evaluation of FLPF was then applied to 20 normal volunteers (N), and 25 patients affected by biopsy-proven liver cirrhosis (C); some of them had been also submitted to preliminary experiments.

All subjects gave their informed consent.

Chemicals and Chemical Analysis. The pyrogen-free and sterile 10% solution of S was prepared in pharmacy. Each solution was concurrently controlled with plasma samples. Sorbitol concentration in plasma (1 ml of heparinized blood samples) and urine (1 ml of 10% diluted urine) was determined by an enzymatic-spectrophotometric method previously described (6). Sorbitol dehydrogenase (SDH) and nicotinamide adenine dinucleotide (NAD) were commercial preparations from Boehringer-Biochemia, Mannheim (West Germany). Analytical grade reagents were used.

Plasma deproteinization must be performed immediately. The extracts can be stored at +4° C for at least 24 hr or at −20° C for several days.

The assay was reliable between 1 and 10 mg/dl with a mean coefficient of variation of ±6% (10 determinations for 10 levels). Below 1 mg/dl, concentrations were given as the mean of three determinations. Above 10 mg/dl, samples were suitably diluted. Errors in estimation of plasma concentrations were found to be proportional to their true value.

S Clearance After Bolus Injection. Basal blood and 60-min urine samples were taken before the test in fasting and resting subjects. Sorbitol was intravenously administered in 1 min. Blood samples were taken at 2- to 3-min intervals up to 20 min and then every 5 min up to 60 min. Urine was spontaneously collected at 120 min. All measured concentrations were corrected by subtracting the basal level. Total (CL_t) and renal (CL_r) clearances were calculated from the dose (D), the cumulative 2-hr urinary output, and the area under the concentration/time curve extrapolated to infinity (AUC), according to standard equations ($CL_t = D/AUC$, $CL_r = U/AUC$). The hepatic clearance of S (CL_h) was the difference between total and renal clearances.

S Clearance After Continuous Infusion. After taking basal blood and 60-min urine samples, S was infused at a rate of 54 mg/min. To shorten the time taken to achieve the steady-state level, a 2-g intravenous bolus of S was given before the infusion. Blood samples were taken at 10-min intervals and urine was collected between 60 and 120 min. The specific criteria used to define the steady-state level were that the concentrations of three consecutive plasma samples were ±0.1 mg/dl of the mean. Both measured plasma concentrations and urinary outputs were corrected for basal values.

The steady-state total ($CL_{ss,t}$), renal ($CL_{ss,r}$), and hepatic ($CL_{ss,h}$) clearance values were calculated from the infusion rate (I), the mean urinary output (U), and the steady-state concentration (C_{ss}), according to standard equations ($CL_{ss,t} = I/C_{ss}$; $CL_{ss,r} = U/C_{ss}$; $CL_{ss,h} = CL_{ss,t} - CL_{ss,r}$).

Statistical Evaluations. Statistical differences between mean values were assessed by Student's t test for paired data when comparing two procedures in the same subjects, and by Student's t test for unpaired data when comparing pathologic group to controls.

Hepatic Extraction of S. Eleven patients examined by angiography prior to surgery were studied: six were affected by nephrovascular hypertension with no detectable liver alteration, and five were cirrhotic patients with portal hypertension.

Paired blood samples were taken simultaneously from catheters positioned in the femoral artery (C_i) and in the right hepatic vein (C_o) before and between 10 and 20 min after the injection of a 2-g dose of S. The extraction ratio, $E = (C_i - C_o/C_i$, was computed as the mean of the values obtained from five paired samples of each curve.

Controls. To determine dose-dependence of S clearance, 10 subjects (five normal and five cirrhotics) were submitted on successive days to two determinations of S total clearance for the standard (2 g) and a double (4 g) dose, respectively. Moreover, different amounts of S were administered by bolus intravenous injection on successive days in one normal subject (20, 50, 100, and 250 mg/kg body wt) and one cirrhotic patient (12, 20, 33, and 45 mg/kg body wt), and the plasma disappearance rates were measured between 10 and 20 min by linear least-squares regression analysis of the logarithmic concentration–time curves.

To determine sorbitol diffusion into erythrocytes, the possible *in vivo* loss from plasma of injected S by diffusion into erythrocytes was tested in four normal and four cirrhotic subjects. To this extent S concentrations in the hemolysate of red cells were determined before and 20 and 60 min after the intravenous injection of a 2-g dose.

To determine glucose and fuctose interference, the total plasma clearance of S was evaluated for a 2-g dose in two normal subjects and four cirrhotic patients before and after the intravenous injection of 25 g of glucose or fructose (10% water solution).

To test day-to-day reproducibility, in nine subjects (six normals and three cirrhotics) a double test was performed on successive days by injecting a 2-g dose of S and measuring the total plasma clearance.

For comparison between the hepatic clearance values obtained by different methods, bolus intravenous injection and constant infusion tests were performed on successive days in 10 normal subjects and three cirrhotic patients.

Evaluation of FLPF. According to the results of preliminary experiments the FLPF was evaluated in 20 normal subjects and 25 cirrhotic patients by measuring the hepatic clearance of S (CL_h) after bolus intravenous administration of a standard 2-g dose.

RESULTS

Sorbitol Kinetics. The plasma disappearance of S after bolus intravenous injection of a single 2-g dose was found to follow a multiexponential decay

TABLE 1. LIVER EXTRACTION OF S (2 g BY INTRAVENOUS INJECTION) IN 6 NORMAL (N) AND 5 CIRRHOTIC SUBJECTS (C)

Subject	*Condition*	E*
O.C.	N	0.98
C.M.	N	0.96
C.L.	N	0.98
G.P.	N	0.98
S.G.	N	0.98
L.V.	N	0.93
D.Z.	C	0.33
B.V.	C	0.60
A.P.	C	0.90
M.M.	C	0.85
B.M.	C	0.65

*The extraction ratio (E) was computed as the mean value obtained from five paired samples in each curve.

curve. The mean 2-hr urinary elimination was 8.2 ± 1.8% of the administered dose in controls, and 13.3 ± 8.0% in cirrhotic patients. During constant infusion, the steady-state level was always reached at the end of the first hour.

Hepatic Extraction. When given at a 2-g dose, S was almost completely extracted in normal subjects with a mean (± SD) E value of 0.97 (± 0.02) (Table 1). By contrast, in the five cirrhotic patients liver extraction was lower.

Controls. Dose dependence of S clearance is shown in Table 2. In both normal and cirrhotic subjects, no significant difference was found when measuring S clearance with the standard 2-g dose and a double one. Moreover, as shown in Figure 1, the plasma disappearance rate was found to be dose-independent in the range of the amounts used.

Sorbitol diffusion into red cells is shown in Table 3. S concentration in erythrocytes remained in the range of basal values up to 60 min.

TABLE 2. SORBITOL TOTAL CLEARANCE (CL_t) MEASURED AFTER ADMINISTERING 2 AND 4 g OF S ON SUCCESSIVE DAYS IN 5 NORMALS (N) AND 5 CIRRHOTICS (C)

		CL_t *(ml/min)**	
Subject	*Condition*	*2 g*	*4 g*
T.M.	N	943	854
G.G.	N	886	866
A.P.	N	1040	1030
P.M.	N	829	830
C.F.	N	934	897
B.V.	C	499	413
B.D.	C	538	494
G.G.	C	402	407
M.P.	C	321	423
D.V.	C	767	792

*No statistically significant difference (Student's t test for paired data).

Glucose and fructose interference are shown in Table 4. The intravenous administration of 25 g of glucose or fructose before S injection did not significantly change the measured values of S clearance.

Day-to-day reproducibility is shown in Table 5. When a 2-g dose of S was repeated in the same subjects on two successive days, clearance values did not significantly change.

Comparison between bolus intravenous injection and constant infusion method is shown in Table 6. Similar CL_h values were obtained in normal and cirrhotic subjects studied with both methods (no statistical difference at the Student's t test).

Evaluation of FLPF. Functional liver plasma flow (Table 7), as evaluated by S hepatic clearance was 911 ± 137 (mean ± SD) and 456 ± 181 ml/min in normal and cirrhotic subjects, respectively. The difference between the two groups was highly significant ($P < 0.001$). Even if the number of studied subjects was relatively small, the diagnostic efficacy of the S hepatic clearance was evaluated. Taking 638 ml/min as a cutoff value (mean − 2 SD of controls), the test specificity and sensitivity were 0.95 and 0.80, respectively.

DISCUSSION

Very similar values were obtained when the hepatic clearance of S was measured by bolus intravenous injection and constant infusion; when considering the inaccuracy due to experimental error, the methods used can be considered as equivalent. In the final protocol, a standard 2-g dose of S was administered, irrespective of body weight, owing to the dose-independence of the measured S clearance.

Since renal output was low in normal subjects, but not negligible in some patients, renal clearance needs to be measured and the hepatic clearance computed as the difference between total and renal clearances. Some inaccuracy might depend on the fact that urine was collected spontaneously, but the corresponding error was so small that urethral catheterization did not appear to be justified.

Since no appreciable diffusion into erythrocytes was detectable during the test, S hepatic clearance was considered as having the dimension of a plasma clearance. When computed from *in vivo* data according to Bass and Winkler (11), the mean (± SD)

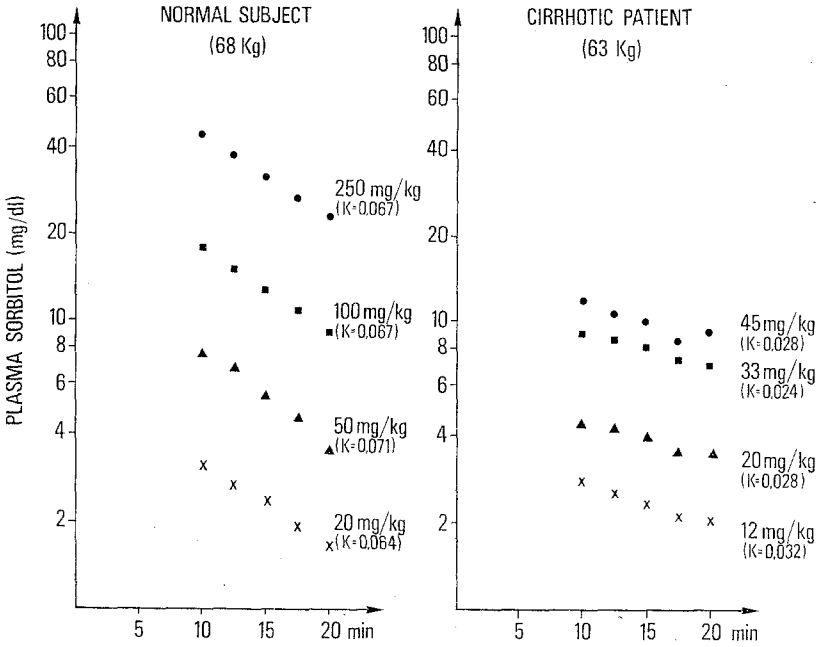

Fig 1. Plasma disappearance rates of S, as measured between 10 and 20 min after bolus intravenous injection of different doses in one normal subject and one cirrhotic patient.

intrinsic clearance of S in the normal subjects was 3925 ± 464 ml/min, largely exceeding the hepatic plasma flow. These data ensure that the S hepatic clearance follows a flow-dependent clearance regimen, so reliably reflecting liver perfusion.

Almost complete liver extraction was observed in normal subjects, directly indicating that in this condition the hepatic clearance of S may be considered a reliable measure of FLPF. By contrast, lower extraction ratios were found in cirrhotics, making questionable the validity of the test in liver patients. Indeed, the impairment of the extraction ratio in liver cirrhosis might depend on some discrepancy between the amount supplied by liver circulation and the residual ability of damaged liver cells to clear it in one passage. In this case, saturation of the metabolic process might occur or, alternatively, a fall of V_{max} out of proportion to the change of K_m might lower the actual intrinsic clearance to such an extent that the condition of flow-limitation might no longer be valid. However, S clearance was found to be dose-independent in cirrhotic patients too, proving that in the range of doses considered S removal still follows first-order

TABLE 3. SORBITOL CONCENTRATION (mg/dl) IN ERYTHROCYTES IN BASAL SAMPLE (B), AT 20 AND 60 MIN AFTER S INJECTION (2 g) IN 4 NORMAL (N) AND 4 CIRRHOTIC SUBJECTS (C)

			*Time (min)**	
Subject	*Condition*	*B*	*20*	*60*
B.M.	N	0.21	0.27	0.27
B.P.	N	0.56	0.59	0.86
F.M.	N	0.19	0.21	0.12
S.E.	N	0.15	0.15	0.19
M.G.	C	0.53	0.44	0.41
A.A.	C	0.59	0.56	0.57
S.V.	C	1.07	0.91	0.92
F.M.	C	0.83	0.89	0.83

*No statistically significant difference (Student's *t* test for paired data).

TABLE 4. SORBITOL TOTAL CLEARANCE MEASURED WITH 2-g DOSE OF S (CL_t) BEFORE AND AFTER INTRAVENOUS INFUSION OF 25 g OF GLUCOSE OR FRUCTOSE IN 2 NORMAL (N) AND 4 CIRRHOTIC SUBJECTS (C)

		CL_t *(ml/min)**		
Subject	*Condition*	*Basal*	*After glucose*	*After fructose*
C.M.	N	999	969	984
P.P.	N	1010	931	997
M.G.	C	535	522	624
A.A.	C	528	619	624
S.V.	C	374	383	365
F.M.	C	677	613	629

*No statistically significant difference (Student's *t* test for paired data).

TABLE 5. DAY-TO-DAY REPRODUCIBILITY OF CL_t MEASURED WITH 2 g DOSE OF S IN 6 NORMAL (N) AND 3 CIRRHOTIC SUBJECTS (C)

		CL_t (ml/min)*	
Subject	*Condition*	*1st test*	*2nd test*
B.M.	N	1047	910
T.M.	N	943	861
P.M.	N	829	799
B.L.	N	1175	1230
F.S.	N	1618	1649
M.L.	N	894	814
B.V.	C	499	425
O.V.	C	327	322
S.C.	C	324	369

*No statistically significant difference (Student's *t* test for paired data).

kinetics. This finding makes the saturation hypothesis untenable, and is also in contrast with the hypothesis of a substantial impairment of the intrinsic clearance, in which case one would expect a parallel reduction of liver extraction and clearance when substrate concentration increases.

Alternatively, according to the "intact hepatocyte theory" (12), the reduced extraction of S might be explained in terms of a reduced number of normally functioning and normally perfused sinusoidal units depending on the development of anatomical or functional intrahepatic shunts. In this case, one could also easily explain the constancy of S hepatic clearance values found in liver cirrhosis

TABLE 6. VALUES OF CL_h OBTAINED WITH DIFFERENT METHODS

		CL_h (ml/min)*	
Subject	*Condition*	*Bolus intravenous injection (2 g)*	*Constant infusion (54 mg/min)*
B.M.	N	976	1068
E.D.	N	1103	964
C.V.	N	826	743
S.E.	N	1001	1050
F.M.	N	864	847
B.P.	N	742	821
G.E.	N	838	756
F.G.	N	995	833
M.A.	N	802	906
R.L.	N	931	769
P.C.	C	497	510
Z.C.	C	662	708
L.L.	C	400	483

*No statistically significant difference (Student's *t* test for paired data).

TABLE 7. MEAN ± SD VALUES (AND RANGES) OF FLPF MEASURED BY CL_h, IN 20 NORMAL SUBJECTS (N) AND 25 CIRRHOTIC PATIENTS (C)

		Mean ± SD (range)	
Condition	*No. of subjects*	*FLPF (ml/min)*	*FLPF (ml/min/kg)*
N	20	911 ± 137 (623–1223)	12.88 ± 2.46 (8.70–17.29)
C	25	456 ± 184 (205–796)	7.38 ± 3.00 (3.42–14.43)

for increasing substrate concentrations, since the fractional amount bypassing the liver cells is constant and independent of the supplied load.

Unpublished theoretical studies further support the use of S clearance in measuring the FLPF: for highly extracted drugs ($E = 0.97$), a 50% decrease of the intrinsic metabolic activity of liver cells gives a relative error in estimated FLPF not exceeding 10%. In normal subjects FLPF values, as given by the hepatic clearance of S, are lower than those for effective liver plasma flow as measured by the low-dose galactose clearance method (13). This discrepancy might be explained in terms of a somewhat larger extrahepatic metabolism of galactose and of the higher extraction ratio of S.

With respect to the liver blood flow estimated by BSP (14, 15) or indocyanine green clearance (16–19), the corresponding values computed from S hepatic clearance are higher but remain in the quite large range of performed measurements. Technical considerations and differences in physiological molecular properties presumably account for the observed discrepancies. Attention should be focused on the fact that minor but different amounts undergo the extrahepatic removal of different substances. Moreover, for anionic dyes, protein binding might influence liver uptake, the hepatic clearance is not flow-dependent, and the extraction ratio probably decreases during the test (17).

Experiments performed in the present study indicate that S hepatic clearance has all the requirements to provide a noninvasive method for evaluating FLPF and its modifications. Moreover, by this test, changes in the systemic bioavailability of highly extracted drugs in pathological conditions might also be easily predicted.

ACKNOWLEDGMENTS

The authors thank Prof. J. Bircher and Prof. K. Winkler for suggestions and comments, and Prof. C. Fava and Miss Adriana Prati for technical assistance.

REFERENCES

1. Waldstein SS, Arcilla RA: Measurement of hepatic blood flow by clearance methods. Am J Dig Dis 3:137–158, 1958
2. Winkler K, Bass L, Keiding S, Tygstrup N: The physiologic basis for clearance measurements in hepatology. Scand J Gastroenterol 14:439–448, 1979
3. Winkler K, Keiding S, Tygstrup N: Clearance as a quantitative measure of liver function. *In* The Liver. Quantitative Aspects of Structure and Function. G Paumgartner, R Preisig (eds). Basel, Karger, 1973, pp 144–155
4. Adcock LH, Gray CH: The metabolism of sorbitol in human subject. Nature 65:554–560, 1957
5. Williams-Ashman HG: D-Sorbitol. *In* Methods of Enzymatic Analysis. HU Bergmeyer (ed). Weinheim, Verlag Chemie, 1975, pp 167–170
6. Bergmeyer HU, Gruber W, Gutmann I: D-Sorbitol. *In* Methods of Enzymatic Analysis. HU Bergmeyer (ed). Weinheim, Verlag Chemie, 1975, pp 1323–1330
7. Blackley RL: The metabolism and antiketogenic effects of sorbitol. Biochem J 49:257–271, 1951
8. Bye PA: The utilization and metabolism of intravenous sorbitol. Br J Surg 56:653–656, 1969
9. Gerlach U, Hiby W: Sorbitol Dehydrogenase. *In* Methods of Enzymatic Analysis. HU Bergmeyer (ed). Weinheim, Verlag Chemie, 1975, pp 569–573
10. Seeberg WP, McQuarrie EB, Secor CC: Metabolism of intravenous infused sorbitol. Proc Soc Exp Biol Med 89:303–305, 1955
11. Bass L, Winkler K: A method of determining intrinsic hepatic clearance from the first pass effect. Clin Exp Pharmacol 7:339–343, 1980
12. Wood AJJ, Villeneuve JP, Branch RA, Rogers LW, Shand T: Intact hepatocyte theory of impaired drug metabolism in experimental cirrhosis in the rat. Gastroenterology 76:1358–1362, 1979
13. Henderson JM, Kutner MH, Bain RP: First-order clearance of plasma galactose: The effect of liver disease. Gastroenterology 83:1090–1096, 1982
14. Bradley SE, Ingelfinger FJ, Bradley GP, Curry JJ: The estimation of hepatic blood flow in man. J Clin Invest 24:890–899, 1945
15. Drapanas T, Kluge N, Schenk WG: Measurement of hepatic blood flow by bromsulphalein and by electromagnetic flowmeter. Surgery 48:1017–1022, 1960
16. Caesar J, Shaldon S, Chiandussi L, Guevara L, Sherlock S: The use of indocyanine green in the measurement of hepatic blood flow and as a test of hepatic function. Clin Sci 21:43–57, 1961
17. Leevy CM, Mendenhall CL, Lesko W, Howard MM: Estimation of hepatic blood flow with indocyanine green. J Clin Invest 41:1169–1179, 1962
18. Daneshmend TK, Jackson L, Roberts CJC: Physiological and pharmacological variability in estimated hepatic blood flow in man. Br J Clin Pharmacol 11:491–496, 1981
19. Bui-Xuan B, Muchada R, Latarjet J, Flandrois C, Motin J: Reproducibilitè du debit sanguin hepatique estimè par la méthode au vert d'indocyanine en perfusion continue chez l'homme. Gastroenterol Clin Biol 5:87–90, 1981