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Effects of hyperglycemia on quantitative liver functions by the galactose load test in diabetic rats

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Abstract

Blood galactose clearance after an intravenous galactose load has been widely used as a quantitative liver function test. We have developed a novel quantitative rat liver function test, the galactose single point (GSP) method, to assess residual liver function with various injuries by measuring single time point galactose concentration in blood after an intravenous bolus injection of galactose. The goal of this study was to evaluate the influence of nonhepatic factors such as hyperglycemia on GSP and galactose elimination capacity (GEC) in rats. Four groups of animal studies were carried out, as follows: (1) normal control (NC), (2) streptozotocin-induced diabetes (DM), (3) carbon tetrachloride–induced hepatotoxicity (CCl_4), and (4) streptozotocin-induced diabetes with CCl_4-induced hepatotoxicity (DM + CCl_4). The serum glucose levels in the diabetic groups (DM and DM + CCl_4) were significantly increased compared with the NC and CCl_4 groups ($P < .001$). A significant increase in hepatic activities of aspartate aminotransferase and alanine aminotransferase was observed in the CCl_4-treated groups (CCl_4 and DM + CCl_4) compared with the NC and DM groups ($P < .001$). In comparison with the NC group, the values of GSP and GEC in the diabetic groups (DM and DM + CCl_4) were significantly reduced ($P < .001$) and increased ($P < .01$), respectively. Galactose single point had highly significant correlations with GEC ($P < .001$). These results suggest that galactose metabolism tests—as quantitative parameters of liver function—should be interpreted with caution in the condition of a significant hyperglycemia.

1. Introduction

Galactose is a naturally forming sugar with a high extraction ratio that is approximately 90% metabolized by the liver in humans and in rodents [1]. Galactose is phosphorylated by galactose kinase, the rate-limiting step in galactose metabolism in the liver; but the epimerization of galactose-1-P to glucose-1-P is catalyzed by UDPglucose-hexose-1-phosphate uridyltransferase [2-4]. At high concentrations in rats and in humans, galactose follows the Michaelis-Menten kinetics [5,6]. The galactose elimination capacity (GEC) test was proposed several years ago as a quantitative test to measure human liver function [7]. However, the multiple blood samples required to establish the decrease of galactose concentration made the test difficult in clinical practice and therefore led to the more practical investigation of the use of galactose single point (GSP) methods in the assessment of human liver function. In patients with chronic hepatitis, cirrhosis, and hepatocellular carcinoma, our laboratory has previously shown that GSP is well correlated with the severity of liver disease [8]. This method has been successfully applied to drugs that are extensively metabolized or excreted from the liver such as promazine and cefoperazone clearance in patients with various liver diseases [9-11]. This GSP method also has been recommended by the US Food and Drug Administration [12] in the guidance for industry pharmacokinetics

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in patients with impaired hepatic function. We have extended the GSP method to rats for quantitative determination of residual liver function based primarily on the marked GSP elevation after carbon tetrachloride (CCl_4)– or isoniazid (INH)-induced extensive and severe hepatic necrosis and also high sensitivity for the detection of even the early stages of liver disease [13]. However, subjects with a recent history of hyperglycemia and/or diabetes mellitus were not enrolled in our previous study. A previous animal study has shown that in the presence of hyperglycemia, the ^{13}C-glucose produced from ^{13}C-galactose metabolism is diluted into the enlarged glucose pool, leading to a major decrease in $^{13}CO_2$ production and thus affecting the ^{13}C-galactose breath test results [14,15]. Thus, diseases that interfere with carbohydrate metabolism, such as diabetes, may produce different effects on GSP and GEC. In an attempt to elucidate this point, we investigated the effects of intravenous galactose load (0.5 g/kg body weight [BW]) on the 4 subgroups of rats aforementioned.

2. Materials and methods

2.1. Materials

All chemicals were of the highest purity available. Streptozotocin (STZ), CCl_4, and corn oil were purchased from Sigma Chemical (St Louis, MO). Galactose injection solution was prepared by Nang-Kuang Pharmaceutical (Tainan, Taiwan, Republic of China) using 400 g galactose (Sigma) dissolved in 1.0 L distilled water with appropriate buffer system and isotonic reagents for injection.

2.2. Animals

Male Sprague-Dawley rats weighing 300 to 325 g were obtained from the National Applied Research Laboratories and National Laboratory Animal Center (Taipei, Taiwan, Republic of China). All of the experiments were performed in adherence to the National Institutes of Health guidelines for the treatment of animals. All of the rats were housed in a room with air/humidity control and with a 12-hour light/dark cycle, and allowed access to food and water ad libitum throughout the experiment. All of the rats were intraperitoneally (IP) anesthetized with sodium pentobarbital at a dose of 50 mg/kg BW. Polyethylene catheters were placed in the right internal jugular vein for the administration of galactose. Catheter insertion was performed by the cut-down technique, and the distal end of the catheter was tunneled under the skin and externalized through an incision in the back of the neck. After the surgery was completed, rats were fasted overnight (approximately 16 hours) during recovery but were allowed water.

2.3. Induction of experimental diabetes

Diabetes was induced by a single IP injection of STZ (60 mg/kg BW) dissolved in 0.1 mol/L citrate buffer (pH 4.5) after the animals were fasted for 16 hours. An equal volume of citrate buffer was administrated to duration-matched control rats. Three to 4 days after injection of STZ, fasting blood glucose was determined; and the rats with blood glucose more than 300 mg/dL were included in the group of diabetic rats.

2.4. Acute intravenous galactose loading test in control and STZ-induced diabetic rats

To assess the effect of galactose on in vivo glucose metabolism, galactose (0.5 g/kg BW) was rapidly (over a 30-second period) injected intravenously in the control rats (n = 8) and STZ-induced diabetic rats (n = 8). Blood samples were collected from the tail vein into sterilized glass tubes before and 5, 10, 15, 30, 45, 60, 90, and 120 minutes after galactose injection. Thirty minutes after collection, the blood was centrifuged (10 minutes, 5000 rpm) and serum was isolated. Enzymatic measurement of serum glucose using a Synchron LXi 725 system (Beckman Instruments, Palo Alto, CA) was performed.

2.5. Effects of hyperglycemia on galactose load test

To study the influence of hyperglycemia on GSP and GEC tests, the rats were randomly divided into 4 study groups: normal control group (NC, n = 8)—the control rats were given a single IP injection of corn oil vehicle at a volume of 2 mL/kg; STZ-induced diabetic control group (DM, n = 8)—the diabetic rats were given a single IP injection of corn oil vehicle at a volume of 2 mL/kg; control rats CCl_4-treated group (CCl_4, n = 6)—the control rats were given a single IP injection of 2 mL/kg BW of CCl_4-corn oil (1:1 mixture); and diabetic rats CCl_4-treated group (DM + CCl_4, n = 6)—the diabetic rats were given a single IP injection of 2 mL/kg BW of CCl_4-corn oil (1:1 mixture). After 24 hours, blood samples of the experimental animals were collected from the tail vein into sterilized glass tubes before galactose injection. Serum was separated by centrifugation at 5000 rpm for 10 minutes. The serum glucose and the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by the Synchron LXi 725 system (Beckman Instruments).

2.6. Measurement of quantitative liver function

Galactose single point and GEC tests were done on each subject. All study groups underwent 30 seconds of rapid intravenous administration of 0.5 g/kg BW galactose solution (0.4 g/mL). A colorimetric galactose dehydrogenase method was used to measure galactose levels using a modification of the neonatal screening test (Interscientific GAL 570 nm, Hollywood, FL). It is sensitive, quick, and inexpensive and has high clinical resolution. Dried blood specimens were taken from the tail vein at 5, 10, 15, 30, 45, and 60 minutes after injection. The concentration range of the calibration curve was 50 to 1000 μg/mL. Within-day variations were evaluated using SD and percentage coefficient of variation for each concentration. A maximal of 10%

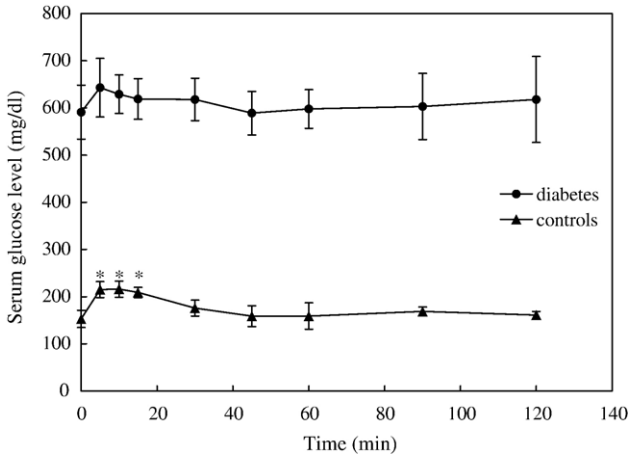

Fig. 1. Effect of quick intravenous injection of galactose (0.5 g/kg BW) on the serum glucose level in control rats and STZ-induced diabetic rats. All data are expressed as mean ± SD. *P < .001 compared with the fasting values.

coefficient of variation was permitted. Day-to-day variation was also checked by comparing the slope and intercept of the calibration curves. The GEC values were calculated according to the following formula, which is modified from the equation of Tygstrup [16]:

$$\mathrm{GEC} = \frac{D}{T_{C=0} + 7}[\mathrm{mg}/(\mathrm{kg} \cdot \mathrm{min})]$$

where D is the injected amount of galactose; $T_{c\ =\ 0}$ is the amount of time required for the concentration to reach zero, which is extrapolated from a linear regression of the blood concentration-time curve from 20 to 60 minutes after injection; and 7 is the empiric correction for uneven distribution in the body. The GSP is the galactose blood concentration at 60 minutes after 30-second infusion stopped.

2.7. Statistical analysis

All data were expressed as mean ± SD. The results were analyzed for statistical significance by 1-way analysis of variance test using the Statistical Package for the Social Sciences program (Version 13, SPSS, Chicago, IL). The least significant difference post hoc test of multiple comparisons was used subsequently to identify significant differences among groups. Group means were considered to be significantly different at P < .05.

3. Results

3.1. Blood glucose value during galactose load test

After the intravenous injection of the galactose (0.5 g/kg BW), the mean serum glucose concentration in STZ-induced diabetic rats showed a slight increase from 591 ± 57 mg/dL (0 minute) to 643 ± 62 mg/dL (5 minutes) and returned to the fasting value of 589 ± 46 mg/dL at 45 minutes. There were no significant variations of serum glucose concentrations in STZ-induced diabetic rats from zero after 120 minutes (P > .05). On the other hand, in the control rats, galactose injection produced an increase in the mean serum glucose concentration, which rose to a maximum of 216 ± 17 mg/dL at 10 minutes, when compared with the fasting value of 153 ± 18 mg/dL (P < .001), and had also returned to near fasting values at 45 minutes (159 ± 22 mg/dL, P = .48) (Fig. 1).

3.2. Effect of STZ-induced hyperglycemia

As shown in Table 1, the serum glucose levels of the NC group were at very constant levels of about 141 ± 27 mg/dL. Although a slight decrease of about 124 ± 20 mg/dL was noted in the CCl_4 group compared with the NC group, this was not statistically significant (P = .46). The administration effects of STZ on the serum glucose levels in the DM and DM + CCl_4 groups are shown in Table 1. Serum levels of glucose were 567 ± 59 and 540 ± 47 mg/dL in the DM and DM + CCl_4 groups, respectively. A highly significant difference was observed when comparing glucose values from the NC group with respect to the DM and DM + CCl_4 groups (P < .001 and P < .001, respectively). In the 2 diabetic groups (DM and DM + CCl_4), the concentrations of serum glucose were not significantly different. The DM group showed no significant increase in the serum AST and ALT activities (AST from 103 ± 16 to 143 ± 10 IU/L; ALT from 48 ± 6 to 59 ± 13 IU/L).

Table 1
Values and statistical results of glucose, AST, ALT, GSP, and GEC (mean ± SD) in NC, CCl_4, DM, and DM + CCl_4 groups after 0.5 g/kg galactose was intravenously administered

Subject	NC (n = 8)	CCl_4 (n = 6)	DM (n = 8)	DM + CCl_4 (n = 6)	Statistical analysis: ANOVA and LSD					
					NC-CCl_4	NC-DM	NC-DM + CCl_4	CCl_4-DM	CCl_4-DM + CCl_4	DM-DM + CCl_4
Glucose (mg/dL)	141 ± 27	124 ± 20	567 ± 59	540 ± 47	NS	<.001	<.001	<.001	<.001	NS
AST (IU/L)	103 ± 16	1570 ± 336	143 ± 10	1695 ± 451	<.001	NS	<.001	<.001	NS	<.001
ALT (IU/L)	48 ± 6	829 ± 76	59 ± 13	877 ± 66	<.001	NS	<.001	<.001	NS	<.001
GSP (μg/mL)	365 ± 98	609 ± 15	208 ± 47	230 ± 22	<.001	<.001	<.001	<.001	<.001	NS
GEC (mg/[min kg])	5.0 ± 1.0	3.1 ± 0.2	6.8 ± 0.7	6.1 ± 0.4	<.001	<.01	<.01	<.001	<.001	NS

For detailed group divisions, see Materials and methods. ANOVA indicates analysis of variance; LSD, least significant difference; NS, not significant.

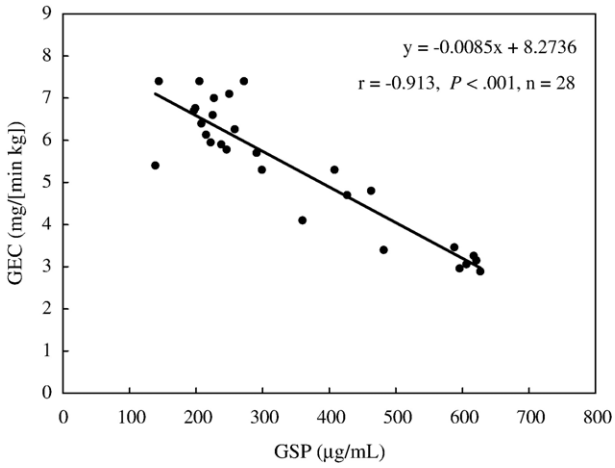

Fig. 2. The correlation between GEC and GSP among the NC, CCl_4, DM, and DM + CCl_4 groups. For detailed group division, see Materials and methods.

3.3. Effect of CCl4-induced hepatotoxicity

The AST and ALT are hepatic enzymes that are released into the bloodstream when liver cells are damaged. The administration effects of CCl_4 on the serum AST and ALT activities in the CCl_4 and DM + CCl_4 groups are shown in Table 1. In comparison with the NC group, the serum AST and ALT activities in the CCl_4 and DM + CCl_4 groups were significantly increased (in international units per liter; AST = 103 ± 16 in the NC group, 1570 ± 336 in the CCl_4 group, and 1695 ± 451 in the DM + CCl_4 group, $P < .001$, respectively; ALT = 48 ± 6 in the NC group, 829 ± 76 in the CCl_4 group, and 877 ± 66 in the DM + CCl_4 group, $P < .001$, respectively). Similar significant results were obtained when comparing serum AST and ALT levels from the DM group with respect to the CCl_4 and the DM + CCl_4 groups. In the 2 CCl_4-treated groups (CCl_4 and DM + CCl_4), the serum AST and ALT activities were not significantly different.

3.4. Effect of hyperglycemia on the GSP method

The GSP value was significantly higher in the CCl_4 group compared with that in the NC group (609 ± 15 vs 365 ± 98 μg/mL, $P < .001$). However, the baseline GSP was significantly lower in the DM group than that in the NC group (GSP, 208 ± 47 μg/mL in the DM group and 365 ± 98 μg/mL in the NC group, $P < .001$) and was similar to that in the DM + CCl_4 group (230 ± 22 μg/mL). In addition, the DM + CCl_4 group presented lower GSP values with respect to the CCl_4 group (230 ± 22 and 609 ± 15 μg/mL, $P < .001$, respectively), indicating a significant increase in galactose metabolism in hyperglycemic rats.

3.5. Effect of hyperglycemia on GEC

The GEC value was significantly lower in the CCl_4 group compared with that in the NC group (3.1 ± 0.2 vs 5 ± 1 mg/[min kg], $P < .001$). Furthermore, the baseline GEC was significantly higher in the DM group than that in the NC group (GEC = 6.8 ± 0.7 mg/[min kg] in the DM group and 5 ± 1 mg/[min kg] in the NC group, $P < .01$) and was similar to that in the DM + CCl_4 group (6.1 ± 0.4 mg/[min kg]). Significantly, the GEC values were increased in the DM + CCl_4 group as compared with those in the CCl_4 group (6.1 ± 0.4 and 3.1 ± 0.2 mg/[min kg], $P < .001$, respectively). The GSP had highly significant correlations with GEC ($P < .001$), with the correlation coefficient being −0.913 (Fig. 2).

4. Discussion

Several tests, using invasive and noninvasive means, have been used to assess rat hepatic function or to monitor the progression of hepatic injury. The most general tests include the measurement of serum liver enzymes named *AST*, *ALT*, and *alkaline phosphatase*, and hepatocyte products such as bilirubin, albumin, and prothrombin time [17-19]. Measuring the metabolic capacity of certain compounds that act as rate-limiting steps in hepatic metabolism makes it possible to obtain a representative value of residual liver function. Because galactose, which is the first step in hepatic galactose metabolism, is rate limiting, measurement of its disappearance can be used as an index of residual liver function [4,20].

Unlike the GEC test, which requires at least 4 blood samples, the GSP method requires only one blood sample to estimate its value. Furthermore, the slope of the galactose concentration-time curve at 20 to 60 minutes after administration, used by the GEC test, does not always represent the saturated portion for normal healthy subjects and may vary with patients [8]. We have reported that the novel GSP method is not only a simple and clinically useful quantitative liver function test for humans [8], but is also a useful tool to evaluate rat liver function with various injuries [13]. However, it was of importance to compare the effect of extrahepatic diseases, such as hyperglycemia and/or diabetes, known for their ability to interfere with galactose metabolism, on in vivo GSP and GEC tests. With regard to the effect of diabetes on galactose metabolism, our data in diabetic rats revealed a decrease in the GSP value. On the other hand, the GEC values seemed to be increased in diabetic rats compared with those in controls. These results indicated that a hyperglycemia produced by STZ can influence galactose metabolism. In healthy humans, an intravenous infusion of glucose raising the glycemia to about 10 mmol/L during 90 minutes significantly increased blood galactose clearance [21]. Shreeve [22] reported preliminary results in 7 diabetic patients with a mean glycemia of 10.2 mmol/L and showed that the results of the ^{14}C-galactose breath test were decreased by 20% compared with those in controls. The phenomenon of the galactose metabolism being influenced by hyperglycemia status was consistent with our findings. One study that investigated the influence of diabetes on in vivo galactose metabolism is by Salaspuro and Kesaniemi [23], who reported that the results of intravenous galactose elimination tests in diabetic subjects were similar to those in controls. The discrepancy between

these 2 studies may be caused by the well- or poorly controlled blood glucose of the study subjects.

Confronting our results with those published in the literature [21,22,24-27], we believe that hyperglycemia and/or diabetes may increase galactose disposal. One possible explanation for the lowering effect that glucose has on serum galactose levels is that glucose can accelerate the metabolism of galactose and thereby increase the removal of galactose from the blood. Another possible explanation is that hyperglycemia may increase galactose incorporation into glycogen or glycoproteins [27], thereby increasing blood galactose clearance. Finally, one cannot rule out the possibility that in diabetic rats, hyperglycemia was associated with an increased amount of galactose excreted in urine after intravenous injection of galactose. For the above-mentioned reasons, further studies in diabetic rats are needed to clarify the urinary excretion of galactose during the tolerance test. Regardless, studies of galactose metabolism to assess quantitative liver function must be interpreted with caution, especially when a significant hyperglycemia is present. Apart from the glucose factor, alcohol has also been shown to be a factor that caused a misinterpretation of the results of the galactose clearance test [14,23]. The effect of alcohol in the galactose load test should be further investigated.

In conclusion, the use of GSP or GEC should be restricted to those with good control of blood glucose. The GSP method has an excellent correlation with the GEC test. The hyperglycemia and/or STZ-induced diabetes affects GSP and GEC results to the same extent, an observation that has been previously too often underestimated. Further clinical studies are needed to clarify the utility of the galactose load test to determine residual liver function with diabetes.

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