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Validation of curve-fitting method for blood retention of ^{99m}Tc-GSA: Comparison with blood sampling method

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We investigated a curve-fitting method for the rate of blood retention of ^{99m}Tc-galactosyl serum albumin (GSA) as a substitute for the blood sampling method. Seven healthy volunteers and 27 patients with liver disease underwent ^{99m}Tc-GSA scanning. After normalization of the *y*-intercept as 100 percent, a biexponential regression curve for the precordial time-activity curve provided the percent injected dose (%ID) of ^{99m}Tc-GSA in the blood without blood sampling. The discrepancy between %ID obtained by the curve-fitting method and that by the multiple blood samples was minimal in normal volunteers 3.1 ± 2.1% (mean ± standard deviation, n = 77 sampling). Slightly greater discrepancy was observed in patients with liver disease (7.5 ± 6.1%, n = 135 sampling). The %ID at 15 min after injection obtained from the fitted curve was significantly greater in patients with liver cirrhosis than in the controls (53.2 ± 11.6%, n = 13; vs. 31.9 ± 2.8%, n = 7, $p < 0.0001$). There was a highly linear correlation between the %IDs of ^{99m}Tc-GSA and the plasma retention rate for indocyanine green ($r = -0.869$, $p < 0.0001$, n = 27). These results indicate that the curve-fitting method provides an accurate %ID of ^{99m}Tc-GSA and could be a substitute for the blood sampling method.

Key words: ^{99m}Tc-galactosyl serum albumin, asialoglycoprotein, blood retention rate, curve fit

INTRODUCTION

HEPATIC BINDING PROTEIN (HBP) resides at the cell surface of hepatocytes recognizing and specifically binding desialylated galactose-terminated asialoglycoproteins.[1–3] There is clear evidences of a reduction in the HBP concentration as a consequence of hepatocellular injury.[4–6] Quantitative evaluation of the disappearance of labeled asialoglycoproteins has provided a means for measuring the specific receptor-binding process and a novel method for assessing liver function.[7–11] ^{99m}Tc-labeled galactosyl serum albumin (GSA) has been developed by Nihon Medi-Physics (Nishinomiya, Japan) and we investigated its significance as a new functional imaging agent for the liver in experimental studies.[8,12,13] After minor modification of this ligand by using diethylenetriaminepentaacetic acid (DTPA) to obtain stable labeling, clinical phase studies in Japan demonstrated its usefulness for assessing hepatic dysfunction in various liver diseases.[14–16] Following those studies, 3 mg of GSA labeled with 175 MBq of ^{99m}Tc (Asialoscinti®) was commercially available in Japan for clinical use since 1992. The blood concentration of ^{99m}Tc-labeled asialo-glycoproteins is important in kinetic analysis based on the compartment model.[7,11] Blood sampling is theoretically a standard technique for this purpose, but it requires a number of procedures for the radioassay of samples and dose standards by using a well-type scintillation counter in each study. These assay procedures could be performed, but is not practical in clinical situations. As a substitute for the sampling method, we have applied a non-sampling, curve-fitting method in a limited number of subjects.[11] This method provides the percent injected dose (%ID) of ^{99m}Tc-GSA in the blood only from the time-activity curves of the heart and lung, without *in vitro* radioactivity assay. In our preliminary study, the validity was investigated by comparison with blood samples for a single dose of administration (1 mg) and only once (60 min after injection) in each scanning.

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The goals of this study were (a) to further evaluate the accuracy of the curve-fitting method by comparing it with multiple blood sampling and (b) to describe the normal and pathological data of the %ID.

MATERIALS AND METHODS

Patient population

In the first stage of this study, 7 healthy subjects underwent examination as subjects in a phase-I clinical study[14] between October and December, 1988. This group consisted of 7 men aged 22–31 years who had no history of hepatobiliary disease and showed no abnormalities in routine laboratory tests. In the second stage of this study, 27 patients with liver disease underwent examination as a routine study by hepatic scintigraphy with ^{99m}Tc-GSA between September, 1992 and August, 1993. The group comprised 4 women and 23 men, aged 19–74 years (mean age, 59.1 years). The subjects in this group had a variety of liver diseases (metastatic liver cancer, n = 7; chronic hepatitis, n = 7; liver cirrhosis without primary liver cancer, n = 4; and liver cirrhosis with primary liver cancer, n = 9). Patients with moderate or massive ascites were excluded from this study because of the possibility of mismatch between the estimated blood volume, which was calculated from body weight and height, and the actual blood volume. All 7 patients with metastatic liver cancer had a single small nodule in the liver and normal biochemical tests, and therefore served as a control group in the second stage of this study. The diagnosis of liver cirrhosis or chronic hepatitis was histologically confirmed in all patients. All subjects received a full explanation of the diagnostic procedure and gave their informed consent.

Radiopharmaceutical preparation and dosage

The ligand was synthesized by the covalent coupling of galactose residues to the primary amino groups of human serum albumin. The molecular weight of GSA was approximately 76,000 and it contained 30–40 galactose residues. DTPA, a strong bifunctional chelating agent, was added to enhance biostability of the labeling. In the first stage of this study, the binding procedure was performed at hospital. Twenty milligrams of GSA-DTPA, 114 μg of $SnCl_2$, and 88 μg of ascorbic acid in dry solid form were labeled with ^{99m}Tc pertechnetate in 2 mL of solution. Radiochemical purity, as measured by thin-layer chromatography, was more than 98%. Injected radionuclide activity was adjusted to 185 MBq in all cases by adjusting the ratio of ^{99m}Tc to GSA. Healthy volunteers were given various doses of ^{99m}Tc-GSA (1 mg, n = 3; 5 mg, n = 2; 10 mg, n = 2).[14] All patients in the second stage were given a single dose of 3 mg of ^{99m}Tc-GSA that was labeled by the manufacturer (Nihon Medi-Physics). No subjects received repeated administration of ^{99m}Tc-GSA in both stages. The internal radiation absorbed dose per 185 MBq of ^{99m}Tc-GSA was 0.085 cGy for the entire body, 1 cGy for the liver, and 0.1 cGy for the red marrow.

Data acquisition

The subjects were placed in the supine position beneath a large-field-of-view gamma camera (GCA-90B, Toshiba Medical Systems, Japan) connected to a data processor (GMS-55A, Toshiba Medical Systems, Japan). Computer acquisition of gamma camera data was performed at a rate of 20 seconds per frame for 60 min after injection in the first stage and 10 seconds per frame for 40 min in the second stage. Time-activity curves were recorded over the precordium and right peripheral lung field.

Blood sampling method

Multiple blood sampling was simultaneously performed with a gamma camera study. The radioactivity in the syringe before and after injection was measured with a dose calibrator for the exact amount of injection. Venous blood samples were taken 3, 4, 5, 7, 10, 15, 20, 30, 40, 50 and 60 min after injection in healthy volunteers (11 points in a study), and 3, 5, 10, 20 and 40 min after injection in patients (5 points in a study). Radioactivity in the blood sample (1 mL) and in a diluted standard of the labeled product (1 : 5,000) were measured with a well-counter. The %ID in total circulating blood was calculated by the following formula: 100 × radioactivity in 1 mL of the blood sample × total blood volume (TBV) / total radioactivity administered. TBV was estimated according to the following formulae, which are based on the subject's body weight and height:[17]

$$\text{TBV (L)} = 0.1682 \times \text{Height (m)}^3 + 0.05048 \times \text{Weight (kg)} + 0.4444 \text{ for men}$$

$$\text{TBV (L)} = 0.2502 \times \text{Height (m)}^3 + 0.06253 \times \text{Weight (kg)} - 0.662 \text{ for women.}$$

These radioactivity assays and estimations of TBV were essential to the blood-sampling method, but not necessary for the curve-fitting method to be performed.

Curve analysis

Regions of interest were drawn manually over the entire heart and right peripheral lung away from the liver or large vessels in a size of 2 × 4 matrix. After decay correction of ^{99m}Tc, the time-activity curve of the lung was subtracted from that of the heart to correct for the background radioactivity. Between 2 and 60 (40) min after injection, this corrected heart curve was precisely expressed as a biexponential function. This regression function is written as follows: $y(t) = A1 \times e^{-k1t} + A2 \times e^{-k2t}$, where $y(t)$ is the background-subtracted precordial radioactivity, $A1$ and $A2$ are the y-intercepts, $k1$ and $k2$ are the regression coefficients for the corresponding exponential functions, respectively, and t is the time after injection. We use our original program, NLFIT on GMS-55A. The unit for the heart curve was converted from counts per minute to %ID

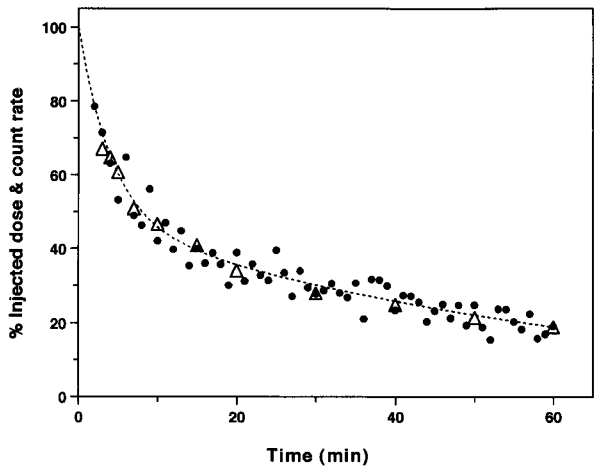

Fig. 1 An example of 5 mg of ^{99m}Tc-GSA data in a normal volunteer. Small dots and dotted curve represent precordial counts after subtraction of lung counts and corresponding fitted curve, respectively. Dotted triangles represent data of the percent injected dose of ^{99m}Tc-GSA obtained by blood sampling method. The heart and corresponding fitted curve are rescaled to demonstrate the result of blood sampling.

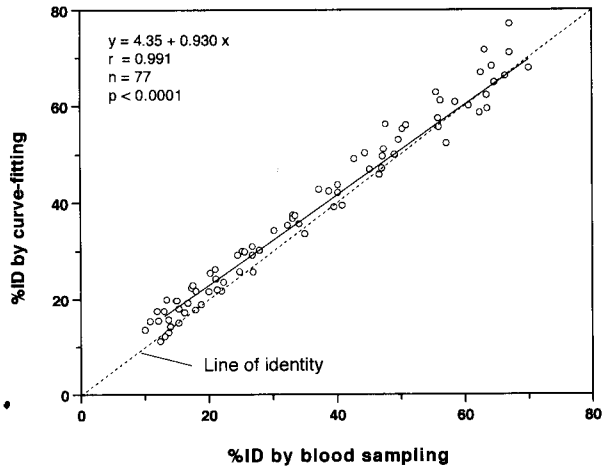

Fig. 2 Comparison of the percent injected dose of ^{99m}Tc-GSA obtained by the curve-fitting method and that obtained by the blood sampling method. Data are obtained in 7 normal volunteers for three doses of ^{99m}Tc-GSA (1 mg in 3 subjects, 5 mg in 2 subjects, and 10 mg in 2 subjects).

of ^{99m}Tc-GSA, based on the assumption that the height of the *y*-intercept is equivalent to the total injected dose, 100%. The %ID was calculated by this method and compared with the actual %ID determined by blood sampling at the corresponding time-point (Fig. 1). The %IDs at 15 min (GSAR15) in each study were also extracted from the regression curve to establish criteria for normal and pathological results.

Assessment of clinical significance

Indocyanine green (ICG, 0.5 mg/kg body weight) test was

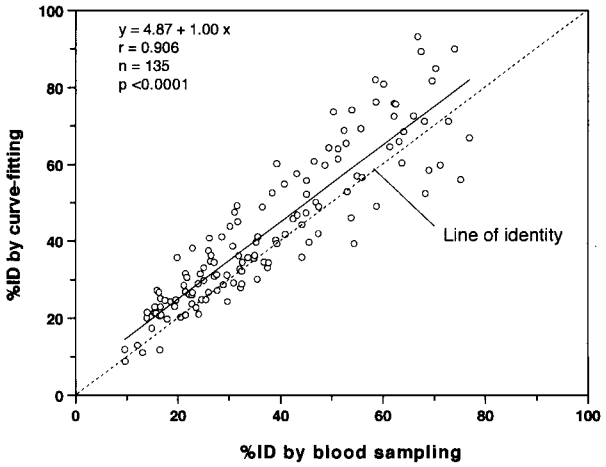

Fig. 3 Comparison of the percent injected dose of ^{99m}Tc-GSA obtained by the curve-fitting method and that obtained by the blood sampling method for 3 mg administration in 27 patients with liver disease.

employed to compare with ^{99m}Tc-GSA results. Blood samples were taken 5, 10, and 15 min after injection, and the plasma retention rate (ICGR15) and plasma disappearance rate (kICG) were obtained.

Statistical analysis

All data were expressed as the mean plus or minus the standard deviation. Correlations between the %ID of ^{99m}Tc-GSA obtained by the curve-fitting method and those obtained by blood sampling or results of the ICG test were analyzed by linear regression and correlation coefficients. Comparisons of %IDs between groups were performed with a combination of one way factorial ANOVA and Scheffe's multiple comparison test, and unpaired t-test.

RESULTS

Agreement of %ID

In normal volunteers, the %ID obtained by the curve-fitting method showed a good linear correlation with that obtained by the blood sampling method ($r = 0.991$, $p < 0.0001$) (Fig. 2). The absolute difference between the two methods in the %ID was $3.1 \pm 2.1\%$ ($n = 77$ samplings). The close relation between the linear regression line and the line of identity in Figure 2 strongly suggests that the curve-fitting method is identical with the blood sampling method. This similarity was consistent with the wide range of doses of ^{99m}Tc-GSA employed (1, 5 or 10 mg). The %ID obtained by both methods also showed a good linear correlation in 3 mg administration study for the patients ($r = 0.906$, $p < 0.0001$) (Fig. 3) but the absolute difference in the %ID in patients ($7.5 \pm 6.1\%$, $n = 135$ samplings) was statistically greater (t-test, $p < 0.0001$) than that in healthy volunteers.

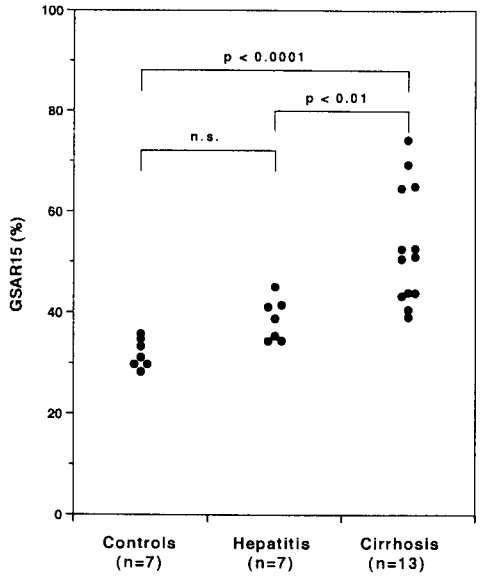

Fig. 4 Group comparison of the percent injected dose of 3 mg of ^{99m}Tc-GSA in 27 patients with liver disease. Date were calculated from fitted curve 15 min after administration.

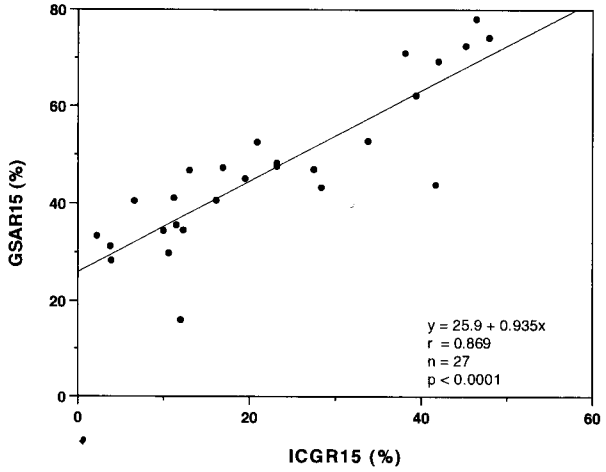

Fig. 5 Correlation between percent injected dose of ^{99m}Tc-GSA and indocyanine green 15 min after administration in 27 patients with liver disease.

Intergroup differences in %IDs

The GSAR15 was higher in cirrhosis (53.2 ± 11.6%, n = 13) than in functionally normal controls (31.9 ± 2.8%, n = 7; $p < 0.0001$) and in hepatitis (38.7 ± 4.1%, n = 7; $p < 0.01$), respectively (Fig. 4). Although there was no statistically significant difference between controls and hepatitis, a higher value was observed in hepatitis than in controls with minimal overlap with each other.

Comparison of GSAR15 with ICG

The GSAR15 showed an excellent correlation with ICGR15 ($r = 0.869$, n = 27, $p < 0.0001$) (Fig. 5) and a good correlation with kICG ($r = -0.676$, n = 19, $p < 0.002$) (Fig. 6) in patients.

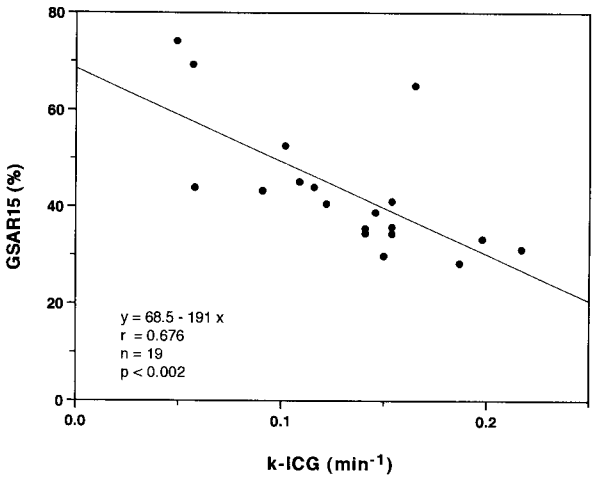

Fig. 6 Correlation between percent injected dose of ^{99m}Tc-GSA and plasma disappearance rate of indocyanine green 15 min after administration in 19 patients with liver disease.

DISCUSSION

It is well recognized that the uptake of GSA is determined by the concentration of HBP, which exists only in hepatocytes. Since there is no target organ except for the liver, the amount of ^{99m}Tc-GSA in the blood is a fundamental indicator for quantitative evaluation of hepatic scintigraphy. There are now two methods of compartment analysis for HBP scintigraphy.[7, 11] Both methods apply a heart curve as an amount of the ligand in the blood, but the calibration procedures for the heart curve are different. Vera's analysis require blood sampling, which is replaced by the curve-fitting method in Kawa's analysis. Because the estimation of %ID from sampling materials is a direct procedure, its data are used as a golden standard in this study. The goal of this study is to validate the curve-fitting method under the assumption that the *y*-intercept (time of injection) for the heart regression curve is equivalent to the total ID (100%). The reliability of the %ID determined by the curve-fitting method has been confirmed by its excellent agreement with that obtained by blood sampling (Figs. 2, 3). This result was consistent for all doses (1–10 mg).

Although there are close relationships between %IDs, we should refer to several differences between these two methods. First, the curve-fitting method does not require venopuncture for blood sampling, preparation of dose standards, or radioactivity assay, which are indispensable in the blood sampling method. The practicality of this method makes it suitable for routine clinical application.

Second, the curve-fitting method is less influenced by the statistical fluctuation in the observed data because a large number of observed points (n = 174 in volunteers, n = 228 in patients) are used to generate the regression curve. The conversion of radioactivity by means of one samplimg cannot avoid this potential error. Similar statis-

tical fluctuation would also exist in the blood sampling data. Taking this into account, we performed several samplings in this study to reduce error by fluctuation. Vera's analysis is vulnerable to this problem since it applies only one sampling in each scanning.

Finally, the estimation of TBV may be another source of error. We observed a statistically greater difference in %ID in patients (7.5 ± 6.1%) than in normal subjects (3.1 ± 2.1%). While the final proof of this difference is still lacking, it is partly explained by the possible deviation of the TBV in the pathophysiological state in patients with liver disease. With the blood sampling method, the TBV is estimated from the patient's body weight and height according to a proposed equation in the literature.[17] This equation is based on a normal subject but it is well known that cirrhotic patients have an increased plasma volume.[18] Conversely, the use of diuretics, which is prescribed in a part of cirrhotic patients as in our subjects, may often invite a dehydrated state. Deviation of the TBV related to these factors inevitably results in a discrepancy between the estimated and real TBV. We excluded a patient with moderate or massive ascites from this study to avoid discrepancy in the TBV but hepatic scan for such a patient is also ordered in a clinical setting and quantitative analysis should be performed. The curve-fitting method applied in this study does not require an estimated TBV, and it is free from this discrepancy. We therefore consider that the curve-fitting method has a number of advantages over the blood sampling method, especially in patients with liver disease.

Theoretically, hepatic uptake may be a direct parameter for quantifying the amount of ligand binding to the HBP but its estimation is not precise without an attenuation correction for inside the body. This still remains a challenge in single photon radionuclide scanning. Furthermore, liver counts also include counts from intrahepatic blood. Since the %ID of ^{99m}Tc-GSA in blood is inversely related to the amount of HBP, the sensitivity of hepatic uptake as an index of liver function would be reduced if a correction were not performed for blood activity in the liver.

The lack of equipment of the algorithm for fitting on computer is a limitation of the curve-fitting method. Although our program, NLFIT, is freely available, it does not work universally in any data processing environment. It is desirable that a biexponential fitting tool be in general use in the Department of Nuclear Medicine.

In conclusion, the curve-fitting method is practical in a clinical setting and provides a reliable estimation of the blood retention rate for ^{99m}Tc-GSA. This would be a substitute method for blood sampling.

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