Compartmental analysis of asialoglycoprotein receptor scintigraphy for quantitative measurement of liver function: a multicentre study

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Abstract. A multicentre study on multicompartmental analysis of hepatic scintigraphy using technetium-99m labelled galactosyl serum albumin (GSA), which binds to the asialoglycoprotein receptor, was carried out at seven institutions in Japan. Seventy-four patients with liver disease received 3 mg (185 MBq) of ^{99m}Tc-GSA by intravenous injection. Sequential scanning was performed 30 min after injection to obtain anterior images of the heart and liver, followed by single-photon emission tomography (SPET). The indices included in this analysis were hepatic blood flow (Q) and maximal receptor binding rate (R_{max}), which showed a good correlation with semiquantitative ratio indices for ^{99m}Tc-GSA, namely the retention rate in blood (HH15) and the hepatic uptake rate (LHL15). Q and R_{max} also showed a significant correlation with other measures of hepatic function. When patients were grouped according to the severity of chronic liver damage (hepatocellular functional damage), Q was reduced in the moderate and severe groups, while R_{max} was reduced in proportion to the functional stage. Both parameters showed no inter-institution difference using analysis of co-variance with the functional stage as a co-variant. With regard to the hepatic uptake rate, anterior planar images and SPET images gave similar results for Q and R_{max}. Acquisition times of 15 or 30 min provided the same results. The multicompartmental model analysis permitted comparable results to be obtained at institutions using different gamma cameras, and is therefore considered a universally applicable method. These results indicate that Q and R_{max} are useful general indices for evaluating the functional reserve capacity of the liver.

Key words: Liver scan – Technetium–99m – Asialoglycoprotein – Multicentre study

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Introduction

The asialoglycoprotein receptor (AGR) is present only in hepatocytes, and its activity is known to fall as a result of liver dysfunction [1–3]. There have been a number of reports describing basic and clinical research on technetium-99m labelled radiopharmaceuticals that bind specifically to AGR [4–10]. Hepatic scintigraphy using such radiopharmaceuticals not only permits images to be acquired for visual evaluation, but also allows quantitative analysis of clearance from the blood and uptake by the liver, thus providing information concerning the binding capacity of hepatic receptors. Several ligands for AGR and corresponding methods for compartmental analysis have been described [6, 9, 11]; however, these techniques have found restricted use: there has been little work using a common analytical method in different institutions.

^{99m}Tc-galactosyl serum albumin (GSA) is a newly developed radiopharmaceutical for hepatic scintigraphy. This radiopharmaceutical has been proven to be suitable for use in patients with liver diseases [12–14] and has been commercially available in Japan since September

1992. If an analytical method for this ligand could be effectively and consistently employed at any institution, it would permit larger numbers of clinical cases to be evaluated, thus clarifying the clinical value of GSA scintigraphy, as well as its differences from conventional liver function tests. For this purpose, we performed this prospective, multicentre study of assess the feasibility of the proposed analytical method for GSA scintigraphy; another major goal was to examine whether the results obtained with the same analytical method using different gamma cameras at several clinical institutions would prove consistent.

Materials and methods

Compartment theory

Kawa's method of multicompartmental analysis has been previously described [9]. A general outline of this method is given below.

Basic concept. The method employs a five-compartment model (extrahepatic blood, intrahepatic blood, receptor, interstitial and urine) to assess ^{99m}Tc-GSA dynamics in vivo. ^{99m}Tc-GSA in sufficient amount shows non-linear binding with AGR. Since the amount of AGR is limited, hepatic removal of GSA is a saturable process. This relationship is described by the following equation of Michaelis-Menten [15]: $R_{obs}=R_{max}\times D/(K_m+D)$, where R_{obs} is the observed binding rate, R_{max} is the maximal binding rate, K_m is the Michaelis constant, and D is an injected dose. Since this equation is applied in the model as a rate of transfer of GSA from the intrahepatic blood to the AGR, this method provides R_{max}, which is the rate of hepatic GSA uptake when all receptors are complexed with GSA.

Analytical methods. Regression analysis is applied to the time-activity curve for the heart to obtain a biexponential function. The zero time of that regression curve (y-intercept) is normalized to a value of 100%, and the ^{99m}Tc-GSA curve in blood is obtained as a unit of mg GSA, since mg equivalent of injected dose (mg/MBq) is prescribed in the case of this ligand. Regression analysis is also applied to the time-activity curve for the entire liver to obtain a biexponential function. Based on standard measurement, calibration is performed for the liver to convert the unit of counts to the mg amount of GSA. Based on the assumption that the zero time of the GSA curve for the liver reflects only the amount of GSA in hepatic blood, in this study the total hepatic uptake was divided into two compartments: an intrahepatic blood compartment and a receptor-bound compartment. The renal excretion rate was negligible, since the observed urinary content was less than 1% of the injected dose 1 h after injection. The interstitial compartment was calculated based on the assumption that it corresponds to the difference between the injected dose and the other three compartments. The input/output equation for ^{99m}Tc-GSA in each compartment was described by four differential equations with six variables in total. The approximate solutions for these equations were obtained using the Runge-Kutta-Gill method and the damping Gauss-Newton method of least-squares fitting [16]. Iteration was performed while parameters were changed, and the optimum parameters were determined from the result in which the sum of the squares of the deviation between the approximate solutions obtained and the input data for GSA was minimum. Hepatic blood flow (Q) and maximal receptor binding rate (R_{max}) were determined.

Subjects

Between July 1993 and September 1993 ^{99m}Tc-GSA studies were performed at seven institutions on seven normal subjects and 67 patients with liver disease. Forty-three were men and 31 were women, and their ages ranged from 34 to 76 years (mean, 59 years). The institutions in question were Kansai Medical University (Okaka) (n=31), Kobe City General Hospital (Hyogo) (n=7), Osaka Koseinenkin Hospital (Oksaka) (n=8), Showa University Fujigaoka Hospital (Kanagawa) (n=8), Yamanashi Medical University (n=6), Fukui Medical School (n=6) and Ehime University School of Medicine (n=8). Clinical diagnoses were chronic hepatitis (n=23) and liver cirrhosis (n=44). Selection criteria for patients with liver disease were confined to chronic viral hepatitis type B or type C. Alcoholic injury, acute hepatitis, fulminant hepatitis, autoimmune hepatic disease, primary biliary cirrhosis and biliary obstructive disease were all excluded. Patients with a liver tumour were included if the tumour was solitary and less than 3 cm in diameter, and no treatment had been performed prior to ^{99m}Tc-GSA scintigraphy. There was no severe abnormality in patients except for liver disease. A patient without any abnormal findings on routine blood chemistry or any physical disorders was employed as a normal control. Laboratory tests were obtained within a week from the day of scintigraphy. Numerical severity scores were calculated as established by the Liver Cancer Study Group of Japan [17] based on five designated clinical and laboratory parameters: ascites, serum bilirubin, albumin (Alb) level, prothrombin time (PT) and plasma retention rate of indocyanine green. The total score can vary from 5 (no disorder) to 15 (severe liver disease). The patients were classified as having mild (stage I), moderate (stage II) or severe (stage III) liver damage on the basis of their severity scores. Normal controls were assigned to stage 0.

Radiopharmaceuticals

We employed Asialoscinti injectable, ^{99m}Tc-GSA (Nihon Medi-Physics Co., Nishinomiya, Japan). GSA was obtained by combining 1 molecule of human serum albumin with 30–44 molecules of galactose, and diethylene triamine penta-acetic acid (DTPA) was used as a chelating agent for labelling with ^{99m}Tc. Radiochemical purity was confirmed by thin-layer chromatography and was always found to be 98% or higher.

Hepatic scintigraphy protocol

The standard form of each test was as follows. The patient was administered a bolus of ^{99m}Tc-GSA (3 mg, 185 MBq) via a peripheral vein, immediately followed by a 20-ml saline flush. The syringe was measured with a curie-meter before and after injection to obtain the precise injected dose in each study. Sequential anterior images of the chest and abdomen were acquired in a 64×64 matrix, at 10 s/matrix for 30 min. Regions of interest (ROIs) were selected for the liver and heart fields. Data were used to generate liver and heart time-activity curves to assess the blood retention rate and hepatic uptake of ^{99m}Tc-GSA. HH15 and LHL15 [13, 14], representing retention of the tracer in blood and

uptake in the liver, respectively, were calculated from these curves as follows:

HH15=count for the heart at 15 min/count for the heart at 3 min

LHL15=count for the liver at 15 min/sum of the counts for the heart and liver at 15 min

Immediately after the dynamic data acquisition, SPET scanning was performed to obtain % injected dose for the liver. The liver counts on SPET images were evaluated by a threshold method using a cut-off value (45% of maximal counts). This value was determined from results of the following phantom experiments.

A known dose of ^{99m}Tc-GSA (measured using a curie-meter) was poured into the water in a liver phantom. The liver counts in the phantom were measured by the planar and SPET methods employing a camera setting consistent with that used in patient studies. These measurements provided counts/dose factors at each institution, which permitted calibrations of the liver counts to the dose of ^{99m}Tc-GSA in two ways.

Absorption or scatter correction for the liver counts was not performed in this study.

Data analysis

Compartment analysis for all kinetic data was performed using a program "Fitas ver1.20" on an MS-DOS system. This program is available free from the corresponding author (S.K. Ha-Kawa). The operator was not informed of the clinical information for patients. Q and R_{max} were compared with conventional hepatic functional indices [Alb, PT, hepaplastin test (HPT), plasma disappearance rate of indocyanine green (ICG-PDR), and retention rate of ICG 15 min after injection (ICG-R15)] and the severity score estimated from the five categories [17].

Statistical analysis

Correlations between indices related to ^{99m}Tc-GSA and each liver function test were examined using standard Pearson's correlation analysis. The statistical significance of the observed differences between groups was evaluated by Student's t test (two-tailed, unpaired). The inter-institution difference in Q and R_{max} was analysed by means of analysis of co-variance (ANCOVA) with the functional stage as a co-variant using the SAS GLM procedure. A value of $P<0.05$ was considered statistically significant.

Results

Figure 1 presents representative observed data and analytical results for a control and for patients with moderate and severe liver dysfunction. In each instance, the theoretical curve obtained from compartmental analysis corresponds closely to the observed curve. As the disease progresses, the blood retention rate of ^{99m}Tc-GSA increases and the hepatic uptake rate, Q and R_{max} decrease.

A significant reverse correlation ($r=0.852$, $P<0.001$) was observed between LHL15 and HH15 (Fig. 2). Both HH15 (Fig. 3) and LHL15 (Fig. 4) showed a reasonable correlation with Q and a significant correlation with R_{max}. R_{max} was linearly related to HH15, but showed a non-linear relationship to LHL15. Similar results were observed with regard to the functional severity score (Fig. 5). The distribution of R_{max} tended to be wide in normal or mild cases, in which the scores were lower. R_{max} showed a stronger correlation ($r=-0.871$) with the functional severity score than did HH15 ($r=0.783$) and LHL15 ($r=-0.829$).

The results for HH15, LHL15, Q and R_{max} in different functional stages are shown in Table 1. HH15-increased and LHL15 and R_{max} decreased with advancing stage, showing significant differences between groups from stage I to stage III. Q remained the same in con-

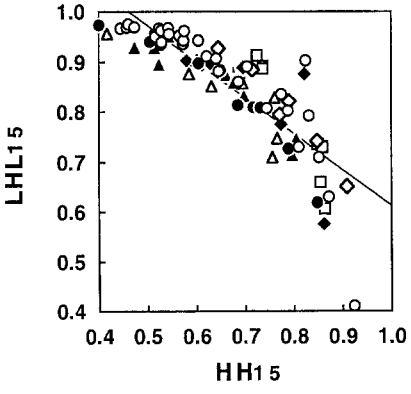

Fig. 2. Correlation between LHL15 and HH15. The different symbols indicate the data obtained at each institution $y=-0.718x+1.331$; $r=0.852$

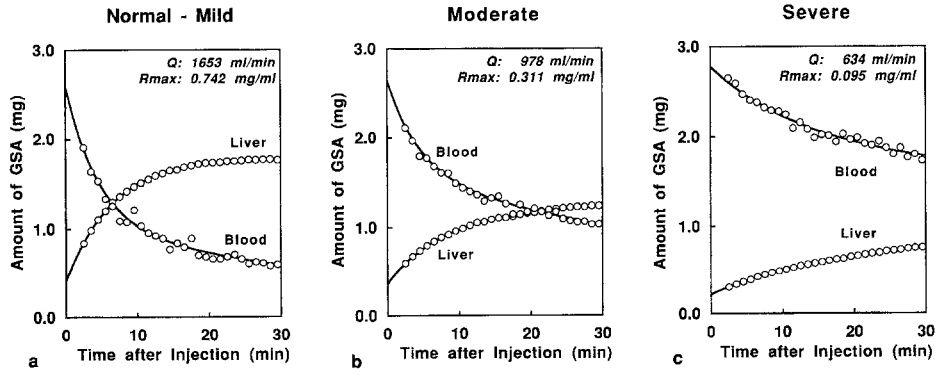

Fig. 1a–c. Representative time course of ^{99m}Tc-GSA for the heart and liver. Observed values (*dotted data*) and the calculated result (*solid line*) are depicted for a control and patients with moderate and severe liver dysfunction

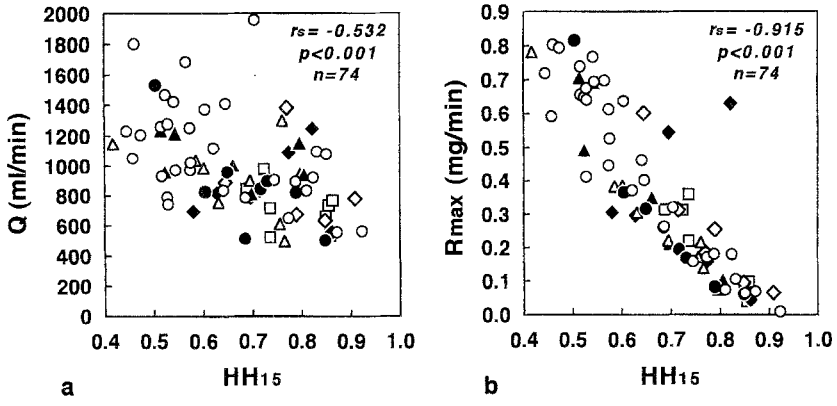

Fig. 3. Correlations between HH15 and Q (**a**) and R_{max} (**b**). The different symbols indicate the data obtained at each institution

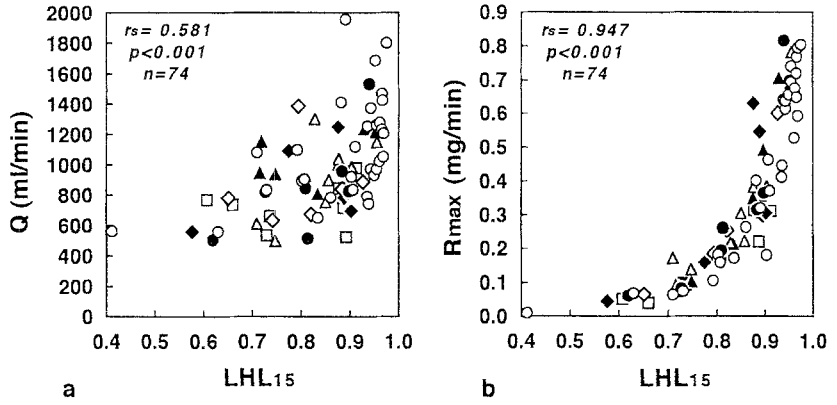

Fig. 4. Correlations between LHL15 and Q (**a**) and R_{max} (**b**). The different symbols indicate the data obtained at each institution

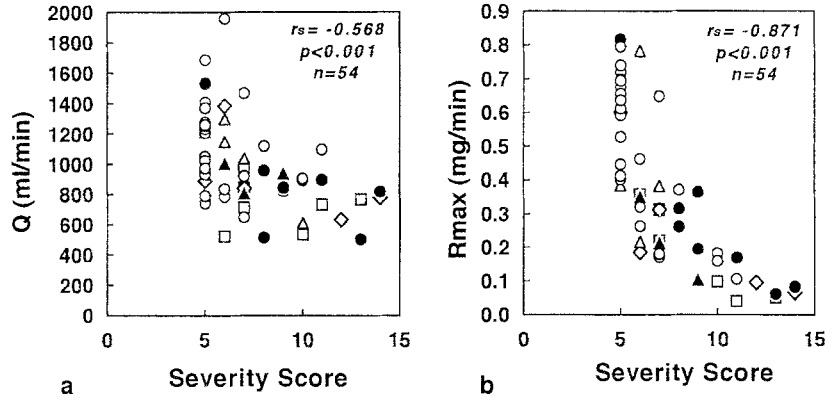

Fig. 5. Correlations between functional severity scores and Q (**a**) and R_{max} (**b**). The different symbols indicate the data obtained at each institution

Table 1. Results obtained with ^{99m}Tc-GSA in different functional stages

Functional stage	No. of patients	HH15		LHL15		Q (ml/min)		R_{max} (mg/min)	
0	7	0.529±0.044	P=0.072	0.950±0.015	P=0.253	1158±229	P=0.997	0.650±0.120	P=0.064
I	26	0.597±0.107	P<0.001	0.917±0.046	P=0.001	1159±338	P=0.001	0.532±0.195	P<0.001
II	20	0.702±0.092	P<0.001	0.849±0.07	P<0.001	847±231	P=0.272	0.265±0.130	P<0.001
III	17	0.831±0.054		0.688±0.101		770±118		0.089±0.050	

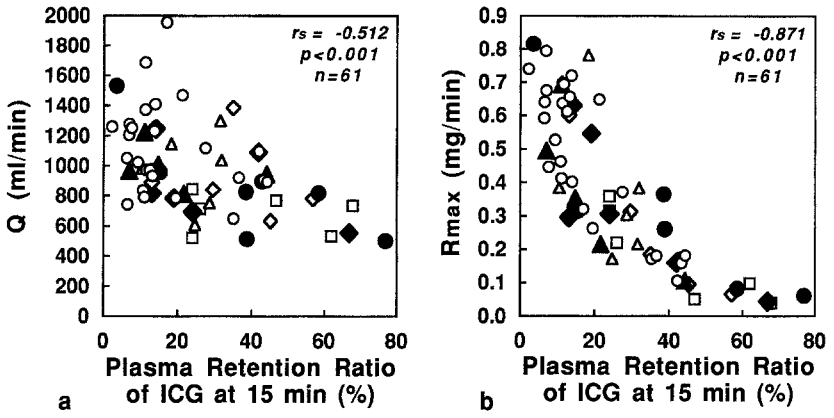

Fig. 6. Correlations between ICG-R15 and Q (**a**) and R_{max} (**b**)

Table 2. Correlations of Q and R_{max} with conventional liver function tests

		Albumin (g/dl)	Prothrombin time (%)	Hepaplastin test (%)	ICG-R15[a]	ICG-PDR[b]
Q (ml/min)	r_s[c]	0.497	0.424	0.515	0.512	0.495
	P[d]	<0.001	<0.001	<0.001	<0.001	<0.001
	n[e]	74	63	49	61	40
R_{max} (mg/min)	r_s	0.749	0.811	0.857	0.871	0.891
	P	<0.001	<0.001	<0.001	<0.001	<0.001
	n	74	63	49	61	40

[a] Plasma retention ratio of indocyanine green at 15 min (%)
[b] Plasma disappearance rate of indocyanine green (min^{-1})
[c] Spearman's correlation coefficient
[d] Probability
[e] Number of patients

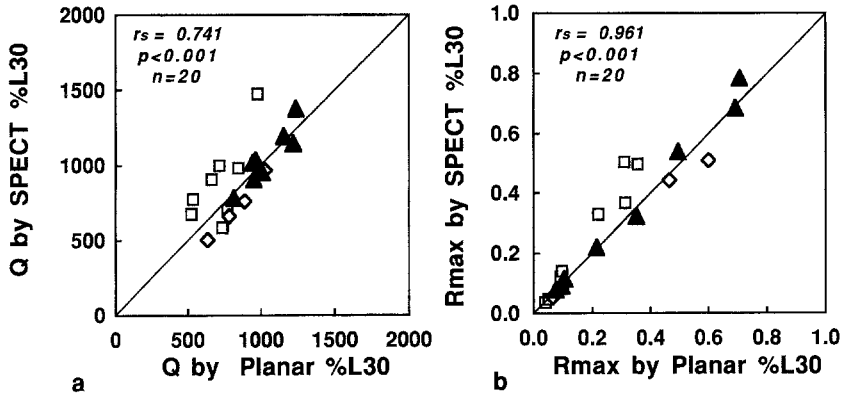

Fig. 7. Comparison of Q (**a**) and R_{max} (**b**) values using two different hepatic uptake rates, obtained from planar or SPET images

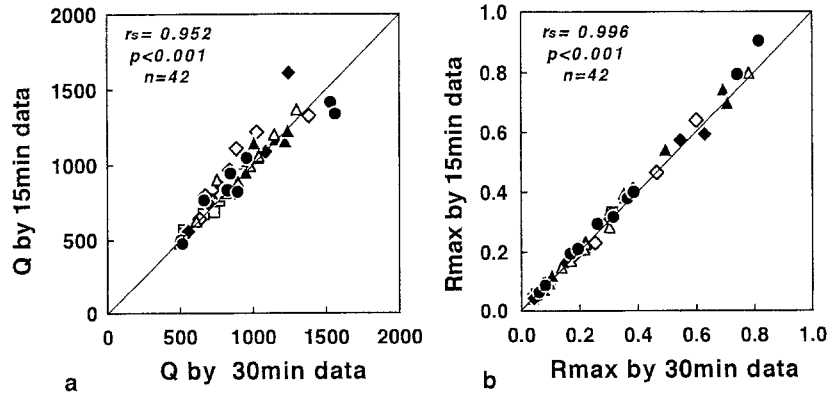

Fig. 8. Comparison of Q (**a**) and R_{max} (**b**) values obtained with the measurement times of 15 min and 30 min. The different symbols indicate the data obtained at each institution

trols and stage I, and decreased significantly in stages II and III.

The ANCOVA statistics showed consistent differences in Q (P=0.0005) and R_{max} (P<0.0001) according to functional severity. There were no statistically significant differences between institutions in respect of either parameter. ICG-R15 showed a moderate correlation with Q and a strong correlation with R_{max} (Fig. 6). The relationship between R_{max} and ICG-R15 was non-linear, and R_{max} showed a wide distribution in the normal range (<10%) of ICG-R15. Alb, PT, HPT and ICG-PDR showed moderate correlations with Q and strong correlations with R_{max} (Table 2). Figure 7 shows the results for Q and R_{max} using different %ID of the liver with planar and SPET methods. Data were scattered around the line of identity and significant correlations were obtained for both Q (r=0.741), P<0.001) and R_{max} (r=0.961, P<0.001). This indicates that both planar and SPET images give consistent estimations of liver uptake, thereby permitting compartmental analysis. When we performed analysis using the data measured for up to 15 min, Q and R_{max} were both distributed around the line of identity (Fig. 8). Reducing the examination time to 15 min did not affect the results of the analysis.

Discussion

^{99m}Tc-GSA is currently available in Japan, and hepatic scintigraphy using this ligand is becoming widely used in nuclear medicine departments. A phase III study [14] involving 460 cases has demonstrated the clinical effectiveness of ^{99m}Tc-GSA emplyoing the parameters of HH15 and LHL15. The AGR in the liver has a different binding capacity in each individual, and the maximal binding rate of GSA is limited by the amount of the AGR. A characteristic of Kawa's compartment analysis [9], employed in this multicentre study, is a setting of transfer velocity from the hepatic blood space to the hepatocytes. A number of experimental studies indicate that asialoglycoprotein binds to the AGR according to Michaelis-Menten kinetics in vitro [18, 19] and in vivo [20]. This kinetics explains the maximal rate of transfer as a function of the amount of receptor in the membrane. Kawa's method calculates the maximum velocity (R_{max}) that characterizes the behaviour of a ligand following Michaelis-Menten kinetics. This is an essential criterion for establishing the process of receptor-mediated binding.

HH15 and LH15, which are simple and convenient indices of GSA scanning, have been clinically confirmed to be useful parameters for assessing hepatic function [13, 14]. The values of Q and R_{max} obtained in this study agree well with HH15 and LHL15, indicating their potential usefulness in the evaluation of hepatic function. Although there was a good correlation between LHL15 and other liver function tests [21], the value in the normal state and mild dysfunction is close to the theoretical maximal value (1.0). The present study also showed the values of LHL15 in the normal state (0.950) and in stage I liver damage (0.917) to be near to each other. Suppose a 10% functional improvement were to be obtained after medical care in a patient with an LHL15 value of 0.917, a concordant increase of LHL15 would not be obtained because it would exceed 1.0. LHL15 is one of the indicators of hepatic uptake, whereas its denominator (H15+L15) is not equivalent to the total injected dose of ^{99m}Tc-GSA. When the volume of the cardiac blood pool is compared with that of systemic blood, it is certain that H15, counts from the cardiac blood pool, represents only a small part of the total counts in systemic blood. Therefore, the value of LHL15 is not equivalent to the actual uptake of ^{99m}Tc-GSA in the liver. This factor might interrupt linear correlation with actual liver uptake. HH15 is also not an absolute value; however, we observed an excellent correlation between HH15 and % blood retention (data not shown).

Hepatic uptake is determined by two factors (the hepatic blood flow and the amount of AGR). These cannot be assessed separately in HH15 or LHL15 estimates. Compartmental analysis based on pharmacokinetic modelling permits absolute physiological estimates to be obtained independently for ligand transfer (i.e. hepatic blood flow) and uptake (i.e. binding ability). This method is easily understandable for clinicians, biochemists, and pharmacologists, who may be unfamiliar with nuclear medicine techniques. Q and R_{max} have also shown good correlations with biochemical tests with regard to the synthetic capacity of the liver, thus suggesting that they may be useful for assessing hepatic function. In particular, they show the strongest correlation with ICG, which provides the best estimate of the hepatic reserve capacity among the conventional tests. Hepatic blood flow and the reserve capacity of the liver are factors that underlie the pharmacokinetics of ICG. This might be reflected in the observation in this study that both Q and R_{max} showed a moderate to strong correlation with ICG.

When Q and R_{max} were compared, the correlations with other liver function tests were found to be higher for R_{max} than for Q. Q showed a statistically significant reduction in stages II and III compared with the value in normals and stage I. However, there was no difference between Q in normals and stage I, nor between Q in stage II and stage III. Hepatic blood flow measured by the clearance technique has been found to be moderately reduced in cirrhosis [22], whereas very similar results were obtained between patients with non-cirrhotic liver disease and controls [23]. Our results in respect of Q are in agreement with these findings. On the other hand, R_{max} declined in accordance with the stage. This difference would explain why R_{max} showed better correlations with conventional liver function tests than did Q.

The present study showed a value for R_{max} of 0.65 mg/min±0.12 (mean±SD, n=7) in normal controls, which was higher than the value of 0.547 mg/min±0.069 (n=3) found in a previous study [9] using the same ana-

lytical method. Although the difference is not statistically significant (P=0.21, Student's t test), it was presumably due to the difference in the administered dose of ^{99m}Tc-GSA: in the previous study a dose of 1 mg of^{99m}Tc-GSA was used in a phase I or early phase II trial, whereas in the present study the current standard dose of 3 mg was employed. One would expect competition between a physiological asialoglycoprotein [24] and an extrinsic ligand (such as ^{99m}Tc-GSA) because both substances bind to the same receptor and the amount of binding receptor would be the same even after the administration of the extrinsic ligand. It is natural that administration of a higher dose of ^{99m}Tc-GSA would be less extensively affected by the physiological asialoglycoprotein than a lower dose. Therefore, the R_{max} following administration of 3 mg, as in the present study, would be expected to be higher than in the previous study in which 1 mg was administered.

R_{max} was linearly related to HH15 in almost all ranges, but non-linearly related to LHL15. In particular, the normal range of R_{max} showed a wide distribution. This result suggests that R_{max} can adequately demonstrate individual differences among normal subjects.

Comparison with other methods for determination of the hepatic blood flow would be required to confirm the reliability of Q. Since invasive methods are needed for accurate measurement of hepatic blood flow, the results of this study do not permit direct comparisons to be made. However, the mean Q value in normals (1158 ml/min±229) is in close agreement with direct measurements of hepatic blood flow (1229 ml/min±230) by means of hepatic vein catheterization [25].

With regard to the measurement of the hepatic uptake rate, absorption correction was not performed in this study. The planar anterior images and SPET images of a standard liver phantom served as the basis for calculations using the calibration coefficient. The difference in absorption efficiency due to each patient's constitution obviously affects the observed hepatic uptake rate. However, even when these differences were ignored, the Q and R_{max} values obtained in this study correlated well with the results of conventional liver function tests. The development of precise absorption correlation techniques in the future will minimize the variations in the hepatic uptake rate, which is expected to improve the accuracy of the analytical indices.

The standard acquisition time for the time-activity data was set at 30 min in this study. Nevertheless, analysis based on 15-min data provided almost identical Q and R_{max} values. Therefore, we have concluded that the data acquisition time can be reduced to 15 min, which would increase the number of patients examined in actual clinical situations.

Statistical analysis using ANCOVA proved that there was no inter-institutional difference in either Q or R_{max}. Scatter plots against HH15 or LHL15 (Figs. 3–6) showed no significant differences between the various institutions. These results demonstrate the feasibility of the analytical method for GSA scintigraphy applied in present study.

Kinetic analysis for labelled asialoglycoprotein has been performed with the use of a private software program [6, 11], which is one reason why kinetic analysis is not widely employed in other institutions. This study demonstrates the feasibility of employing an analytical program in public use for asialoglycoprotein scanning.

Our method needs a number of arithmetic processing steps for solving differential equations. However, actual curve processing is fully automated with the use of a macro-program. Thus, no knowledge or previous experience with data analysis is requied to perform this method. The time required for calculation is about 2 min on a personal computer, and stable results have been obtained in all cases. No cases have shown divergence due to failure to achieve approximation.

In conclusion, our method of ^{99m}Tc-GSA analysis is suitable for use at virtually all medical institutions. Q and R_{max} have been found to be the most standard analytical indices for ^{99m}Tc-GSA hepatic scintigraphy.

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