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Abstract

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COMPARISON OF ESTIMATION METHODS OF LIVER MAXIMUM REMOVAL RATE OF INDOCYANINE GREEN

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Abstract. Three linear plots by which the liver's maximum removal rate (Rmax) of indocyanine green (ICG) and the Michaelis constant (Km) can be calculated were compared in a microcomputer simulation study. The widely-used Lineweaver-Burk plot (1/V vs. 1/S; V, ICG initial removal rate (mg/kg/min); S, ICG loading dose (mg/kg)) presented the greatest bias and variance. There was no remarkable difference in bias between the S/V vs. S plot and the V vs. V/S plot, but the latter possesed a smaller variance. Therefore, the V vs. V/S plot was considered the best for estimating Rmax. The best combination of three ICG loading doses was 0.5, 2, and 5 mg/kg. This combination was selected by comparison of the Rmax estimated from three points with that estimated from six points (0.5, 1, 2, 3, 4 and 5 mg/kg).

Key words : indocyanine green, liver's maximum removal rate, liver function test, simulation study.

The liver's maximum removal rate (Rmax) of indocyanine green (ICG) is important in the evaluation of liver function, especially in deciding on the operability of hepatocellular carcinoma complicated by liver cirrhosis (1). However, ICG Rmax values are difficult to estimate reliably for a number of reasons (2, 3). Various combinations of ICG loading doses have been used in different institutes (1-6). Rmax is usually calculated using a Lineweaver-Burk plot (12), which is known not to be the best method for the analysis of enzyme-substrate relationships (8-10). Therefore, standardization of loading doses along with accurate calculation of Rmax is necessary to establish universality. In this study, calculation methodologies and combinations of loading doses were investigated by simulated data in order to derive the most accurate and reliable method of determining of ICG Rmax.

METHODS

ICG Rmax was determined by the following equation (7):

$$\text{Rmax} = \frac{V\,(\text{Km} + S)}{S} \qquad \text{(A)}$$

V is the initial removal rate (mg/kg/min) when the ICG dose is S; thus, V equals the product of the percent disappearance rate times S. Km represents the affinity of hepatic receptors for

ICG and equals the dose at which V = Rmax/2 (11). In the calculation of Rmax and Km, three linear plots were employed: 1/V vs. 1/S (A plot), S/V vs. S (B plot), and V vs. V/S (C plot) (12-14). These were compared in a simulation study of normal and liver cirrhosis groups. The normal values of Rmax and Km were set at 3.0 mg/kg/min and 18.0 mg/kg/min respectively (2, 3). Equation (A) becomes:

$$V = \frac{3.0 \times S}{18.0 + S} \quad \text{(B)}$$

For liver cirrhosis (LC) patients 0.4 mg/kg/min and 5.0 mg/kg were used as representative values for Rmax and Km (1-5):

$$V = \frac{0.4 \times S}{5.0 + S} \quad \text{(C)}$$

"True" values of V were calculated from equations (B) and (C) for six ICG-doses (S); these doses were assumed to be error-free.

S:	(mg/kg)	0.5	1	2	3	4	5
true V in normal:	(mg/kg/min)	0.081	0.158	0.300	0.429	0.545	0.652
true V in LC:	(mg/kg/min)	0.036	0.067	0.114	0.150	0.178	0.200

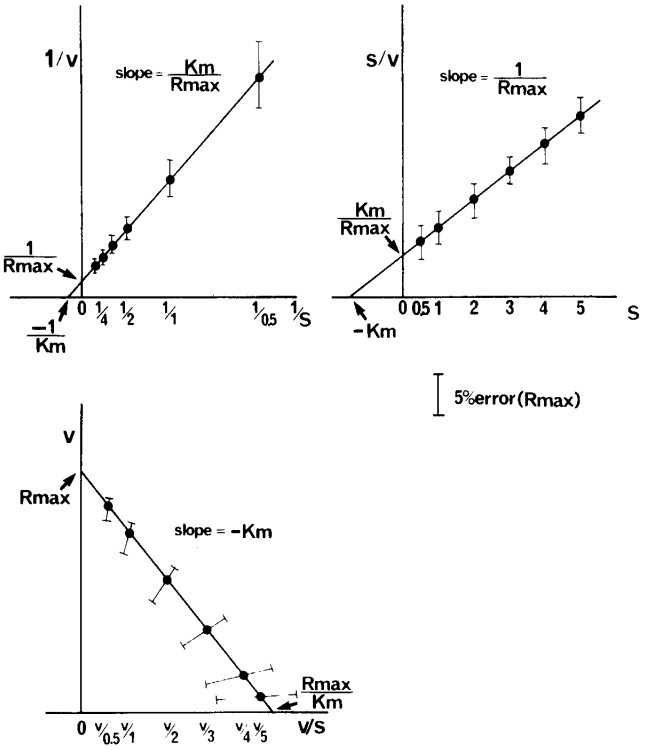

Fig. 1. Six points corresponding to different doses (0.5, 1, 2, 3, 4 and 5 mg/kg) were plotted with a 5 % error range in Rmax. Upper left - 1/V vs. 1/S (A plot, The Lineweaver-Burk plot); upper right - S vs. S/V (B plot); and lower left - V vs. V/S (C plot).

Inversely, to calculate "sample" Rmax and Km values from 6 sets of S and "sample" V, 3 types of errors were introduced to convert a "true" V to a "sample" V: (a) a constant absolute error (S.D. = ±0.01), (b) a constant relative error (S.D. = ±5.0 %) and (c) a inconstant relative error (S.D. = ±0.005 and S.D. = ±2.5 %). The first two errors (a and b) gave a sample V population which distributed normally around the "true" V with a prescribed standard deviation (PC-8801 microcomputer, NEC, Tokyo). The last error (c) contained both components of (a) and (b) and resembled the error of clinical estimates (9). Five hundred sets of 6 subsets of S and "sample" V were generated by adding the 3 types of errors. Each set was plotted by each of the linear plots. Regression lines were established by the least squares method. Rmax and Km were obtained from the abscissas and the slope of the line (Fig. 1).

To simplify the ICG-Rmax test, the most accurate combination of 3 loading doses was selected from 6 doses; 0.5, 1, 2, 3, 4 and 5 mg/kg. As 0.5 mg/kg ICG is widely used in usual liver function testing, this doses was adopted as a constant element. Two remaining doses were selected from 1, 2, 3, 4 and 5 mg/kg so as to minimize the difference between the value of Rmax obtained from 3 doses and that from 6 doses using the most accurate plot (C plot), as described below.

RESULTS

Comparison at the three linear plots. Five hundred Rmax and Km values were obtained for each plot for each type of error in both normal and liver cirrhosis groups

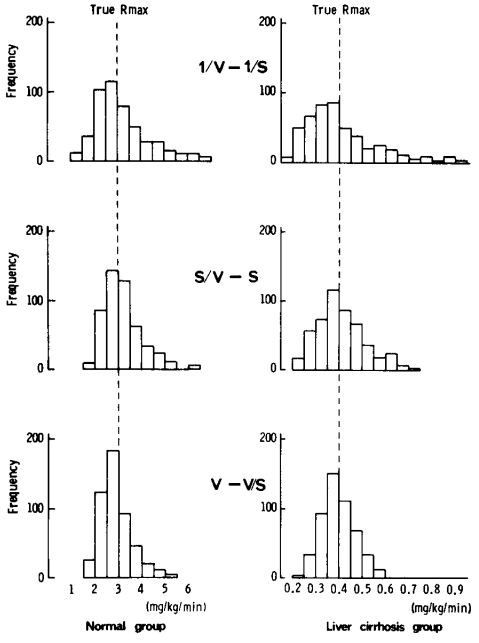

Fig. 2. Frequency distribution of 500 Rmax values calculated by 6-point analysis with an inconstant relative error (c). Left column - normal group, true Rmax = 3.0 mg/kg/min; right column - liver cirrhosis group, true Rmax = 0.4 mg/kg/min.

TABLE 1. COMPARISON OF RMAX AND KM VALUES IN 3 LINEAR PLOTS, WITH 3 KINDS OF ERROR IN NORMAL AND LIVER CIRRHOSIS GROUPS

Error of V	Linear plot axes	Normal		Liver cirrhosis	
		Rmax (true value = 3.0)	Km (true value = 18)	Rmax (true value = 0.4)	Km (true value = 5.0)
		Mean S.D.	Mean S.D.	Mean S.D.	Mean S.D.
Constant absolute error (S.D. = ±0.01)	1/V vs. 1/S	7.83 ±62.5	48.6 ±326	0.439 ±1.10	5.75 ±21.7
	S/V vs. S	3.15 ± 1.28	19.3 ± 8.59	0.425 ±0.0913	5.55 ± 2.01
	V vs. V/S	2.90 ± 0.963	17.7 ± 3.22	0.403 ±0.0633	5.12 ± 1.38
Constant relative error (S.D. = ±5.0%)	1/V vs. 1/S	3.93 ±11.7	24.4 ± 81.3	0.407 ±0.509	5.15 ± 0.970
	S/V vs. S	3.44 ± 1.74	21.2 ± 12.2	0.405 ±0.468	5.12 ± 0.886
	V vs. V/S	2.56 ± 0.71	15.0 ± 4.87	0.395 ±0.431	4.83 ± 0.816
Inconstant relative error (S.D. = ±0.005) +(S.D. = ±2.5%)	1/V vs. 1/S	3.60 ± 6.93	22.2 ± 48.6	0.517 ±1.11	5.82 ±22.5
	S/V vs. S	3.23 ± 2.21	20.1 ± 10.5	0.410 ±0.415	5.05 ± 8.75
	V vs. V/S	2.87 ± 0.667	17.2 ± 4.68	0.398 ±0.107	4.96 ± 2.21

(Table 1, Fig. 2). The variance of both Rmax and Km as demonstrated by their standard deviation decreased in the order of A, B and C plots and constant absolute error (a), constant relative error (b) and inconstant relative error (c) (Table 1). The variances were less in the cirrhosis group in all tests. For example, in the normal group with error (c), the mean of Rmax was 3.60, 3.23, and 2.87 in the A, B and C plots, respectively, whereas the true Rmax was 3.00. Therefore, the C plot was the most accurate and the A plot was the least. This order of accuracy was the same in all segments.

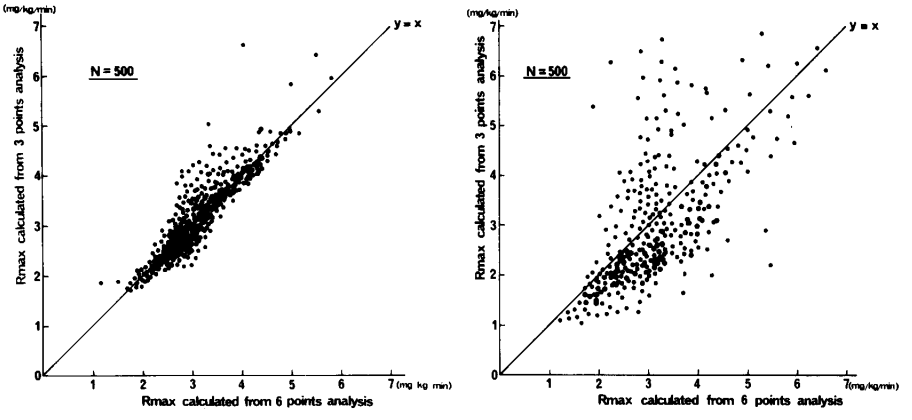

Fig. 3. Correlation between Rmax calculated from 6 points (S = 0.5, 1, 2, 3, 4 and 5 mg/kg) and that from 3 points by the V vs. V/S plot with an inconstant relative error (c) in the normal group. Left - the best combination (S = 0.5, 2, 5 mg/kg); right - the worst combination (S = 0.5, 1, 2 mg/kg).

TABLE 2. SUM OF DISTANCES BETWEEN RMAX OBTAINED BY 3 DOSES AND THAT BY 6 DOSES FOR SELECTION OF THE BEST COMBINATION OF 3 DOSES FOR RMAX ESTIMATION

Combination of 3 loading doses (3 points analysis)		Normal group	Cirrhosis group
0.5, 1, 2	mg/kg	1.60	1.28
0.5, 1, 3	〃	1.35	1.24
0.5, 1, 4	〃	0.871	0.991
0.5, 1, 5	〃	0.660	0.628
0.5, 2, 3	〃	0.996	1.25
0.5, 2, 4	〃	0.658	0.871
* 0.5, 2, 5	〃	0.421	0.303
0.5, 3, 4	〃	0.829	0.982
0.5, 3, 5	〃	0.695	0.649
0.5, 4, 5	〃	0.493	0.395

*best combination

Selection of the best combination of three loading doses. The sum of distances between Rmax obtained from 3 doses and from 6 doses was smallest with the combination of 0.5, 2 and 5 mg/kg and the largest with 0.5, 1 and 2 mg/kg (Fig. 3). This was the situation in both normal and liver cirrhosis groups (Table 2).

DISCUSSION

The best linear plot for the calculation of Rmax and Km was the C plot. The B plot was a close second. The Lineweaver-Burk plot (A plot) presented the greatest degrees of bias and variance. This result was surprizing as this plot is the are generally used (1-7). The Lineweaver-Burk plot posseses a defect in that the smallest value of V is the major determinant of the regression line because reciprocals are plotted. Thus, when S is 0.5 mg/kg, 1/V varies greatly even with a small sample error and, accordingly, Rmax ranges widely (Fig. 1). At high doses, S being 3 to 5 mg/kg, the three derived points cluster near the origin and figure only slightly in the calculations (Fig. 1). In both the B and C plots, all 6 dosage, points are distributed almost evenly, with the variance of each point being similar (Fig. 1). Moreover, in the C plot, Rmax is estimated at a point distant from the origin, minimizing error.

Rmax and Km determinations utilize 3 loading doses (1-6). In our survery of the literature we found numerous combinations; 0.5, 2 and 5 mg/kg (3); 0.5, 3 and 5 (2); 0.5, 1 and 2 (1, 5); and 0.5, 1 and 5 (4, 6). In this study, Rmax and Km obtained by the dose combination of 0.5, 2 and 5 mg/kg proved to approximate most closely those obtained from 6 points.

Loading doses both below and above Km should be used to estimate Rmax and Km accurately (8, 9). However, ICG toxicity prohibits high loading doses such as near the normal Km (18 mg/kg) (15). Therefore, the development of a

less toxic test substance is necessary for accurate estimations of total liver capacity in normal persons. ICG-Km in liver cirrhosis is relatively low, around 5 mg/kg, which is clinically safe even in cirrhotic patients. Therefore, Rmax may be estimated with good accuracy in liver cirrhosis. In conclusion, the best combination of 3 ICG loading doses for the calculation of ICG Rmax and Km was 0.5, 2 and 5 mg/kg. Rmax and Km was obtained most accurately by plotting V vs. V/S.

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