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Liver uptake function measured by IODIDA clearance rate in liver transplant patients and healthy volunteers

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Summary

Hepatobiliary scintigraphy with $^{99}Tc^{m}$-diethyl-iodo-HIDA (IODIDA), an iminodiacetic acid derivative, is used to assess hepatocyte function and to visualize the hepatobiliary system. The aim of this study was to evaluate whether it is possible to describe liver function by calculating the clearance rate of IODIDA from the blood. Clearance rate was evaluated in 18 liver transplant patients (28 studies) and 11 healthy volunteers (11 studies). Two different clearance rates were calculated: the clearance of IODIDA from the blood due to liver uptake and the total clearance of IODIDA from the blood due to all possible routes of elimination. Both for the healthy controls and the liver transplant patients, there was an excellent correlation between these two methods ($r = 0.92$ and $r = 0.93$, respectively), indicating that the liver is the only essential pathway for elimination of IODIDA from the blood. The difference in clearance rate between healthy controls and liver transplant patients was highly significant ($P < 0.01$), corresponding to the clinical condition of the two groups. We conclude that the clearance rate of IODIDA, based on a simple measurement from the time–activity curve derived from a blood pool region of interest, is a reliable test of liver function.

Introduction

Hepatobiliary scintigraphy is performed in liver transplant patients to assess the functional and morphological status of the graft, including structural complications (infarction, abscess, bile leakage, bile duct obstruction). Morphological information can be gained by evaluating sequential static images [1–3]. The assessment of liver function is more difficult and has been the subject of several clinical and experimental reports [4–8]. It has been suggested that the ideal way to describe liver function is by measuring the hepatic clearance of a test substance [9]. In order to develop a reasonable model for calculation, the test substance should be eliminated via the hepatobiliary system and have no alternative routes of elimination, even with deteriorating liver function.

Hepatobiliary scintigraphy with $^{99}Tc^{m}$-diethyl-iodo-HIDA (IODIDA) allows imaging to be performed in patients with high plasma bilirubin levels [1, 3, 6]. Earlier iminodiacetic derivatives, such as *N*-2,6,-diethyl-phenyl-carbamoyl iminodiacetic acid (HIDA), with a molecular weight of approximately 300 Da, provided useful information only when serum bilirubin was less than 100 μmol l^{-1} [1]. At higher bilirubin levels, HIDA is excreted to a significant extent via the kidneys and hepatobiliary imaging is inadequate. IODIDA, on the other hand, with a molecular weight of 500 Da, is only excreted to a minor extent by the kidneys [1, 4, 6]. Thus it would seem that IODIDA is cleared from the blood pool mainly by the liver, even when serum bilirubin reaches very high levels (> 1000 μmol l^{-1}). When hepatocyte uptake is impaired, IODIDA remains in the blood pool for a longer period of time, thus reflecting impaired hepatic clearance of IODIDA from the blood.

The aim of this study was to evaluate the possibility of describing liver uptake by calculating the clearance rate of IODIDA from the blood. This was done by investigating whether pathways other than the liver contribute to

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a significant extent to the clearance of IODIDA from the blood. Two different clearance rates were calculated: the clearance of IODIDA from the blood due to liver uptake (LClr) and total clearance of IODIDA from the blood due to all possible routes of elimination (BClr). The two rates were calculated and compared among a group of liver transplant patients and a group of healthy volunteers.

If it can be shown that the increase in activity in the liver corresponds to a reduction in activity in the blood pool, it can be concluded that there are no other pathways than the liver for the elimination of IODIDA from the blood, making measurement of urinary excretion unnecessary.

Patients and methods

Patients

The study group consisted of 18 consecutively scanned patients (10 females, 8 males) who had undergone liver transplantation. The mean age of the patients was 42 (range 17–60) years (Table 1). Transplantation had been performed 8 days to 2 years prior to scanning. Hepatobiliary scintigraphy was either performed electively as part of the routine follow-up or requested due to the suspicion of some complication. In total, 28 studies were performed in the liver transplant patients: 13 patients had one study each, 2 patients were scanned twice, 2 patients three times and 1 patient five times (Table 1).

Controls

The control group comprised 11 healthy volunteers (5 females, 6 males) with neither a history of previous liver disorder nor abnormal liver tests. The mean age of the control subjects was 40 (range 28–67) years. The controls had one scan each.

Scintigraphy

Data acquisition for hepatobiliary scintigraphy was the same for the patients and controls. After an overnight

Table 1. Results of routine liver biochemistry, BCLr and LCLr.

Patient	*Age (years)*	*Sex*	*Bil*	*ASAT*	*ALAT*	*ALP*	*BClr*	*LClr*	*Comments*
1	61	M	120	1.60	8.70	5.3	8	6	Rejection
			74	0.64	3.30	4.6	17	16	After treatment
2	59	F	26	0.84	0.81	3.9	14	14	No complications
3	57	F	7	0.44	0.51	3.1	24	21	No complications
4	53	M	30	1.07	1.45	10.0	14	12	No complications
5	52	M	45	0.81	0.48	18.0	18	15	Bile leakage
			51	1.20	0.71	15.0	11	8	Bile leakage
			57	1.30	0.79	13.0	13	14	Bile leakage
			76	1.00	0.69	18.0	7	4	6 days after bile duct repair
			15	0.53	0.30	5.4	16	13	3 months after bile duct repair
6	52	F	23	0.39	0.24	2.1	14	15	No complications
7	50	F	10	0.41	0.36	2.1	14	16	No complications
8	50	M	6	0.26	0.21	1.5	18	17	No complications
			40	1.50	3.50	2.6	15	13	Bile leakage
9	49	M	33	0.77	4.50	4.6	11	12	No complications
10	46	F	17	0.76	0.36	17.0	13	12	No complications
11	43	M	11	0.46	0.49	4.0	16	15	No complications
12	41	F	41	0.58	1.00	3.8	13	11	No complications
13	37	F	16	0.71	0.68	2.4	19	17	No complications
14	35	F	38	0.49	2.30	9.0	15	11	Bile leakage
			50	0.63	1.10	8.1	11	10	Bile leakage
			23	0.93	1.50	7.4	13	13	Spontaneous recovery
15	32	M	43	2.10	4.00	14.0	7	6	Bile duct stenosis
16	30	M	14	0.77	0.76	2.1	14	14	No complications
17	23	F	56	0.79	1.40	1.6	13	11	No complications
18	17	F	66	8.30	1.00	4.0	12	12	No complications
			160	2.90	6.30	8.2	6	6	Rejection
			57	1.30	1.50	21.0	6	6	Rejection

ASAT reference value < 0.7 μkat l^{-1}; ALAT reference value < 0.7 μkat l^{-1}; ALP reference value < 5.0 μkat l^{-1}; bilirubin reference values 3.4–21.0 μmol l^{-1}.

fast, they were positioned supine with the gamma camera centred over the upper abdominal region. The heart, mediastinum, liver and part of the intestinal tract were included in the field of view (FOV) of the gamma camera. Then, 200 MBq IODIDA was administered to the patients and 50 MBq to the controls by bolus injection, and acquisition was begun simultaneously.

A computerized Elscint gamma camera (Apex 415), equipped with a low-energy, general-purpose collimator (FOV 40 cm) was used. Dynamic acquisition was performed at 10 s per frame for 30 min post-injection in a 64×64 matrix (zoom factor = 1.0). The dynamic study was followed by one static antero-posterior view of the abdomen and one right lateral view. The delayed static images were acquired in a 128×128 matrix.

One region of interest (ROI) was drawn over the liver, another over part of the heart and large vessels in the mediastinum (i.e. the blood pool), and a third enclosing the whole frame (i.e. the FOV; see Fig. 1). As the heart is very close to the liver, the ROI representing the blood pool did not include the entire heart, but just part of it together with part of the large vessels, in order to avoid scatter from the liver. Based on the liver, blood pool and FOV ROIs, three time–activity curves were generated (Fig. 1).

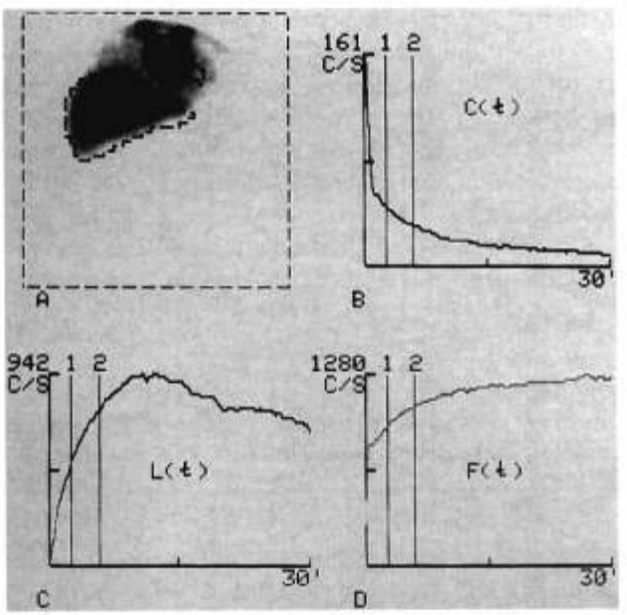

Fig. 1. (A) Three ROIs are shown; one ROI over part of the heart and mediastinal structures representing the blood pool, another over the liver and a third enclosing the whole frame. (B) The time–activity curve from the blood pool ROI, $C(t)$. (C) The time–activity curve from the liver ROI, $L(t)$. (D) The time–activity curve from the entire field of view, $F(t)$. Markers 1 and 2 denote $t_1 = 150$ s and $t_2 = 350$ s, respectively.

Liver tests

On the day of the study, blood samples were drawn for routine laboratory liver biochemistry, including aspartate aminotransferase (ASAT, reference value < 0.7 μkat l^{-1}), alanine aminotransferase (ALAT, reference value < 0.7 μkat l^{-1}), alkaline phosphatase (ALP, reference value < 5.0 μkat l^{-1}) and bilirubin (reference values 3.4–21.0 μmol l^{-1}). The study was approved of by the hospital's ethical and isotope committees.

Clearance rate calculations

The clearance (Cl) of a substance is defined as the volume of a pool of the substance that is cleared per time unit (ml min^{-1}). Clearance rate (Clr) is defined as the fraction, or percentage, of a pool of the substance that is cleared per time unit:

$$\text{Clr} = (\text{Cl}/V) \times 100 \ (\% \ \text{min}^{-1})$$

where V is the volume of the pool (ml).

Two different clearance rates were calculated: (1) the clearance of IODIDA from the blood due to liver uptake, based on increasing activity in the liver (LClr), and (2) the total clearance of IODIDA from the blood due to all possible routes of elimination, based on decreasing activity in the blood pool (BClr).

LClr

The amount of activity that has accumulated in the liver at a given time is denoted $L(t)$. The amount of activity that accumulates in the liver from time t_1 to t_2 can be written:

$$L(t_2) - L(t_1) = \int_{t_1}^{t_2} \text{LCl} \ C(t) \ dt \qquad (1)$$

where LCl is liver clearance due to liver uptake (ml min^{-1}), $C(t)$ is the concentration of circulating activity (Bq ml^{-1}), which is a function of the total circulating activity in the blood pool, $A(t)$, and the volume of the blood pool, V. This gives:

$$C(t) = \frac{A(t)}{V} = A(t_1)\frac{C_{\text{norm}}(t)}{V} \qquad (2)$$

where $A(t_1)$ is the total amount of circulating activity at time t_1, and $C_{\text{norm}}(t)$ is $A(t)$ normalized to unity at time t_1, reflecting changes in concentration in the blood pool. Equation 1 can be rewritten:

$$\text{LCl} = \frac{L(t_2) - L(t_1)}{\frac{A(t_1)}{V}\int_{t_1}^{t_2} C_{\text{norm}}(t) \ dt} \qquad (3)$$

Hence:

$$\text{LClr} = \frac{\text{LCl}}{V} = \frac{L(t_2) - L(t_1)}{A(t_1)\int_{t_1}^{t_2} C_{\text{norm}}(t)\,dt} \quad (4)$$

Equation 4 is used to estimate LClr. First, however, an expression for $A(t_1)$, the total amount of circulating activity at t_1, has to be found.

The activity registered by the gamma camera (i.e. the total activity in the FOV at time t) is denoted $F(t)$. If there is no escape of activity from the FOV and compensation for activity decay is performed, then:

$$A(t_1) = F(\infty) - L(t_1) \quad (5)$$

where $F(\infty)$ equals the total injected activity. $F(\infty)$ also equals the sum of activity inside the frame, $F(t)$, and outside the frame, $g \times C_{\text{norm}}(t)$, for any t:

$$F(\infty) = F(t) + g \times C_{\text{norm}}(t) \quad (6)$$

where g is an unknown constant.

Combining equations 5 and 6 for $t = t_1$ and $t = t_2$ gives the following equations:

$$\begin{aligned} A(t_1) &= F(\infty) - L(t_1) = F(t_1) + g \times C_{\text{norm}}(t_1) - L(t_1) \\ A(t_1) &= F(\infty) - L(t_1) = F(t_2) + g \times C_{\text{norm}}(t_2) - L(t_1) \end{aligned} \quad (7)$$

Eliminating g gives the following estimate of $A(t_1)$:

$$A(t_1) = F(\infty) - L(t_1) = \frac{F(t_2) - L(t_1) - [F(t_1) - L(t_1)]C_{\text{norm}}(t_2)}{1 - C_{\text{norm}}(t_2)} \quad (8)$$

$L(t)$ is described by the time–activity curve based on a ROI over the liver (Fig. 1); $C(t)$ is described by the time–activity curve based on a ROI over the blood pool (Fig. 1); $F(t)$ is described by the time–activity curve based on a ROI enclosing the whole frame (Fig. 1) and $C_{\text{norm}}(t) = C(t)/C(t_1)$.

It was assumed that the tracer is distributed evenly in the blood pool by 150 s post-injection; therefore, t_1 was chosen to be 150 s. Time t_2 was chosen to be 350 s in order to avoid errors due to the escape of the radiopharmaceutical from the liver ROI. With these definitions, LClr can be estimated (equation 4).

Estimating $A(t_1)$ using this model (equations 5–8) leads to an underestimation of LClr if there are pathways of elimination of IODIDA other than the liver.

BClr

$C(t)$ denotes the concentration of circulating activity (Bq ml^{-1}) in the blood pool at time t. If a single-pool kinetic model is used, $C(t)$ can be written:

$$C(t) = C_0\, e^{-\lambda t} \quad (9)$$

where C_0 is the initial concentration of circulating activity. By using equation 1 and our definition of Clr, it can be shown [10] that:

$$\text{BClr} = \lambda \quad (10)$$

Thus BClr can be estimated from a simple mono-exponential fit and is equal to λ. A mono-exponential fit was performed on the time–activity curve, $C(t)$, between 150 s (t_1) and 350 s (t_2). $C(t)$ is described by a time–activity curve based on the blood pool ROI (Fig. 1).

Statistics

The results are presented as the mean ± S.D. The Wilcoxon rank test was used to analyse differences between the groups and methods of calculating clearance rate. The relationship between BClr and LClr, as well as BClr and biochemistry, was analysed by least square linear regression analysis.

Results

Table 1 shows the results of the routine liver biochemistry tests, blood clearance (BClr) and liver clearance (LClr) rate calculations for the liver transplant patients. The patients had a lower IODIDA clearance rate compared with the controls, irrespective of the calculation method used (Table 2). There was a significant correlation between BClr and LClr for the patients ($r = 0.93$) and for the controls ($r = 0.92$) (Fig. 2). The intercept of the line of the linear fit was 1.7 ± 1.6 (90% confidence interval) for the transplant patients and 4.2 ± 6.8 (90% confidence interval) for the controls. The low value of the intercept for the patient group indicates that the liver is the only essential pathway for the elimination of IODIDA from the blood. There was a significant negative correlation between BClr and serum bilirubin (Fig. 3) and alkaline phosphatase, but not between BClr and serum aminotransferase. The LClr and BClr values correlated well with the clinical condition of the patients (Table 1).

Table 2. BClr and LClr calculated from scintigraphy performed on control subjects and liver transplant patients.

	Mean BClr (% min^{-1})	*Mean LClr (% min^{-1})*
Controls ($n = 11$)[b]	31±6	30±6
Range	17–42	16–43
Liver transplant ($n = 28$)[b]	13±4	12±4
Range	6–24	6–21

[a]Significant difference between the clearance rates of the patients and the controls ($P < 0.01$).
[b]The number of scintigraphic studies. For controls, the number of scintigraphic studies = number of subjects.

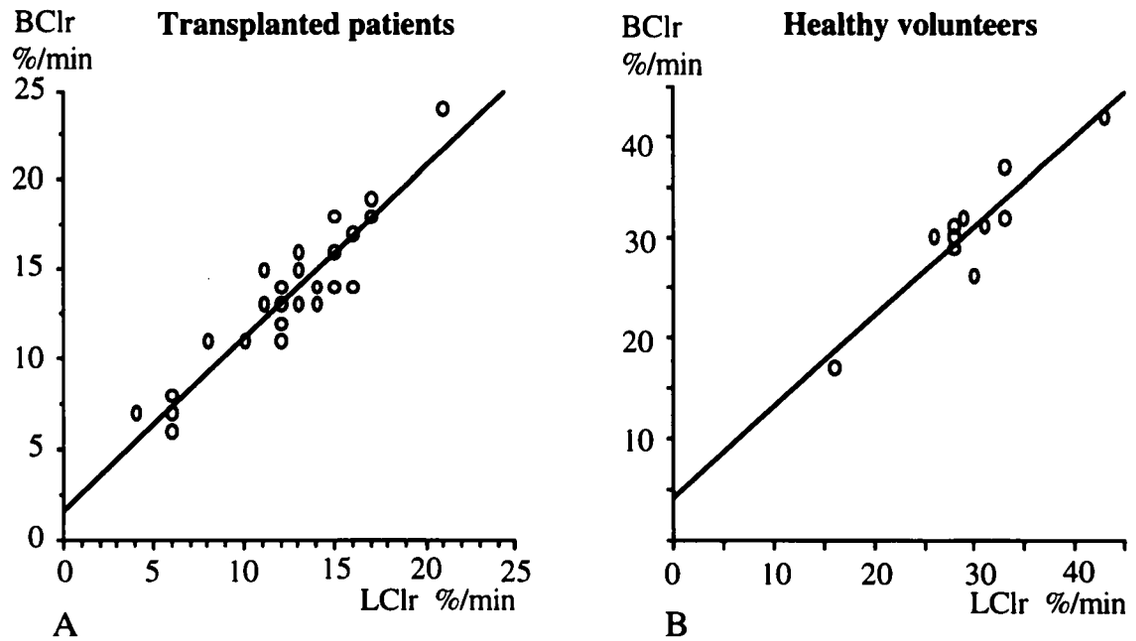

Fig. 2. The relationship between LClr and BClr, as estimated by linear regression analysis, in (A) the liver transplant patients ($y = 1.7 + 0.97x$; $r = 0.93$, $P < 0.001$) and (B) the healthy volunteers ($y = 4.2 + 0.89x$; $r = 0.92$, $P < 0.001$).

Discussion

Organ uptake function can be described by calculating the elimination of a substance from the blood. This is well established in nephrology, where ^{51}Cr-EDTA clearance is widely accepted as a means of measuring glomerular filtration rate [11]. Techniques for calculating plasma clearance of a substance using data acquired from a routine gamma-camera study have also been presented. Methods are also available for evaluating renal function (e.g. by measuring ^{123}I-hippuran clearance [12] or ^{99}Tc^{m}-DTPA clearance [13]) based on cardiac curves from gamma-camera renography.

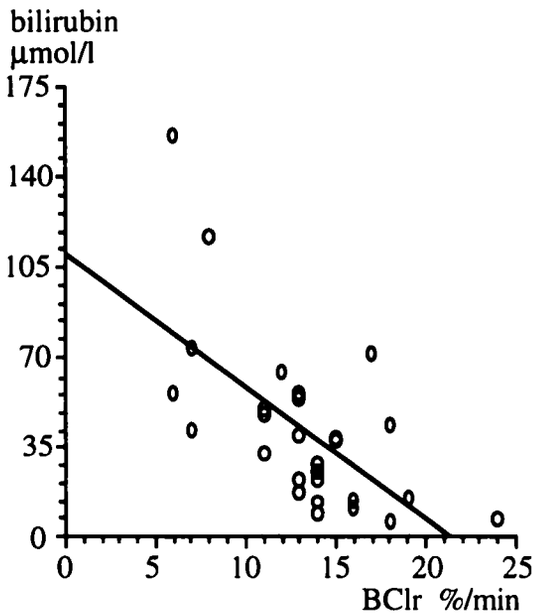

Fig. 3. The relationship between BClr and serum bilirubin, as estimated by linear regression analysis, in the liver transplant patients ($y = 114 - 5.3x$; $r = 0.64$, $P = 0.001$).

Substances that are eliminated from the blood by the liver can be used in a similar way to describe liver function. Examples include ^{99}Tc^{m}-neoglucoalbumin for hepatocyte [14] and ^{99}Tc^{m}-colloids for Kupffer cell function [15], where the cardiac time–activity curve from a routine gamma-camera study of the liver is used to estimate clearance rate. ^{99}Tc^{m}-neoglucoalbumin is specifically trapped in the hepatocyte by a receptor-mediated uptake mechanism and colloids are trapped in the Kupffer cells by phagocytosis. The uptake mechanisms of these substances make them of no value if the biliary system needs to be visualized.

In hepatobiliary scintigraphy, iminodiacetic acid derivatives are used which give information about the morphological as well as the functional status of the liver parenchyma and the biliary tree. A problem with the earliest iminodiacetic acid derivatives was that they were excreted in part by the kidneys and, with deteriorating liver function, renal excretion was considerable, making it impossible to obtain adequate images of the

hepatobiliary system. Since the introduction of IODIDA, this problem has been overcome. In an experimental study, IODIDA was shown to have minimal urinary excretion [8]. The absence of renal excretion and the possibility of obtaining reliable images in patients with high hyperbilirubinaemia has often been confirmed [16].

In this study, we calculated the clearance rate of IODIDA in a group of healthy volunteers and a group of liver transplant patients. Our calculations are based on data acquired from routine hepatobiliary gamma/camera studies with IODIDA. Two different clearance rates were compared: (1) the clearance of IODIDA from the blood due to live uptake, based on increasing activity in the liver (LClr), and (2) the total clearance of IODIDA from the blood due to all possible routes of elimination, based on decreasing activity in the blood pool (BClr). To ensure we were beyond the initial period of uneven distribution immediately post-injection and within the rapid phase of elimination, we chose to perform the calculations on values acquired between 150 and 350 s post-injection. Although the elimination of IODIDA from the blood is at least bi-exponential [17], performing a mono-exponential fit gives a good model of $C(t)$ in the chosen interval.

By adopting the definitions given here, BClr can be used to calculate the total elimination of tracer from the blood. That is, BClr is the weighted average of the rate of elimination of IODIDA from the blood via different pathways [e.g. liver and kidney as well as dilution to the extracellular compartment (ECV)]. LClr, on the other hand, can be used to calculate liver uptake. LClr is the weighted average of the rate of uptake by the liver. If there are other routes of elimination of IODIDA in addition to the liver, LClr will underestimate liver uptake function.

Therefore, based on these definitions, it is concluded that the intercept of the linear fit between BClr and LClr provides information regarding organs other than the liver for elimination of IODIDA from the blood pool. For the patient group, the intercept was less than 3.3% min^{-1} (90% confidence interval). Thus it can be concluded that there are no significant alternative pathways of elimination other than the liver. The number of control subjects was small, making the confidence interval of the intercept too wide for any conclusions to be drawn about the intercept for this group.

The correlation coefficients between LClr and BClr for the controls ($r = 0.92$) and the liver transplant patients ($r = 0.93$) further strengthen the assumption that the liver is the only pathway of elimination of IODIDA from the blood, making measurements of urinary excretion unnecessary.

Carlsen *et al.* [17] determined the clearance rates of IODIDA and HIDA as part of a routine hepatobiliary gamma-camera study. Their calculations were performed using a cardiac curve, which was decomposed into a bi-exponential curve and a constant. They showed that, in normal subjects, the clearance rate of HIDA was slower than that of IODIDA. Their patient group, which consisted of patients with liver tests outside the reference ranges, was examined with HIDA only. Carlsen *et al.* noted a reduction in the rate of HIDA in the patient group compared with normal individuals. The decrease was not very impressive and perhaps renal excretion of HIDA is one explanation for the moderate decrease. When there is impaired liver function, the kidneys take over the excretion of HIDA, and clearance rate calculations on cardiac time–activity curves reflect not only liver function but the sum of liver and kidney function. Had they calculated IODIDA clearance rate in a group of patients with impaired liver function, they may have observed a larger difference between the normal subjects and patients.

Our results, which show that IODIDA is eliminated in the main via the hepatobiliary system, are in accordance with those of other studies [1, 3, 6]. The alternative routes of excretion are so insignificant that it is justifiable to describe liver uptake function by determining BClr. BClr is easy to calculate, whereas LClr is based on elaborate mathematical formulae and therefore complicated to calculate. In a nuclear medicine department with facilities for hepatobiliary scintigraphy, the BClr of IODIDA can easily be determined. The assessment of IODIDA BClr (i.e. liver uptake function) could be of importance in liver transplant patients as well as other patients with liver dysfunction.

In our liver transplant patients, there was no correlation between the clearance rate of IODIDA and aminotransferase. Routine liver tests are non-specific; for example, transaminases reflect liver cell damage and do not necessarily reflect total liver function. We found a correlation between bilirubin levels and IODIDA clearance rate, which may reflect the involvement of similar uptake mechanisms. The estimation of the clearance rate of IODIDA as described in this paper probably provides better information regarding liver function than routine liver biochemistry tests. This conclusion is supported by the observation that BClr was very low in patients 1 and 18 who were experiencing continuing rejection, whereas BClr was initially uninfluenced by leakage in patients 5, 8 and 16.

We can only speculate on the reasons for the reduced BClr and LClr in the liver transplant patients with stable liver graft function when compared with the controls. The immunosuppressive drug cyclosporine is cholestatic

and could influence not only excretion of bile [18, 19] but also uptake of bilirubin and IODIDA.

It has to be stressed that the clearance rate calculations, as described in this study, were based on data acquired from 150 to 350 s after tracer injection and that clearance rate calculations based on other time intervals may be different. Furthermore, BClr represents liver uptake function only when a substance is chosen that is mainly excreted via the hepatobiliary system, even when liver function is severely impaired. IODIDA fulfils this requirement, whereas the earlier iminodiacetic acid derivatives (e.g. HIDA, DISIDA, BIDA) do not. Mebrofenin, being a bromo-substituted IDA compound, could possibly be used, as it has been shown to have very low renal excretion [20]. Mebrofenin as well as IODIDA has a halogen atom on the phenyl ring. This increases the molecular weight of the substance, which in turn probably contributes to the negligible renal excretion even in cases with very high bilirubinaemia.

Doo *et al.* [21] have estimated the hepatic extraction fraction (HEF) of mebrofenin. We estimate liver uptake function by clearance rate calculations. By combining clearance rate and HEF calculations, it should be possible to calculate blood flow rate to the liver; that is, flow rate = Clr/HEF.

Conclusion

Liver uptake function can be assessed by IODIDA clearance rate calculations. These calculations are performed on a simple mono-exponential fit of the time–activity curve based on a blood pool ROI from a routine hepatobiliary scintigraphic study.

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