CHOLESCINTIGRAPHY IN JAUNDICED PATIENTS

Comparison of diethyl iminodiacetic acid (IDA) with p-butyl-IDA

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Abstract

CHOLESCINTIGRAPHY IN JAUNDICED PATIENTS: COMPARISON OF DIETHYL IMINODIACETIC ACID (IDA) WITH p-BUTYL-IDA.

Synthesis of the inactive compounds was carried out with a modification of the method of Callery and Lofgren. After $^{99}Tc^{m}$ labelling, radiochemical purity control was performed with ITLC, TLC, PC and HPLC. The kinetic behaviour of diethyl-IDA (de-IDA) and p-butyl-IDA (pb-IDA) and also their ability for sequential imaging was compared in 8 jaundiced patients (serum bilirubin: 3–25 mg/dl). Blood clearance curves were biexponential with half-times for the fast component of 4.1 and 4.9 min for de-IDA and pb-IDA respectively. Cumulative urinary excretion was higher for de-IDA. pb-IDA demonstrated a slower liver uptake and elimination. Thus on sequential images pb-IDA proved to exhibit relatively prominent hepatic uptake compared with de-IDA, whereas gall bladder filling and intestinal excretion were delayed, and depiction of the biliary system failed in 5 patients using pb-IDA. The delayed imaging characteristics using pb-IDA offer no diagnostic advantage over de-IDA. Misinterpretation of the scintigrams may occur more often with pb-IDA especially if the observation time is not adequately prolonged. The data of the authors indicate that de-IDA seems to be superior to pb-IDA in its diagnostic usefulness, also in patients with severe hyperbilirubinaemia.

INTRODUCTION

Advances in radioisotope imaging and in radiopharmacology have increased the potential for visualization of the biliary system by non-invasive techniques. Synthesis of IDA derivatives labelled with $^{99}Tc^{m}$, which are taken up by the liver

and excreted into the bile, have improved the accuracy for the differential diagnosis of jaundice [1, 2].

Hepatic uptake and excretion take place even with high serum levels of alkaline phosphatase and bilirubin when radiological contrast methods cannot be applied. However, when the degree of liver dysfunction and hyperbilirubinaemia increases, a diminished uptake will lead to inconclusive results.

From previously reported animal studies [3] and from limited clinical experience in patients with jaundice [4], it was suggested that among the IDA derivatives p-butyl-IDA (pb-IDA) best maintains biliary exretion despite hepatic dysfunction and rising bilirubin levels, and offers some advantage over diethyl-IDA (de-IDA). But as there are considerable interspecies differences [5], and no direct comparison was made between de-IDA and pb-IDA, it might be important to obtain data in man and to compare them in patients with varying degrees of liver dysfunction.

PREPARATION OF RADIOPHARMACEUTICALS

Synthesis of the inactive compound (in a modification of the procedure of Lofgren [6] and Callery [7], and labelling with $^{99}Tc^{m}$ was carried out as previously described [8]. Radiochemical purity control was performed in four independent chromatographic systems [8]: (a) thin-layer chromatography (TLC) on Merck cellulose plates developed in acetone; (b) TLC on cellulose using the solvent n-butanol : acetic acid : water (20 : 5 : 5); (c) paper chromatography on Whatman No.1 developed in acetonitrile : water (3 : 1); (d) high-pressure liquid chromatography (HPLC) on a reversed phase column (Merck LiChrosorb RP18, 7 μm, 4.6 X 250 mm) using an eluent composed of 0.01M phosphate buffer pH 6 and 20–100% (vol./vol.) methanol in a linear 20-min gradient, flow rate 1 ml/min. Technetium-99m activity in the effluent was measured by a flow through the scintillation detector and 254 nm UV absorption was also monitored. Radioactive TLCs and PCs were scanned on a thin-layer scanner.

Free pertechnetate can be quantified by system (a) (less than 2% of the total activity); an independent radiochemical purity control is provided by systems (b) and (c) which separate hydrolysed reduced TC from $^{99}Tc^{m}$-IDA plus TcO. System (d) shows that the $^{99}Tc^{m}$-IDA complex is a molecule with a different chemical identity from the inactive IDA derivative. The chemical structure of $^{99}Tc^{m}$-IDA is assumed to be an octahedral complex with 2 IDA ligands co-ordinating one technetium ion. This complex is therefore larger and more lipophilic than the unlabelled IDA derivative.

PATIENTS AND METHODS

Eight patients who had given informed consent were investigated, the six males and two females ranged in age from 38–66 years. Serum bilirubin levels were 3–4 mg/dl

TABLE I. COMPARISON OF DIETHYL-IDA WITH p-BUTYL-IDA IN JAUNDICED PATIENTS (n = 8; $\bar{x} \pm s$)

		Diethyl-IDA	p-Butyl-IDA
Uptake index	5 min	1.25 ± 0.58	1.04 ± 0.58[a]
	60 min	2.07 ± 1.32	3.26 ± 2.26[a]
Retention index	30 min	0.97 ± 0.03	Not calculable
	60 min	0.81 ± 0.07	Accumulation curve not calculable
Liver peak time	(min)	20.60 ± 5.6	>60[a]
Cumulative urinary excretion (% dose) t = 180 min		19.60 ± 9.2	7.40 ± 4.1[a]

[a] $p < 0.01$, significantly different from diethyl-IDA (paired data).

in three patients, 10.6 – 14 mg/dl in another three, and 19 and 25 mg/dl in the remaining two. There was no significant change in biochemical parameters which were evaluated before each investigation. Diagnosis was established by liver biopsy, sonography, PTC, autopsy in one, and operation in two of the patients. Two patients had primary biliary cirrhosis, three cholostatic alcoholic liver cirrhosis, one carcinoma of the liver. In the remaining two there was obstructive jaundice due to stones associated in one patient with cystic duct obstruction.

All diagnostic procedures were carried out on fasting patients at a three-day interval. The activity administered was 0.06 – 0.07 mCi/kg bw.[1] Sequential samples for blood clearance were obtained by means of an indwelling intravenous catheter up to 180 min following injection. Urine was collected for 3 h after initiation of the study. Scintifotos were made at 15-min intervals for 1 h and after 3 h. Time activity curves were obtained over regions of interest (ROI) during a 60-min period after injection using a DEC PDP 11.

The hepatic uptake of de-IDA and pb-IDA was assessed by means of an 'uptake index' [9], obtained by taking a ratio of the activity in the periphery of the right liver lobe over that from the heart both at 5 and 60 min post injection.

The liver's excretory capacity was measured by two values of a 'retention index' [9], defined as the ratio of the activity in the right lobe at 30 (or 60) min over that at its maximum.

[1] 1 Ci = 3.70×10^{10} Bq.

TABLE II. TIME (min) FOR VISUALIZATION OF THE GALL BLADDER (GB), COMMON BILE DUCT (CBD) AND INTESTINE (I)

Diagnosis	Serum bilirubin (mg/dl)	Alkaline phosphatase (U/l)	Diethyl-IDA			p-butyl-IDA		
			GB	CBD	I	GB	CBD	I
Prim. biliary cirrhosis	25	254	Failure to accumulate in the liver					
Prim. biliary cirrhosis	19	250	–	–	180	–	–	180
Cholostatic cirrhosis	14	570	45	–	–	–	–	–
Cholostatic cirrhosis	10.8	255	30	30	30	60	60	60
Cholostatic cirrhosis	10.6	330	30	60	30	60	–	60
Carcinoma of the liver	4.2	263	30	60	60	180	–	180
Cholelithiasis	3.4	1320	–	30	30	–	60	60
Choledocholithiasis	3.2	890	30	20	30	45	–	–

RESULTS

After the initial mixing phase the blood clearance curve can be adequately approximated as biexponential. The initial fast component was faster for de-IDA than for pb-IDA, with half times of 4.1 + 0.7 min and 4.95 + 0.7 min respectively.

The mean cumulative urinary excretion of de-IDA was significantly higher than that of pb-IDA (Table I).

Using de-IDA, the time activity curve generated over the right liver lobe reached a maximum at 20.6 ± 5.6 min after injection. In comparison, maximum liver uptake was markedly delayed for pb-IDA, where time activity curves in most patients exhibited an accumulation pattern during the first 60 min.

The hepatic uptake index was higher for de-IDA at 5 min but was less pronounced at 60 min compared with pb-IDA.

A faster elimination was found for de-IDA as demonstrated by the retention index, which could not be calculated for pb-IDA because of its increasing activity up to 60 min after initiation of the study.

In one patient with primary biliary cirrhosis and a serum bilirubin level of 25 mg/dl, jaundice was so pronounced that no hepatic uptake or excretion could be observed. Time activity curves over ROI of the liver were similar to background curves for both substances, thus de-IDA and pb-IDA failed to accumulate in the liver.

In the remaining seven patients $^{99}Tc^{m}$ de-IDA imaging studies showed that after an initial increase liver activity gradually diminished. It increased on the images obtained up to 60 min using pb-IDA.

Gall bladder filling was observed in five patients with de-IDA, but only in four using pb-IDA (Table II).

Visualization of the biliary tract was possible in five patients between 20–60 min post injection, but delineation of the common bile duct failed in those patients with the highest bilirubin level (14 and 19 mg/dl). Activity within the intestinal tract was demonstrable in six patients, but up to 3 h after intravenous injection of de-IDA no appreciable gut entry was seen in one (bilirubin 14 mg/dl).

Depiction of the common bile duct was markedly delayed for pb-IDA and could be seen only in two patients (Figs 1 and 2). Activity in the bowel was demonstrable in five, but failed to be detected in two (bilirubin 14, 3 mg/dl) until 3 h post injection (Fig.3).

DISCUSSION

Although de-IDA has proved its diagnostic usefulness for cholescintigraphy [1, 9], in severely jaundiced patients the degree of uptake diminishes and the results are of limited value. Therefore other compounds are being investigated

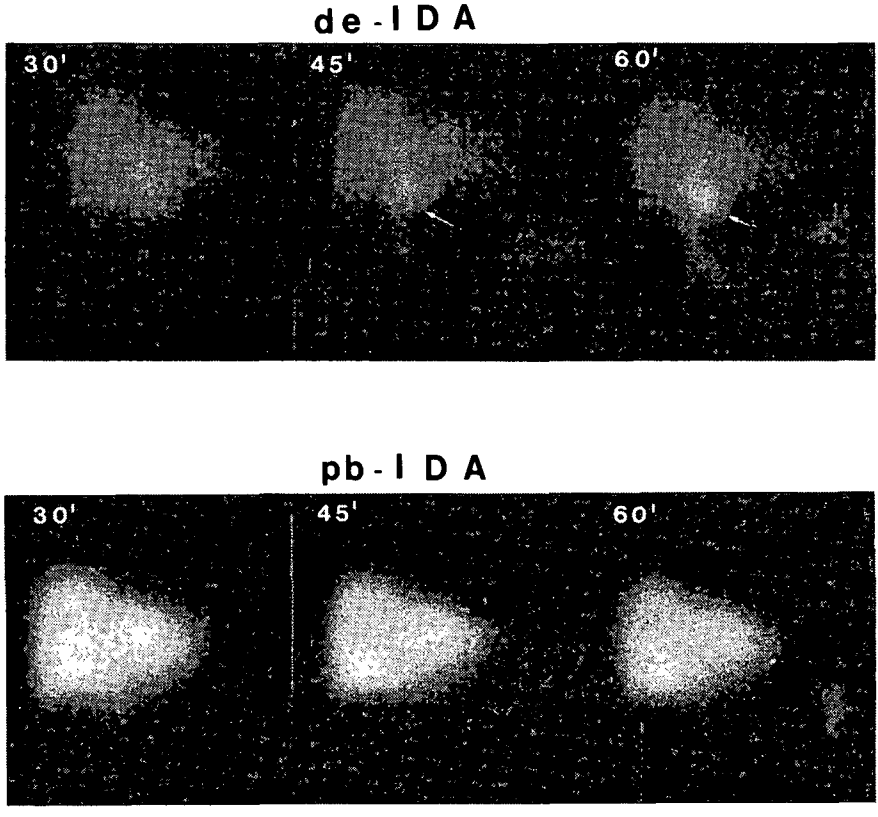

FIG.1. Scintifotos from a patient with stones in the bile duct associated with cystic duct obstruction (serum bilirubin 3.4 mg/dl), illustrating the absence of tracer in the gall bladder and pile-up of radioactivity at the porta hepatis. In comparison, depiction of the bile duct is faint and delayed on images after injection of pb-IDA.

to increase the diagnostic capabilities for investigations of patients with marked hyperbilirubinaemia.

From the kinetic data one can assume a multicompartment distribution of the radiopharmaceuticals. The blood clearance, which can be approximated as biexponential, showed only slight differences between de-IDA and pb-IDA. The distribution phase was slightly prolonged for pb-IDA, and the second slope was not significantly different for both substances. This would be in agreement with an assumption of a delayed liver uptake of pb-IDA, consistent with the findings observed.

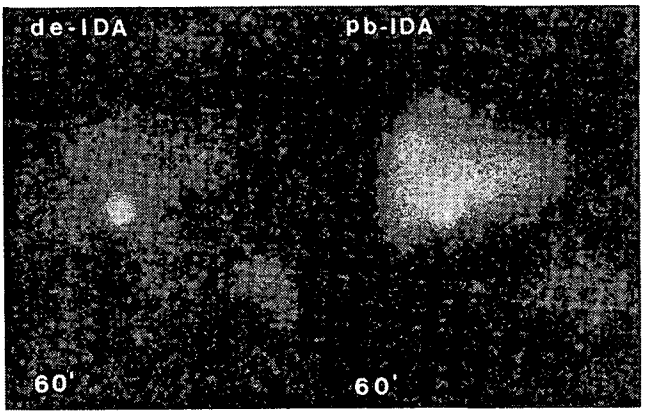

FIG.2. Comparison of images obtained using de-IDA and pb-IDA in a patient with cholestatic cirrhosis (serum bilirubin 10.6 mg/dl). Whereas there was a much greater activity in the liver at 60 min using pb-IDA, depiction of the gall bladder and visualization of intestinal activity was more pronounced using de-IDA.

In contrast to de-IDA, it has been shown that pb-IDA exhibits a high degree of protein binding [10]. This may possibly account for the low renal excretion of pb-IDA despite its slower hepatic uptake. The analogues of N-substituted IDA derivatives varied in their transit time through the liver and in the degree of renal excretion. Urinary excretion was significantly higher using de-IDA. However, there was no clear relation between the degree of hyperbilirubinaemia and the amount of the tracer excreted with the urine, but in patients with severe liver disease an altered renal function has to be considered [11].

Although the qualitative pattern of uptake of IDA derivatives is similar, considerable differences in turnover rates exist which might be due to molecular size, protein binding, type of lipophilicity, and position of substitution [5]. The slower liver uptake and elimination of pb-IDA, which is reflected by the uptake and retention index calculated, was also demonstrable on scintifotos (Figs 1–3). In agreement with animals studies, pb-IDA proved to exhibit relatively prominent hepatic uptake compared with de-IDA. But because of its longer hepatic transit time excretion was delayed. Gall bladder filling and visualization of activity within the intestine occurred later, and delineation of the biliary system was rarely successful.

In our study there were only minor differences between the results obtained with de-IDA and pb-IDA investigations, e.g. complete obstruction of the common bile duct was assumed using pb-IDA, and only incomplete obstruction with de-IDA,

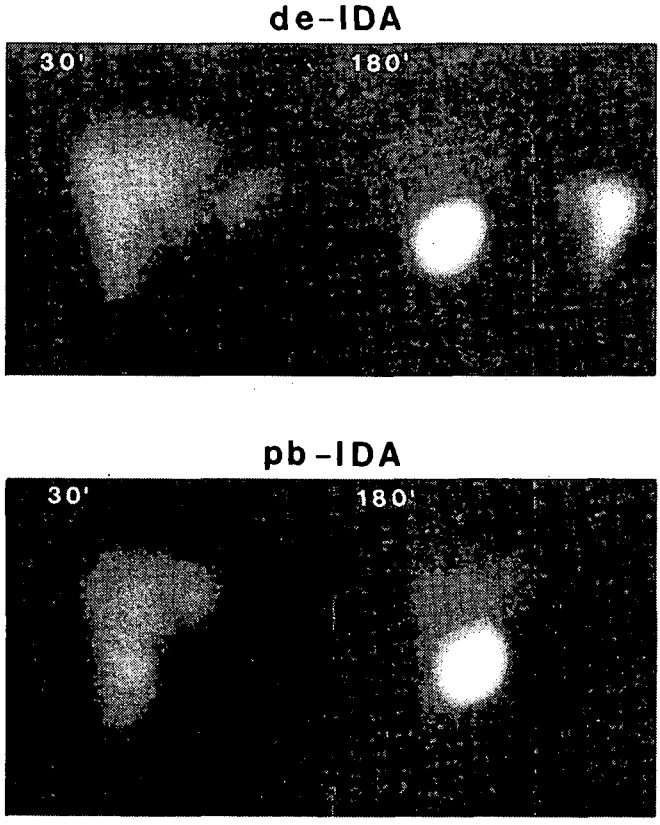

FIG.3. Sequential images of a patient with incomplete obstruction of the common bile duct from a stone (serum bilirubin 3 mg/dl). Gall bladder visualization can be demonstrated with either de-IDA or pb-IDA, whereas activity in the gut, and a faint delineation of the upper part of the bile duct, is only possible with de-IDA. This later finding would be consistent with the diagnosis of incomplete obstruction.

which was consistent with the diagnosis. The delayed imaging of the gall bladder and the biliary tract using pb-IDA may sometimes lead to misinterpretations of the scintigrams.

Our data indicate that de-IDA seems to be superior to pb-IDA in its diagnostic usefulness, also in patients with severe hyperbilirubinaemia. A virtue of de-IDA is its rapid appearance in the biliary tract. Since the primary value of $^{99}Tc^{m}$-labelled IDA derivatives is to evaluate the dynamic function, and not to visualize the liver, pb-IDA offers no diagnostic advantage for studying jaundiced patients.

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