Evaluation of ^{131}I (monoiodide) BSP for Clinical Studies*

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In 1925 Rosenthal and White[1] introduced a bromosulfophthalein (BSP) dye retention test as a sensitive indicator of liver function. Even now it is regarded as one of the most sensitive agents for the detection of non-icteric liver disease (liver cirrhosis, early stage of acute hepatitis and hepatic tumor). BSP accumulates in the liver cells, conjugates with glutathione and is excreted into the bile. Therefore, a disorder in its excretion is due to a disturbance of one of these processes. Since bilirubin and BSP compete for uptake by the liver and increased serum bilirubin interferes with the colorimetric determination of BSP, it has been considered that BSP test is inappropriate for the differential diagnosis of jaundice conditions. It has been generally said[3-5] that when jaundice is present, the BSP test is useless and should not be performed.

In 1955, Taplin et al.[6] labeled rose bengal, a dye similarly metabolized in the liver as BSP, with ^{131}I and measured the hepatic excreation of this dye by external monitoring. Later, Blahd et al.[7] applied this method to the determintion of the peripheral pool, succeeding in the diagnosis of chronic and subacute hepatic diseases without colorimetry.

In 1968, Yamada, Taplin et al.[8] suggested the possibility of differentiating so-called medical jaundice from surgical jaundice (Fig. 1) by scanning the subjects during 24 to 48 hours following intravenous injection of ^{131}I-labeled rose bengal.

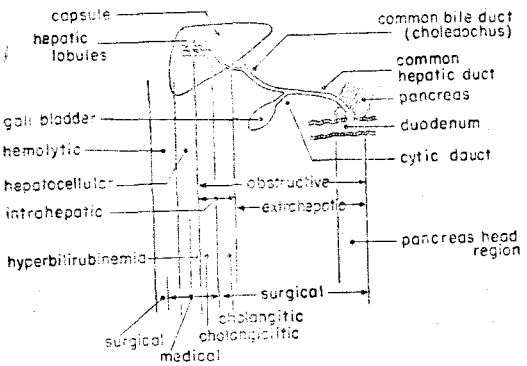

Fig.1 Definition of Jaundices

As mentioned before, many authorities hold the opinion that BSP is not proper for the differential diagnosis of jaundice states. Some have tried to diagnose biliary tract obstruction by a malignant tumor by measuring BSP excretion into duodenal fluid[9] and others by quantitating changes in serum levels of conjugated and free BSP[10]. Furthermore, Burton et al. reported that in patients with extrahepatic obstructive jaundice, BSP retention was observed for 24 days after its administration. From a consideration of all these findings we came to

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a conclusion that the differential diagnosis of various jaundice states, (medical, surgical and constitutional) is possible by sequential scanning with radioisotope-labeled BSP, as with rose bengal, in accordance with procedures described by Yamada, Taplin et al[8]. The evidence suggested that labeled BSP might make a more important contribution than rose bengal.

Material and method

^{131}I-BSP monoiodide and diiodide were experimentally prepared by Dainabot Radioisotope Laboratories by a modification of the procedure introduced by Tubis and Nordyke[12] in 1961. The position of the label was determined by nuclear magnetic resonance at the Institute of Applied Microbiology, University of Tokyo. The specific activity was 300 uCi/mg.

The stability of ^{131}I-BSP during storage and in serum was investigated and chromatographic analysis with various developing solvents was performed.

A does of 2.5 uCi of ^{131}I-BSP was intravenously injected into 24 rats, and three animals were sacrificed 20 and 40 minutes, 2, 6 and 24 hours, and 8 days after the injection respectively to measure the distribution of radioactivity to the liver, kidney, intestinal tract, and remaining carcass.

A cannula was inserted into the biliary tract of six dogs, collecting bile every 15 minutes following an intravenous injection of 1.0~1.9 mCi of ^{131}I-BSP. Biliary excretion of BSP was evaluated with respect to time of appearance and metabolic products.

In order to study metabolism in man, 350 uCi (1.64~7.34 mg) of ^{131}I-BSP was intravenously injected into 110 subjects. Blood samples were collected at 5, 7, 9, 12, 15, 30, 45, 60 and 90 minutes and 3, 5 to 6 and 24 hours after injection. Scanning was performed to measure disappearance from the blood and distribution in the body. In some cases, 5 mg/kg of cold BSP was then intravenously injected and blood samples were collected in order to study metabolism.

In the same study, ^{35}S-BSP (20 uCi), the chemical structure of which is the same as BSP, was mixed with non-radioactive BSP and was intravenously injected in the doses of 0.1 mg/kg, 1.0 mg/kg, 2.5 mg/kg and 5.0 mg/kg respectively (33 subjects). Blood clearance was measured with a liquid scintillation counter and compared with that of ^{131}I-BSP in tracer doses.

Results

1. Chemical Structure of ^{131}I-BSP (monoiodide and diiodide)

Two labeled preparations were made by Dainabot Radioisotope Laboratories. The first lot was prepared according to the method by M. Jirsa et al[3]. by reacting ICl (8.5 mol.) with BSP. The second lot was prepared by reacting KI, KIO and HCl with BSP.

Both preparations were analysed by nuclear magnetic resonance and elementary analysis.

The first preparation was found to be BSP diiodide ($C_{20}H_8O_{10}S_2Br_4I_2$) as is shown in Fig. 2 a. The second preparation was found to be BSP monoiodide ($C_{20}H_9O_{10}S_2Br_4I$) which contains one iodine in one side of the phenyl group (Fig. 2b).

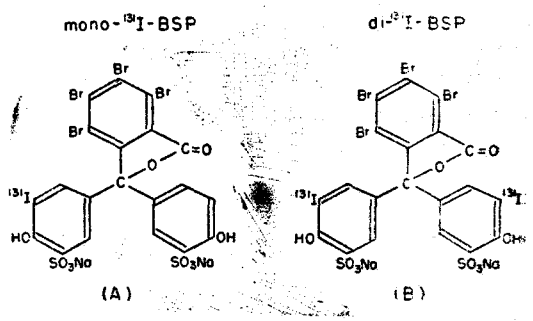

Fig. 2.

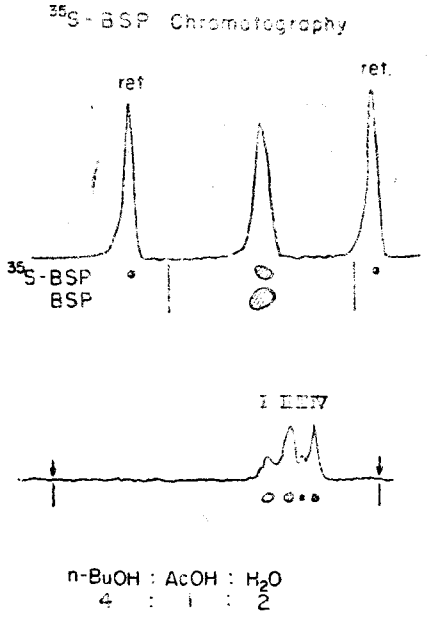

Fig. 3.

The latter is available now as BSP-131 commercially. More detailed analysis was performed on the BSP-monoiodide.

2. Stability

^{131}I-BSP preparations made by Dainabot Radioisotope Laboratories demonstrated a constant Rf value, without separation of free iodine, even after four weeks' storage in a refrigerator. There was no evidence of metabolic degradation in serum samples following injection. This is an important point of superiority when compared with the instability of other preparations which the authors have previously encountered. Such stability is required to make the compound clinically useful.

3. Chemical investigations on ^{131}I-BSP

Rf values for ^{131}I-BSP (monoiodide) using various solvents are summarized in Table 1. A system consisting of butanol, acetic acid and water (4 : 2 : 1, or 1.48 : 1 : 0.37) was excellent for sharp separation of $Na^{131}I$ and ^{131}I-BSP.

As expected, ^{35}S-BSP prepared by synthesis showed the same Rf value as that of BSP (Fig. 3). ^{131}I-BSP (monoiodide Rf 0.77) exhibited a different peak from that of BSP. The influence of this difference on metabolism was studied when ^{131}I-BSP was compared biologically with BSP and ^{35}S-BSP.

Table 1. Chromatographies of ^{131}I BSP

Solvent System (Parts)	Rf	
	BSP	NaI
n-BuOH : H_2O : Acetic Acid		
1.48 : 1 : 0.37	0.85	0.39
50 : 47 : 3	0.83	0.51
4 : 2 : 1	0.80	0.32
tert-BuOH : H_2O		
17 : 1	0.98*	0.78
n-Propanol : Acetic Acid : H_2O		
10 : 1 : 5	0.97*	0.71

Table 2. Clearance of ^{131}I and ^{35}S Labeled BSP

	Dose	# of Exam.	Average	Range
^{131}I-BSP	0.66~2.0mg /whole body	7	0.139	0.099~0.193
^{35}S-BSP	0.1 mg/kg	14	0.222	0.140~0.256
	1.0	5	0.162	0.144~0.173
	2.5	6	0.136	0.107~0.173
	5.0	8	0.098	0.075~0.154

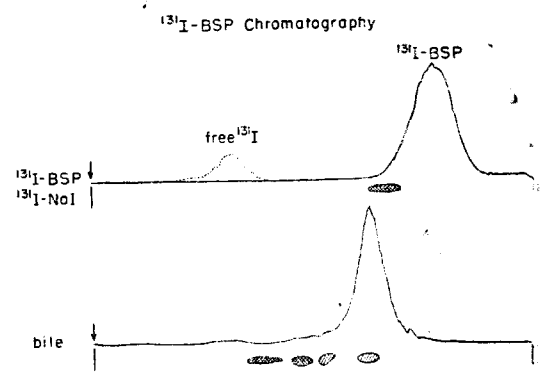

Fig. 4.

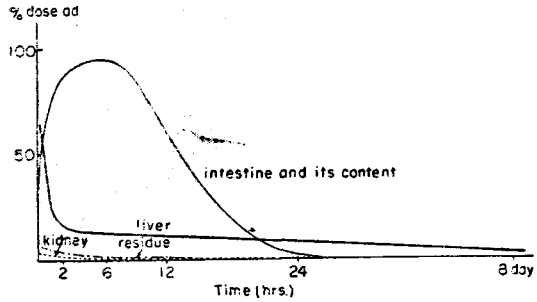

Fig. 5. Distribution of ^{131}I BSP in rat

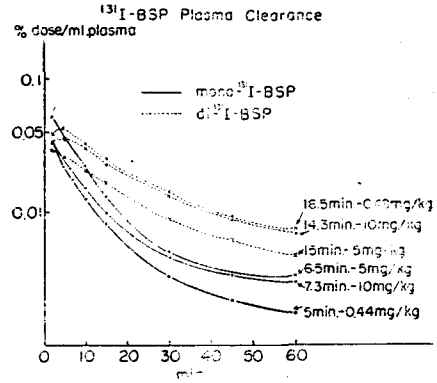

Fig. 6.

4. Biological study of ^{131}I-BSP

a. Intracorporeal distribution in the rat

As indicated in Fig. 5, ^{131}I-BSP(monoiodide) possesses a very strong affinity for the liver, as does BSP. ^{131}I-BSP is quickly taken up by the liver and excretion into the intestine through the biliary tract reaches its peak in six hours, being almost complete in 24 hours. In the liver, several percent of ^{131}I-BSP administered remains even eight days after injection. This may be due either to the storage of BSP or to hepato-intestinal circulation, or to both. However, the contribution of hepato-intestinal circulation is considered to be negligible because of the very small dose adminstered.

b. Metabolism in the canine liver

After an intravenous injection in dogs of 0.44, 5 and 10 mg/kg doses of ^{131}I-BSP (monoiodide and diiodide), and a 5 mg/kg dose of ^{35}S-BSP, blood and bile were collected and BSP clearance and excretion were studied.

BSP monoiodide was cleared more rapidly

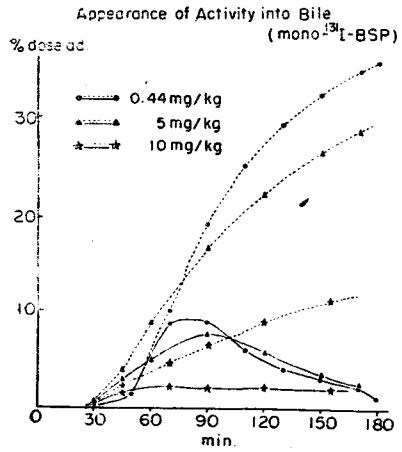

Fig. 7. a

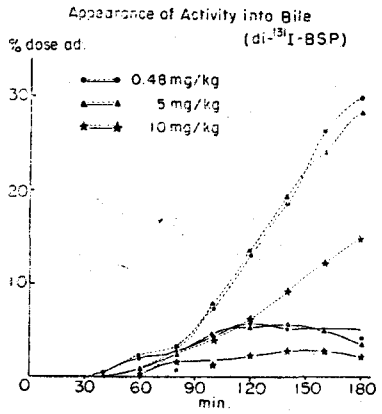

Fig. 7. b

than the equivalent dose of BSP diiodide (Fig. 6) and appearance in the bile was also more rapid than the diiodide (Figs. 7a, 7b).

^{131}I-BSP appeared in the bile within 15 minutes after intravenous injection, and secretion steadily increased for 60 to 90 minutes. The half times of blood clearance of a tracer dose of monoiodide and diiodide were six minutes and 10 minutes respectively. Biles thus obtained were evaluated chromatographically. As shown in Fig. 3, ^{35}S-BSP in dog bile revealed four bands, I, II,, III and IV, indicating the presence of three conjugated BSP's and one free BSP. Colors developed and the energy radioactive peak of these bands exactly corresponded with those of BSP, indicating identical metabolic behavior for ^{35}S-BSP. On the other hand, ^{131}I-BSP(mono) exhibited only one peak, as shown in Fig. 4, instead of the four divided peaks obtained with ^{35}S-BSP. No separation of ^{131}I during transport through the liver was observed. ^{131}I-BSP(diiodide) also showed no appreciable conjugate formation.

c. Metabolism of ^{131}I-BSP in man

Doses ranging from 0.66 mg to 2.0 mg (200 uCi to 400 uCi) of ^{131}I-BSP were intravenously injected into healthy subjects, and blood clearance and excretion by the liver were determined. As shown in Table 2, the disappearance

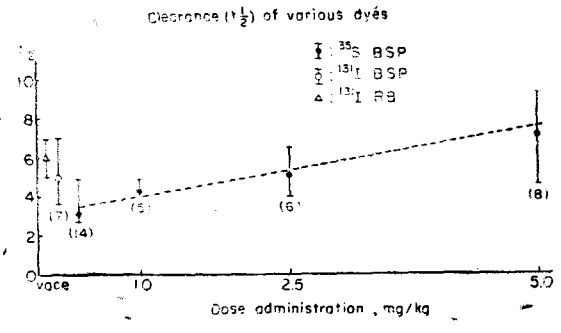

Fig. 8.

constant $k(0.693/t_{1/2})$ was 0.099~0.193(0.139 average). This was significantly lower than the 0.222 value for ^{35}S-BSP at a dose of 0.1 mg/kg and the 0.162 value at a dose of 1.0mg/kg. The blood clearance of ^{131}I-BSP is fairly rapid, yet it is significantly slower than that of BSP (^{35}S-BSP), demonstrating clearly a difference in metabolism between these two dyes. In a comparison with ^{131}I rose bengal, the clearance of ^{131}I-BSP was always faster, as shown in Fig. 8.

Discussion

^{131}I-BSP monoiodide, which was studied in depth in this investigation, is a new dye with a somewhat different metabolic conduct than BSP, ^{35}S-BSP, ^{131}I-BSP diiodide or ^{131}I rose bengal.

The method of labeling BSP with ^{131}I was first introduced by Tubis in 1961[2]. However, the position of the label, the content of ^{131}I and the chemical structure were not fully investigated. In 1966, Jirsa et al.[13] assumed that the preparation was a diiodide with iodine atoms at positions ortho to the phenol-OH groups. We have verified this study using nuclear magnetic resonance determinations and elementary analysis and have found that the preparation prepared by their method is indeed BSP diiodide. Wood et al.[14] also prepared the diiodide (BSPI) for liver scintigraphy and reported that the $t_{1/2}$ for retention by the liver of the diiodide was as long as eight minutes. They claimed that diiodide BSP is adequate for liver scanning because it stays in the liver for a longer period than does ^{131}I-BSP. This property of the diiodide was confirmed in the present study.

However, the newly specifically prepared ^{131}I monoiodide BSP(BSP-131) showed a different chemical and biological behavior than ^{131}I-diiodide BSP. The Rf value is different from that of ^{131}I-BSP diiodide. It is cleared and excreted from

the blood through the liver into the intestine faster than ^{131}I-BSP diiodide and ^{131}I-RB. In a study in patients with constitutional hyperbilirubinemia, ^{131}I-BSP monoiodide showed a different metabolic pattern than ^{131}I-RB, and served for a differential diagnosis[15].

The rapid clearance and excretion of ^{131}I-BSP (mono) were found to be useful in the differential diagnosis of medical and surgical jaundice states and in the detailed study of extrahepatic obstructive jaundice states.

Using the property of slower clearance possessed by ^{131}I-BSP (mono), a tracer dose clearance test was found to be useful for the study of liver cell function[17]. It should be stressed that loading with even a tracer dose of this dye represents liver cell function and not hepatic blood flow. This provides the possibility of a simple retention test with negligible BSP loading and possibly obliterates the problem of BSP allergy or shock. Such reactions are relatively frequent in Japanese patients. The advantages of ^{131}I-BSP(monoiodide) for sequential scanning and retention studies are to be published.

Conclusion

^{131}I labeled monoiodide BSP was prepared. Its structure was confirmed by nuclear magnetic resonance determinations and by elementary analysis. For comparison, ^{131}I-diiodinated BSP, ^{131}I-rose bengal, ^{35}S-BSP and BSP were used. ^{131} I-BSP (mono) retained stability during storage and hepatic transport into bile. No radioisotope was freed from the preparation during these processes. ^{131}I monoiodide BSP showed slower blood clearance than ^{35}S-BSP and BSP. However, the monoiodide was cleared and excreted from the blood through the liver into the bile more rapidly than ^{131}I-diiodinated BSP and ^{131}I-RB. ^{131}I monoiodide BSP was found useful in sequential scanning for the differential diagnosis of medical, surgical and constitutional hyperbilirubinemia and in simplified retention testing. The advantages of this dye are:

1. BSP is a dye well evaluated in the last several decades; 2. becase of its radioactive label the mechanisms of liver uptake and excretion are easily ascertained; 3. a retention test is easily performed with tracer dose loading, even in cases with jaundice.

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