Studies on the Excretory Function of the Liver: A Comparison between Indocyanine green and Sulfobromphthalein sodium

Minoru MIZUTA and Kenzo NAGAI

First Division, Department of Internal Medicine, Yamaguchi University School of Medicine, Ube, Japan

(Received April 14, 1970)

The sulfobromphthalein (BSP) retention test and indocyanine green (ICG) clearance test have been widely used as clinical measurements of the liver excretory function. Some physiological differences were pointed out between both compounds in the steps involved in the movement of dyes from blood to bile following intravenous administration.

It was reported that ICG, when compared with BSP, is less bound to serum albumin[1], is uptaken by the liver more easily[2], is stored in the liver more abundantly[3], is excreted into bile in larger amounts[4][5], is transfered less into the extra-hepatic tissues[4][6][7], is excreted at a lower maximal biliary transport rate[3], is transfered less into the hepatic lymph[6][7], and has no metabolite[4][7].

Schmid and his co-workers[8] demonstrated that sodium salicylate inhibited the bilirubin-binding affinity of plasma albumin, resulting in a distinct decrease of hyperbilirubinemia. In our previous paper[9] a similar mechanism has been demonstrated in the case of biliary excretion of BSP following administration of sodium salicylate. Thus, sodium salicylate is a very important tool for clarifying the mechanism of biliary excretion of dyes, especially at the site where dye binding of plasma protein occurs.

The present studies were undertaken to examine the physiological differences between BSP and ICG, applying the effect of sodium salicylate on the dye-binding affinity of serum albumin.

METHODS

1. Estimation of the maximal biliary transport rate and the hepatic storage of the dye

A polyvinyl tube, 0.62 mm in diameter, was inserted into the common bile duct of adult male Wistar rats anesthetized by the intraperitoneal administration of 10 mg sodium thiopentalate. Using a constant infusion pump, BSP or ICG in normal saline solution was infused intravenously for one hour at a constant rate of 5 to

20 mg of BSP or 0.2 to 3 mg of ICG per hour per 100 gm of body weight.

The maximal biliary transport of BSP or ICG was estimated from the bile obtained 50 to 60 minutes following the beginning of infusion.

The rats were sacrificed through cardiac puncture 60 minutes after the start of the infusion, blood was obtained for determination of serum dye concentration, and the livers were removed for determination of their dye content.

2. Biliary excretion of the dye following a single intravenous administration

A polyvinyl tube, 0.62 mm in diameter, was inserted into the common bile duct of anesthetized rats. Either 10 mg BSP or 1 mg ICG per 100 gm of body weight was administered intravenously to the rats and bile was collected for one hour. Prior to the administration of the compound, 6 mg of sodium salicylate per 100 gm of body weight was administered intravenously. The amount of the compound excreted in the bile was determined spectrophotometrically and its percentage to the total amount injected was calculated.

3. Estimation of BSP

1) Concentration of BSP in serum was estimated by Gaebler's method[10].

2) For analyzing the BSP in bile, samples were made alkaline with 10 ml of 0.1 N KOH and diluted 1,000 or 2,000 times with distilled water. Then the concentration of BSP in these diluted samples was determined by the same method that was used for the determination of serum BSP.

3) For analyzing BSP in the liver, triplicated samples of liver weighing approximately 1 gm each were homogenized with 2 ml of ice cold acetone in a homogenizer with a glass pestle. Three milliliters of 80 per cent acetone were added to the contents of each tube and the contents were then transfered to a glass-stoppered conical centrifuge tube. The contents were thoroughly mixed and the tube was centrifuged for 10 minutes at 45,000 rpm. The supernate was removed and the precipitate was extracted twice with 80 per cent acetone. The supernates were pooled and the volume recorded. Duplicated aliquots of the combined acetone extract were mixed with 0.1 N KOH to a final volume of 4.15 ml, and the optical density was determined using 0.1 N KOH as a blank in a spectrophotometer first at 580 mμ and then at 620 mμ. The optical densities of 150 μl of BSP standards mixed with 4 ml of 0.1 N KOH were similarly determined. The alkalinized acetone extracts exhibited varying amounts of turbidity. The optical density at 580 mμ of similarly turbid alkalinized acetone extract of homogenate averaged 1.20 times the optical density at 620 mμ. Thus BSP extracted per gram of liver wet weight was calculated as follows:

$$\frac{\text{O.D. } 580 - 1.2 \times \text{O.D. } 620 \text{ unknown}}{\text{O.D. } 580 - 1.2 \times \text{O.D. } 620 \text{ standard}} \times \frac{\text{Volume of standard } (150\mu\text{l}) \text{ in } 4.15 \text{ ml}}{\text{Volume of unknown (in } \mu\text{l}) \text{ in } 4.15 \text{ ml}} \times$$

Concentration of standard in mg per 100 ml $\times$
Volume of acetone extract in fractions of 100 ml $\div$
weight of liver sample in grams

The data were expressed as mg of BSP contained per gm of liver wet weight per 100 gm of body weight. Since recovery of unknown amounts of BSP from the liver averaged 70 per cent, the above values were multiplied by 100/70.

4. Estimation of ICG

1) One milliliter of serum sample was diluted with 2 ml of normal saline solution, the optical density at 805 mμ was read spectrophotometrically and the concentration of ICG in the serum was estimated. The standard curve was made separately, diluting ICG in an albumin solution.

2) Concentration of ICG in bile was estiamted by using the same method as described by Wheeler and co-workers.[4]

3) For analyzing ICG in the liver, the extraction was undertaken through the same method as in the case of BSP extraction from the liver. The optical density of the acetone extract of the liver thus obtained was read spectrophotometrically at 780 mμ and the concentration of ICG in the liver was estimated. The turbidity which was exhibited in the acetone extract was corrected in the same manner as in the case of BSP estimation. Since recovery of unknown amounts of ICG from the liver averaged 90 per cent, the above values were multiplied by 100/90.

5. Dye-binding affinity of serum albumin

One hundred milligrams of crystalline bovine serum albumin was dissolved in a 100 ml solution of 1/15 M, pH 7.4 phosphate buffer.

Triplicated equilibrium dialysis was performed by a method similar to that described by Klotz and his co-workers[11]. Cellophane dialysis bags (20/32″, Visking Co.) were filled with 10 ml of the bovine serum albumin solution. Each bag contained 50 ml of BSP or 50 ml of ICG (both being 10 mg per 100 ml) and the bags were suspended in a solution of sodium salicylate. The sodium salicylate solution was of two different strengths, one being 100 mg of sodium salicylate in 1/15 M, pH 7.4 phosphate buffer and the stronger solution being 500 mg of sodium salicylate in 1/15 M, pH 7.4 phosphate buffer.

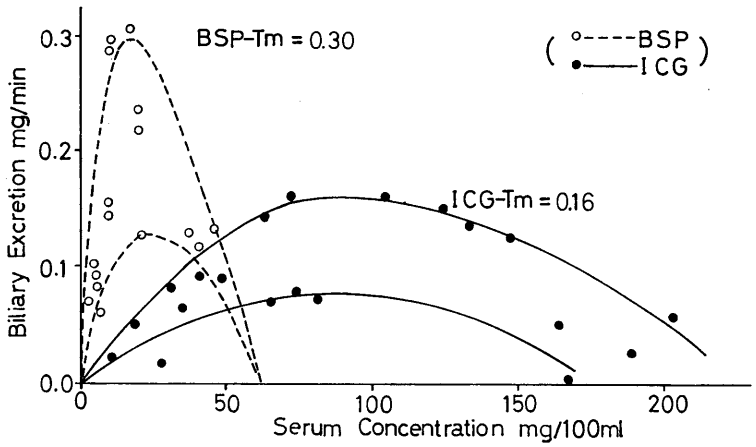
Dialysis was carried out for 17 hours at 4°C with the tubes being shaken constantly by means of a motor-driven shaker. The concentration of dye in the contents of the bags and in the outside buffer solution was determined at the end of the dialysis period. The concentration of protein-bound dye in the bags was calculated by substracting the value of dye concentration in the outside solution from the dye concentration in the solution in the bag.

RESULTS

1. Maximal biliary transport rate of the dye

The relationship between serum concentration of the dye and biliary excretion of the dye is shown in Fig. 1. Biliary excretion of BSP per minute reached a

Fig. 1. Relationship between Serum Dye Concentration and Biliary Dye Excretion



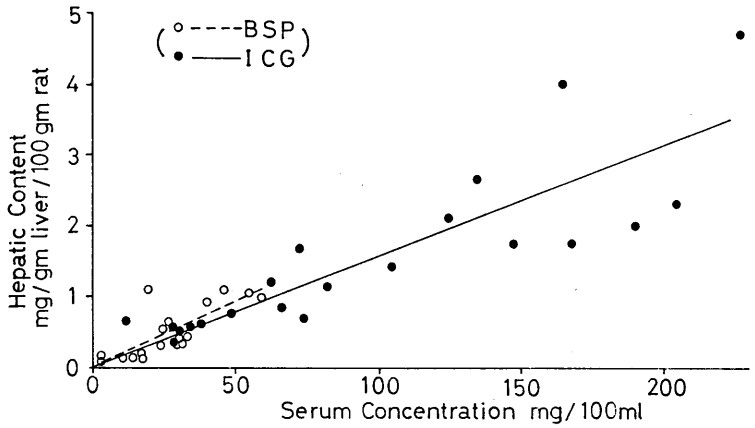
maximum at the serum BSP level of about 20 mg per 100 ml, then it decreased rapidly as the serum BSP level was increased by the administration of larger amounts of BSP. When the maximal point of biliary excretion of BSP was assumed to be the maximal transport rate of BSP into the bile (biliary BSP-Tm), it was estimated to be 0.30 mg per minute.

Biliary excretion of ICG per minute reached a maximum at the serum ICG level of about 100 mg per 100 ml, then it decreased gradually as the serum ICG level was increased by the administration of larger amounts of ICG. The maximal biliary transport rate of ICG was estimated to be 0.16 mg per minute.

2. Hepatic storage of the dye

The relationship between serum concentration of the dye and hepatic content of the dye is shown in Fig. 2. Hepatic content of both BSP and ICG was

Fig. 2. Relationship between Serum Dye and Hepatic Dye Concentrations

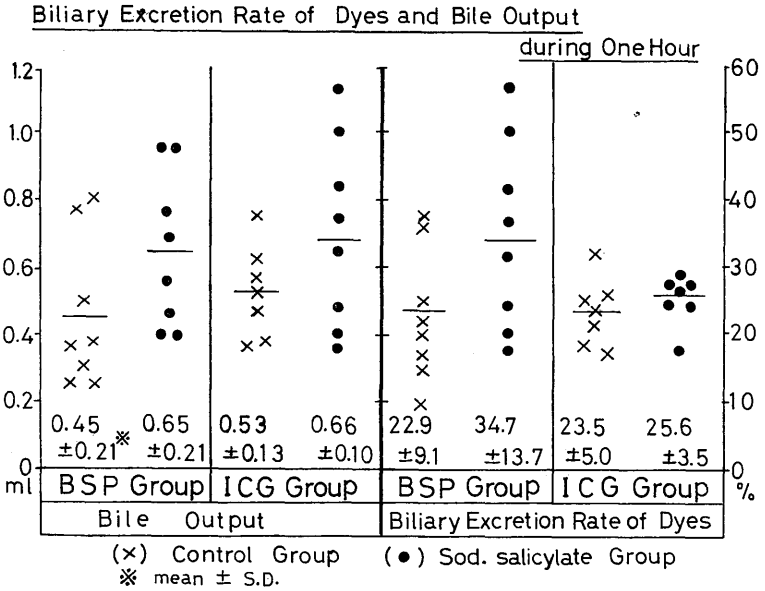


almost directly proportional to the serum concentration and the proportion in both dyes was nearly equal.

3. Biliary excretion of the dye and influence of sodium salicylate thereupon

As shown in Fig. 3, the volume of bile output during one hour in the control rats of the BSP group was $0.45 \pm$ S.D. 0.21 ml and it was increased slightly to 0.65 ± 0.21 ml by the administration of sodium salicylate. The volume of bile output in the control rats of the ICG group was 0.53 ± 0.13 ml and it was increased slightly to 0.66 ± 0.10 ml by sodium salicylate.

Fig. 3.



Biliary excretion rate of BSP during one hour was 22.9 ± 9.1 per cent in the control rats and increased to 34.7 ± 13.7 per cent in the rats treated with sodium salicylate. Biliary excretion rate of ICG was 23.5 ± 5.0 per cent in the control rats and remained at 25.6 ± 3.5 per cent in the rats treated with sodium salicylate.

4. Dye-binding affinity of serum albumin

As shown in Tab. 1, BSP was bound to crystalline bovine serum albumin at the rate of $0.147 \pm$ S.D. 0.004 mg per 1 mg, but ICG was bound to it only at the rate of 0.013 ± 0.001 mg per 1 mg.

The binding affinity of 1 mg of the serum albumin to BSP was reduced to 0.135 ± 0.002 mg in the solution of sodium salicylate of 100 mg per 100 ml, and further reduced to 0.123 ± 0.003 mg in the solution of sodium salicylate of 500 mg per 100 ml.

The binding affinity of 1 mg of crystalline bovine serum albumin to ICG was reduced to 0.007 ± 0.0003 mg in the sodium salicylate solution of 100 mg per

100 ml and was further reduced to 0.002 ± 0.0003 mg in the sodium salicylate solution of 500 mg per 100 ml.

Tab. 1. Dye-binding affinity of crystalline bovine serum albumin

	Compounds (mg) per 1 mg of crystalline bovine serum albumin
BSP in a solution without sodium salicylate	0.147 ± S.D. 0.004
BSP in a solution of sodium salicylate, 100 mg/100 ml	0.135 ± 0.002
BSP in a solution of sodium salicylate, 500 mg/100 ml	0.123 ± 0.003
ICG in a solution without sodium salicylate	0.013 ± 0.001
ICG in a solution of sodium salicylate, 100 mg/100 ml	0.007 ± 0.0003
ICG in a solution of sodium salicylate, 500 mg/100 ml	0.002 ± 0.0003

DISCUSSION

The results that ICG is less bound to serum albumin and excreted at a lower maximal biliary transport rate than BSP were reconciled with facts reported by other authors[1)3)]. Concerning the biliary excretion of the compounds in a limited period, our results could not be compared with the results described by some authors[4)5)], because the amount injected into the experimental animals was different in each case. The hepatic storage of both BSP and ICG was nearly equal. This finding is contrary to that reported by other authors[3)]. The difference between our results and those reported by others can not be discussed here because experimental conditions were too dissimilar for comparison and discussion. Furthermore, our values for BSP-Tm in rats were on the low side. This might be due to a strain difference in rats and differeme in the method of Tm estimation.

In our previous paper[9)], it was reported that sodium salicylate slightly increases the volume of bile output and inhibits the BSP-binding affinity of plasma albumin, resulting in an increase of biliary excretion of BSP. The results obtained in this study have confirmed our previous findings. In comparison to the BSP experiments, an increase of biliary excretion of ICG was not revealed by the administration of sodium salicylate.

For the hepatie uptake of ICG from the blood stream it may be of great advantage that the compound is loosely bound to serum albumin[12)]. Furthermore, it may be beneficial to the biliary excretion, when it is assumed that a similar type of relationship as was seen between ICG and the serum protein exists between ICG and the liver soluble protein.

It may be considered that the effect of sodium salicylate on the dye-binding affinity of serum albumin is insignificant in the steps of biliary excretion of ICG,

because the compound is originally loosely bound to serum albumin.

Thus it was concluded that the problem of the dye-binding affinity of serum albumin is very important for the biliary excretion of BSP and not so important for the excretion of ICG.

In the constant BSP infusion experiments, the biliary excretion of BSP per minute reached a maximum at the serum BSP level of about 20 mg per 100 ml, then it decreased rapidly as the serum BSP level was increased by the administration of larger amounts of BSP. This depressive phenomenon in biliary excretion is incompatible with the theory on the maximal transport rate. As a result it was then assumed that this depressive phenomenon may be brought about by either of the two following means; (A) by some mechanism which resulted from an excess of BSP or (B) by toxicity of BSP itself.

The biliary excretion of ICG per minute reached a maximum at the serum ICG level of about 100 mg per 100 ml, then it decreased gradually as the serum ICG level was increased. Thus it was concluded that ICG is not toxic or else overdosage of ICG has minimal depressive excretory effects.

CONCLUSION

Comparative studies between indocyanine green (ICG) and sulfobromphthalein sodium (BSP) in the steps involved in the movement of compounds from blood to bile following their intravenous administration were carried out in rat experiments.

1) The maximal transport rate of ICG into the rat bile was less than that of BSP.

2) A distinct depression of biliary BSP excretion was induced by an excess dosage of BSP, but this phenomenon was not pronounced in the case of ICG.

3) The hepatic storage of both compounds was nearly equal.

4) Biliary excretion of BSP was increased by the intravenous administration of sodium salicylate, while that of ICG was not influenced by the same treatment.

5) The volume of bile output in both BSP and ICG groups was increased slightly by the intravenous administration of sodium salicylate.

6) Far less ICG was bound to bovine serum albumin than BSP in vitro.

7) Binding affinity of bovine serum albumin to both compounds was reduced by the addition of sodium salicylate.

8) It was confirmed that the binding affinity of serum protein is an important factor in the steps involved in the movement of BSP from blood to bile, but it is not important in the case of ICG.

REFERENCES

1) Baker, K.J.: Binding of sulfobromophthalein (BSP) sodium and indocyanine green (ICG) by plasma α_1 lipoproteins. *Proc. Soc. Exp. Biol. Med.*, **122**: 957, 1966.

2) Leevy, C. M.: Dye extraction by the liver. *Progress in Liver Disease*, **1**: 174, 1961, Grune & Stratton, N. Y.
3) Nanbu, K.: Hepatic clearance of indocyanine green in liver diseases. *Jap. J. Gastroent.*, **63**: 777, 1966.
4) Wheeler, H. O., Cranston, W. I. & Meltzer, J. I.: Hepatic uptake and biliary excretion of indocyanine green in the dog. *Proc. Soc. Exp. Biol. Med.*, **99**: 11, 1958.
5) Rapaport, E., Ketterer, S. G. & Wiegand, B. D.: Hepatic clearance of indocyanine green. *Clin. Res.*, **7**: 289, 1959.
6) Hunton, D. B., Bollman, J. L. & Hoffman, H. N.: Studies of hepatic function with indocyanine green. *Gastroent.*, **39**: 713, 1960.
7) Cherrick, G. R., Stein, S. W., Leevy, C. M. & Davidson, C. S.: Indocyanine green: Observation on its physical properties, plasma decay, and hepatic extraction. *J. Clin. Invest.*, **39**: 592, 1960.
8) Schmid, R., Diamond, I., Hammaker, L. & Gundersen, C. B.: Interaction of bilirubin with albumin. *Nature*, **206**: 104, 1965.
9) Mizuta, M., Murata, K., Nagai, K. & Tamura, K.: Effects of certain drugs on the mechanism of biliary excretion of sulfobromphthalein: Sodium dehydrocholate, sodium hippurate, sodium salicylate and liver hydrolysate. *Bull. Yamaguchi Med. School*, **15**: 113, 1968.
10) Gaebler, O. H.: Determination of bromsulphalein in normal, turbid, hemolyzed or icteric serums. *Am. J. Clin. Path.*, **15**: 452, 1945.
11) Klotz, I. M., Walker, F. M. & Pivan, R. B.: The binding of organic ions by proteins. *Am. Chem. Soc.*, **68**: 1486, 1946.
12) Brauer, R. W. & Pessotti, R. L.: The removal of bromsulphthalein from blood plasma by the liver of the rat. *J. Pharmacol. Exptl. Therap.*, **97**: 358, 1947.