Hiroshima J. Med. Sci.
Vol.39, No.1, 11~14, March, 1990
HIJM 39-3

Effects of ATP Depletion with DL-Ethionine on Biliary Excretion of Indocyanine Green in the Rat

Yoshiki SHIBA and Yoshinobu KANNO

Department of Oral Physiology, Hiroshima University School of Dentistry, 2-3 Kasumi 1 Chome, Minami-Ku, Hiroshima 734, Japan

ABSTRACT

To clarify the energy-dependency of biliary excretion of a diagnostic dye, the effect of ATP depletion on biliary excretion was investigated. Biliary excretion of indocyanine green (ICG) in rat liver reached a maximum 20 min after intravenous application. The level of ATP in the liver was lowered to 35.4% of the control value about 5 h after administration of DL-ethionine (100 mg/100 g body weight). The rate of bile flow and the amount of ICG excreted in initial 10 min into the bile of ethionine-treated rats were lowered to 67.5% and 61.3% of the control values, respectively. The amount of ICG excreted for 10 min from 30 min to 40 min after application of ICG in ethionine-treated rats was comparable to that of the control. The level of ICG in blood 5 min after application of ICG was raised by 59.2% of the control value. These results suggest that ATP depletion in the liver caused by treatment with ethionine suppresses the initial rate of uptake and biliary excretion of ICG.

Key words: *ATP depletion, Biliary excretion, Liver*

The liver takes up exogenous substances in the blood and excretes them into the bile[6,14]. There are three key features of this process: the first is the uptake across the sinusoidal membrane; the second is the intracellular transport from the sinusoidal side to the biliary side; and the third is the excretion across the biliary membrane. Uptake across the sinusoidal membrane is carried out in different mechanisms for anions, cations, and neutral substances in the liver by the carrier transport system[3,6,14]. A specific protein in hepatocytes is thought to play a major role in this intracellular transport[8]. Biliary excretion may also be the result of active transport[1]. However, it is still unclear which is the most energy-dependent process in the biliary excretion of anions.

The study of induced changes in energy metabolism *in vivo* is difficult, but DL-ethionine is a useful drug for such studies in the liver[4]. The concentration of sulfobromophthalein (BSP) excreted into the bile is not reduced by treatment of rats with ethionine[11]. This result is inconsistent with the hypothesis that uptake and excretion are both active processes. The effect of ATP depletion on the biliary excretion of another diagnostic dye might be different since there are differences between the excretion of BSP and that of other diagnostic dyes such as indocyanine green (ICG)[5]. Indocyanine green is an organic anion and is used as a dye in the diagnosis of hepatic disease[16]. This acid dye easily binds to albumin and to other macromolecules in blood and is taken up by the liver[2]. However, the effect of ATP depletion on the total excretion of dye from the liver is as yet unknown. In this study, we examined the effect of ATP depletion induced by DL-ethionine on the uptake and excretion of ICG.

MATERIALS AND METHODS

Female Donryu rats (140 - 190 g) were anesthetized with pentobarbital (4 mg/100 g body weight) which was injected intraperitoneally and warmed by lamp to keep the body temperature in constant through the experimental period. Indocyanine green (ICG, 0.1 mg/100 g body weight; Daiichi Seiyaku Co., Tokyo, Japan), dissolved in physiological saline, was injected into the female vein about 1 h after injection of the anesthetic DL-Ethionine (100 mg/100 g body weight, Katayama Chemical Industries Co., Osaka, Japan) was injected intraperitoneally 4 h before injection of anesthetic. The levels of ATP in the liver were determined by assays with hexokinase and glucose-6-phosphate dehydrogenase[7].

Fine polyethylene tubing was inserted into the common bile duct prior to injection of ICG. Bile was collected at intervals of 10 min and bile volume was measured by micropipette. A blood sample was obtained from the portal vein 5 min after injection of ICG. Measurements of the concentration of ICG in bile and blood were carried out with a spectrophotometer at 805 nm.

RESULTS

Biliary excretion of ICG in the control rats was observed a short time after injection, and the amount and concentration of ICG excreted into the bile reached a maximum 10-20 min after application of ICG (Figs. 1 and 2). In controls, bile flow before injection of ICG was 131.1 ± 2.6 μl/10 min (mean ± standard error, n=5) and did not change during the experiment, regardless of the application of ICG.

Treatment with ethionine decreased the amount of ICG excreted into the bile just after application of ICG, when DL-ethionine was administered intraperitoneally 320 min before measurements were made (Fig. 1). In the initial 20 min, treatment with

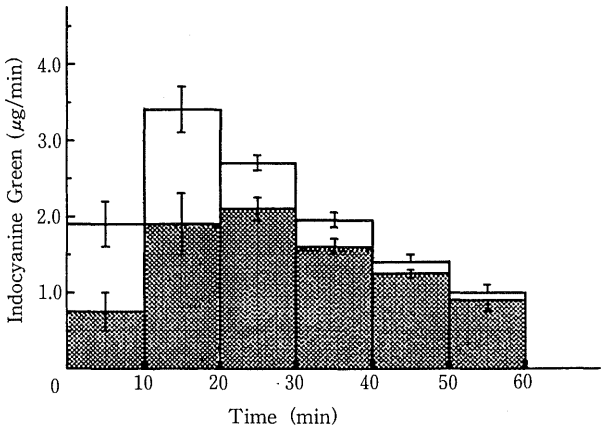

Fig. 1. Effects of treatment with ethionine on the amount of ICG excreted into the bile. ICG was injected intravenously at time 0. The amount of ICG was then determined in samples of bile collected over 10 min periods. Clear bars indicate average biliary excretion by the control rats (mean body weight and standard error, 150 ± 1.6 g; n=5). Hatched bars indicate excretion by the rats (mean body weight, 147.5 ± 4.4 g; n=4), treated with ethionine, which had been administered about 5 h previously. Both bars are superimposed at the same periods of time and both the higher bar and the lower bar are measured from the base of the histogram. Vertical bars show standard errors.

ethionine significantly suppressed the amount of excreted ICG, but did not markedly suppress it 30 min after ICG application. The concentration of dye excreted into the bile in ethionine-treated rats was similar to that excreted by the controls, while ICG concentration in initial period was slightly lower than that of control and ICG concentration in later period was slightly higher (Fig. 2).

The effects of ethionine on excretion of ICG and ATP content are summarized in Table 1. Treatment with ethionine decreased the level of ATP in the liver from 2.56 ± 0.14 (n=3) to 0.86 ± 0.14 μmoles/g liver (n=3). The total amount of ICG excreted for 60 min after injection in the control was 123.6 ± 1.9 μg (n=5) and was 82.4% of the

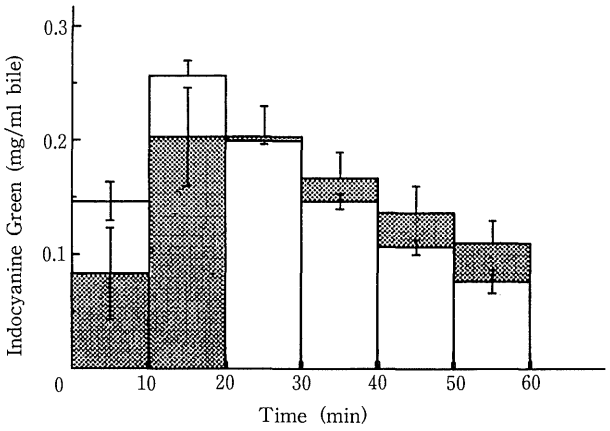

Fig. 2. Effect of treatment with ethionine on the concentration of ICG excreted into the bile. Experimental details were the same as those in the legend to Fig. 1.

Table 1. Effects of ethionine on the ATP level in the liver and the amount of indocyanine green (ICG) excreted for initial 60 min into bile

	Control	Ethionine
ATP level*	2.56 ± 0.14 (3)	0.86 ± 0.14 (3)
Excreted ICG**	123.6 ± 1.9 (5)	84.2 ± 4.1 (4)

Each value shows the mean ± standard error. The number of measurements is shown in parentheses.

* The level of ATP in the liver (μmoles/g liver) was measured 320 min after the administration of ethionine.

** The total amount of ICG (μg) excreted for 60 min after injection of ICG is indicated.

Table 2. Effects of ethionine and ischemia on ATP level, bile flow and biliary excretion of indocyanine green (ICG)

	Control	Ethionine	Ischemia
ATP level*	100% (3)	35.4% (3)	24.7% (3)
Bile flow**	100% (5)	67.5% (5)	8.5% (5)
ICG excretion***	100% (5)	68.1% (4)	—

The number of measurements is shown in parentheses.

* The relative level of ATP in the liver expressed as a percentage of the control was determined from the results obtained 320 min after administration of ethionine or 10 min after ischemia by occlusion of the portal vein.

** The relative bile flow expressed as percentage of control was determined from the results obtained for 10 min from 300 min to 310 min after administration of ethionine or for 10 min after ischemia.

*** The relative amount of ICG excreted for 60 min expressed as a percentage of the control was determined from the results obtained in the rats treated with ethionine.

amount injected. The total amount of ICG excreted by ethionine-treated rats during 60 min was 84.2 ± 4.1 μg (n=4) and was 57.1% of the amount

applied.

Treatment with ethionine decreased the level of ATP to 35.4% of the control value ($p<0.001$) (Table 2). Treatment with ethionine decreased the bile flow to 67.5% of the control value and did not change throughout the period of the experiment. Treatment with ethionine also decreased the total amount of ICG excreted in 60 min to 68.1% of the control ($p<0.001$). The ATP level in the liver decreased to 24.7% of the control value 10 min after ischemia by occlusion of the portal vein, and to 11.9% after 40 min. Ischemia decreased the bile flow in the controls to 8.5% after 10 min. The bile flow stopped completely when ischemia was prolonged beyond 10 min.

To clarify the effect of ethionine on uptake by hepatocytes of the dye, the level of ICG in blood was investigated. The levels of ICG in blood and bile are summarized in Table 3. The level of the dye in blood from ethionine-treated rats was raised by 59.2% of the control value ($p<0.001$). By contrast, the amount of ICG excreted in 10 min into the bile of ethionine-treated rats was reduced by 61.3% of the control value ($p<0.001$).

Table 3. Effects of ethionine on uptake and biliary excretion of indocyanine green (ICG)

	Level of ICG in blood* (mg/100 ml serum)	Level of ICG in bile** (μg/10 min)
Control	0.49 ± 0.02 (6)	19.1 ± 1.3 (5)
Ethionine***	0.78 ± 0.04 (5)	7.4 ± 1.3 (4)

Each value shows the mean ± standard error and the number of measurements is shown in parantheses.

* ICG was injected intravenously, 5 min before measurements, into control rats (156.7 ± 1.0 g; mean body weight and standard error) and ethionine-treated rats (158.0 ± 0.5 g).

** ICG was applied intravenously 10 min before measurements and the total amount of ICG excreted into the bile for 10 min is indicated.

*** Ethionine (100 mg/100 g body weight) was administered intraperitoneally 5 h before application of ICG.

DISCUSSION

In this study, ATP depletion of the liver was brought about by two procedures: the occlusion of the portal vein and the administration of ethionine. Bile flow was decreased by ethionine but was maintained at 67.5% of the control value. By contrast, bile flow was markedly decreased by the onset of ischemia and then ceased completely. Levels of ATP were decreased to 35.4% and 24.7% of control values by ethionine and 10-min ischemia, respectively. In our previous studies[12,13], similar effects of ethionine and ischemia on the membrane potential of hepatocytes were observed. The membrane potential was lowered to 73.3% of the control value by ethionine and was more markedly decreased by 10-min ischemia. These results together suggest that ATP depletion by ischemia markedly decreases the activity of the liver, but ATP depletion by ethionine does not totally eliminate all activity of the liver.

ATP depletion with ethionine decreased the amount of ICG excreted into the bile in the same way as it decreased the flow of bile. Excretion of ICG is not accompanied by the excretion of water, while excretion of taurocholate is associated with the excretion of water[17]. The excretion of ICG may not be coupled with the excretion of water, and the mechanisms of excretion of diagnostic dye and taurocholate are clearly different[9,10,15].

The concentration of ICG excreted into bile was not decreased by treatment with ethionine. This result is consistent with the observation that ethionine also does not influence the concentration of sulfobromophthalein (BSP) excreted into the bile (BSP being an acid dye as is ICG)[11]. This result is reasonably explained by the observation that the amount of ICG excreted into the bile and bile flow were decreased to similar extents in the ethionine-treated rats.

The initial level of ICG in blood of ethionine-treated rats was higher than that of the control. However, the level of BSP in blood of ethionine-treated rats did not change compared with the controls[11]. This discrepancy might be accounted for by the different extents of decreases in levels of ATP: the decrease in levels of ATP in their experiment was smaller than in our experiment, since we used a higher dose and a longer period after application. The amount of excreted ICG after ATP depletion was also markedly suppressed in the initial phase of the excretion, but was not suppressed in the later phase of the excretion. These observations suggest that ATP depletion of the liver suppresses the initial rate of uptake and excretion of the dye.

(Received November 9, 1989)
(Accepted February 27, 1990)

REFERENCES

1. **Brown, W. R., Grodsky, G. M. and Carbone, J. V.** 1964. Intracellular distribution of tritiated bilirubin during hepatic uptake and excretion. Am. J. Physiol. **207:** 1237–1241.
2. **Cherrick, G. R., Stein, S. W., Leevy, C. M. and Davidson, C. S.** 1960. Indocyanine green: observations on its physical properties, plasma dacay and hepatic extraction. J. Clin. Invest. **39:** 592–600.
3 **Cornelius, C. E., Ben-Ezzer, J. and Arias, I. M.** 1967. Binding of sulfobromophthalein sodium (BSP) and other organic anions by isolated hepatic cell plasma membranes *in vitro*. Proc. Soc. Exp. Biol. Med. **124:** 665–667.
4. **Farber, E., Shull, K. H., Villa-Trevino, S., Lombardi, B. and Thomas, M.** 1964. Biochemical pathology of acute hepatic adenosine-triphosphate

deficiency. Nature **203:** 34–40.

5. **Kitani, K., Miura, R. and Kanai, S.** 1978. Difference in the effect of bucolome on the hepatic transport maximum of sulfobromophthalein and indocyanine green. Tohoku J. Exp. Med. **126:** 247–256.
6. **Kupferberg, H. J. and Schanke, L. S.** 1968. Biliary secretion of ouabain-^{3}H and its uptake by liver slices in the rat. Am. J. Physiol. **214:** 1048–1053.
7. **Lamprecht, W. and Trautschold, I.** 1965. Determination with hexokinase and glucose-6-phoshate dehydrogenase, p.543–551. *In* H. U. Bergmeyer (ed.), Methods of enzymatic analysis. Acadamic Press, New York.
8. **Levi, A. J., Gatmaitan, Z. and Arias, I. M.** 1969. Two hepatic cytoplasmic protein fractions, Y and Z, and their possible role in the hepatic uptake of bilirubin, sulfobromophthalein and other anions. J. Clin. Invest. **48:** 2156–2167.
9. **Minder, E. and Paumgartner, G.** 1979. Disparate Na^{+}-requirement of taurocholate and indocyanine green uptake by isolated hepatocytes. Experientia **35:** 888–889.
10. **Reichen, J., Blitzer, B. and Berk, P. D.** 1981. Binding of unconjugated and conjugated sulfobromophthalein to rat liver plasma membrane fractions *in vitro*. Biochem. Biophys. Acta **640:** 298–312.
11. **Schenker, S. and Combes, B.** 1967. Role of hepatic adenosine triphosphate in BSP transport and metabolism *in vivo*. Am. J. Physiol. **212:** 295–300.
12. **Shiba, Y., Muneoka, Y. and Kanno, Y.** 1977. Energy requirement for the maintenance of membrane potential in rat liver cells *in situ*. Jpn. J. Physiol. **27:** 185–193.
13. **Shiba, Y., Muneoka, Y. and Kanno, Y.** 1978. The relationship between membrane potential and ATP content in rat liver during ischemia. Experientia **34:** 57–58.
14. **Solomon, H. M. and Schanker, L. S.** 1963. Hepatic transport of organic cations: active uptake of a quarternary ammonium compound, procaine amide ethobromide, by rat liver slices. Biochem. Pharmacol. **12:** 621–626.
15. **Von Dippe, P. and Levy, D.** 1983. Characterization of the bile acid transport system in normal and transformed hepatocytes: photoaffinity labeling of the taurocholate carrier protein. J. Biol. Chem. **258:** 8896–8901.
16. **Wheeler, H. O., Cranston, W. I. and Meltzer, J. I.** 1958. Hepatic uptake and biliary excretion of indocyanine green in the dog. Proc. Soc. Exp. Biol. Med. **99:** 11–14.
17. **Wheeler, H. O.** 1972. Secretion of bile acids by the liver and their role in the formation of hepatic bile. Arch. Intern. Med. **130:** 533–541.