Application of Indocyanine Green Clearance Test in Dairy Cows

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ABSTRACT. The indocyanine green excretion test was performed in dairy cows, and the usefulness of this test was demonstrated. The results were obtained as follows; (1) Absorption maximum of indocyanine green dye was observed at the wavelength of 805 nm, (2) The dose of indocyanine green dye was varied between 0.25 and 0.75 mg/kg body weight in heifers with no significant ($P>0.05$) difference in plasma clearance, (3) When the dose of 0.25 mg/kg body weight was given to cows, the disappearance was exponential from 2 to 8 min after injection of indocyanine green dye solution in heifers and calvers, and (4) No side effects during and after injection of indocyanine green dye solution were observed.—*Key words:* cow, indocyanine green clearance.

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INTRODUCTION

Indocyanine green was introduced first by Fox and co-workers [4] in 1957 for measurement of blood flow and detection of cardiac malfunction by dilution techniques. Afterwards, it has been widely used as a liver function test in animals such as dogs, rats, rabbits, rhesus monkeys and southdown sheep [7] as well as in human subjects. There is, however, no information available in dairy cows so far, while the use of bromsulphalein tests have been previously reported [1, 2, 9, 18].

Indocyanine green, which is lipoprotein binding-dye [13], might be superior to other protein-binding dyes such as bromsulphalein because of nearly complete recovery from bile [15], single exponential removal pattern [3], distribution limited to the intravascular volume [7], no excretion into the urine [6], no enterohepatic circulation [7], no lymphatic reabsorption [6], and of no placental transfer during labor [11].

Recently, there have been many problems with the dairy cows during the periparturient period, such as milk fever, downer cow syndrome, ketosis, fatty liver, and liver fluke.

Sometimes, several trials were made with the indocyanine green test for the liver function against the diseased cows. However, since there were no standard values of the indocyanine green clearance test for comparison, precise evaluation was hardly obtained.

Therefore, the present study was carried out to determine absorption spectrum of indocyanine green to have the dosage/kg body weight, normal values of half-time, and fractional clearance rate in dairy cows during the periparturient period.

MATERIALS AND METHODS

Animals: Eighteen Heifers at the age of 0.7 to 1.9 years, five 3rd and 4th calvers at the age of 4.0 to 6.0 years, and six 7th and 8th calvers at the age of 7.0 to 13 years were employed. They were all clinically and clinico-pathologically normal Holstein-Friesian heifers and calvers which were kept in the seven standard dairy farms in Hokkaido during the period from 1977 to 1978 were used. They showed the normal range of glutamic oxalacetic transaminase activity i.e., under 80.0 Karmen Unit.

Sampling: Blood samples of heifers were taken from the jugular vein into the 10 ml

heparinized vacuum tube with a 18 gauge needle in the morning before feeding and those from calvers, between 10:00 and 15:00. Samplings of the calvers were done on five occasions; two and one month pre-partum, within 24 hr after calving, half a month and one month post-partum.

Preparation of indocyanine green dye solution for intravenous injections: The body weight of the animals were measured by a platform scale for heifers, and by the body weight measuring tape (Fujihira Industrial Co., Ltd., Japan) for calvers. The estimated amount of dye (Daiichi Pharmaceutical Co., Ltd., Japan) was added to a vial, 10 to 15 ml of distilled water was added and then inverted several times for mixing. The indocyanine green dye solution can be stored stably for 48 hr at room temperature or a week at 4°C under the dark condition [5]. The doses of indocyanine green dye were intravenously administered with 0.25, 0.50 and 0.75 mg/kg body weight for heifers, and with 0.25 mg/kg body weight for calvers, respectively.

Determination of indocyanine green dye concentration: The blood for pre-injection control was taken. The needle was introduced into the distended jugular vein and a small amount of blood was drawn into the syringe to avoid the side effects [17]. Several seconds later the dye solution was injected within 5 to 7 seconds.

The timer was started at the midpoint of the injection interval and eight heparinized blood samples were taken into 10 ml vacuum tubes from the opposite jugular vein every two minutes for heifers. An additional sample for calvers was taken at 30 min after the injection.

The heparinized blood samples were centrifuged at 3,000 rpm for 20 min and the plasma was stored in a double-walled box to avoid direct sunlight [5], and under cold storage condition until analysis. Each plasma sample was diluted three times with saline before measuring. All samples were read

against a diluted plasma blank. The standard curve was prepared as previously reported by Ueda *et al.* [14]. In preparing the standard curve, a small amount of plasma was added to the diluted aqueous indocyanine green dye solutions, since indocyanine green is colorimetrically unstable in water. Concentrations of indocyanine green dye solutions were measured with a Hitachi-model 101 spectrophotometer against the control plasma at 805 nm.

Determination of $T_{1/2}$ and fractional clearance rate: The plasma dye concentrations for each experimental animal were plotted on semilogarithmic coordinates. The best straight line was fitted visually, and the $T_{1/2}$ values and fractional clearance rate were calculated from the line. The $T_{1/2}$ value is the time required for the indocyanine green concentration to be reduced to half of its initial values in the plasma. The fractional clearance rate can be calculated from the following formula:

Fractional clearance rate = $\ln 2 / (T_{1/2}(\text{min}))$
The linear curve was extrapolated back to zero time and the dye concentrations at time "0" (R_0) and "t" (R_t) were read. The per cent of retention can be calculated from the following formula:

$$\text{Per cent of retention} = R_0 / R_t \times 100(\%)$$

RESULTS

Determination of dosage/kg body weight, $T_{1/2}$ values and fractional clearance rate: The different doses of 0.25, 0.50 and 0.75 mg/kg body weight for indocyanine green were given to the heifers, and each disappearance curve of different doses is presented in Fig. 1. With those doses, these plasma concentrations of indocyanine green fell exponentially with respect to time during at least the succeeding 8 min. After 10 min, the concentrations of indocyanine green dye in plasma showed a wider range as the time passed. But there was less variation with a dosage of 0.75 mg/kg given.

The $T_{1/2}$ and fractional clearance values of

Fig. 1. Disappearance curves after administration of different doses of indocyanine green.

Table 1. $T_{1/2}$ values and fractional clearance rates after administration of different doses of ICG

Dose of ICG ^{a)} (mg/kg)	Heifer Nos.	$T_{1/2}$ (min)	Fractional clearance rate (min ⁻¹)
0.25	6	2.8 ± 0.5	0.254 ± 0.066
0.75	6	3.4 ± 0.8	0.214 ± 0.052
0.75	6	3.5 ± 0.0	0.197 ± 0.004

a) =Indocyanine green.

heifers after administration of different doses are presented with the means and standard deviations in Table 1. The mean $T_{1/2}$ values increased from 2.8 to 3.5 min and the mean fractional clearance rates decreased from 0.254/min to 0.197/min, respectively. They were, however, not significantly ($P > 0.05$) different among the different doses of indocyanine green dye/kg.

Half-time values and fractional clearance rates in the calves: The curves fell exponentially during the succeeding 8 to 10 min. The $T_{1/2}$ and fractional clearance values were also presented in Table 2. The $T_{1/2}$ values became

larger, and the fractional clearance rates got to be smaller with a greater variation after parturition.

Per cent of retention of indocyanine green: The mean concentration ($\pm$ standard deviations) of indocyanine green at time "0" calculated from the 54 experimental cows in Table 2 was 0.75 ± 0.15 mg/dl, and the coefficient of variation was 20.5%, when the dosage of 0.25 mg/kg body weight was used.

Side effects: No side effects were observed during and after injection of indocyanine green dye solution.

Table 2. $T_{1/2}$ and fractional clearance rates of indocyanine green in cows

Groups	Time (days)	$T_{1/2}$ (min)	KICG (min^{-1})
		n	M $\pm$ SD
3rd and 4th calvers			
Prepartum	57—67	5	4.1 $\pm$ 0.5
	25—36	5	4.2 $\pm$ 0.6
	0	5	5.3 $\pm$ 1.2
Postpartum	13—17	5	7.3 $\pm$ 3.6
	29—30	5	5.2 $\pm$ 1.7
7th and 8th calvers			
Prepartum	41—66	6	4.5 $\pm$ 1.2
	15—30	6	4.6 $\pm$ 1.4
	0	5	5.6 $\pm$ 1.3
Postpartum	14—16	6	4.7 $\pm$ 0.5
	29—31	6	5.9 $\pm$ 2.0
Mean		54	5.1 $\pm$ 1.8
			0.147 $\pm$ 0.044

KICG=fractional clearance rate of indocyanine green.

DISCUSSION

Cherrick *et al.* [3] used absorption maximum of 815 nm for indocyanine green in human subjects, and Wheeler *et al.* [15], that of 810 nm in dogs. Absorption maximum of indocyanine green in our experimental non-lactating and lactating cows was 805 nm, which was similar to those of rabbits [6], dogs [8], and human subjects [6, 9, 10, 14].

It has been reported that the dosage of indocyanine green/kg body weight was 0.4 to 0.6 mg/kg body weight in dogs [6], and 0.25 mg/kg in human subjects [6, 14, 17]. In an attempt to determine the best dosage per unit of body weight, we have given three doses of indocyanine green to fested heifers with dose for human subjects. There were, however, no significant difference in the fractional clearance rates among the dosages of 0.25 mg/kg to 0.75 mg/kg body weight.

Therefore, the dosage of indocyanine green dye/kg body weight would be between 0.25 mg/kg and 0.75 mg/kg for non-lactating and lactating cows.

It has been described that disappearance was exponential for 10 to 15 min and the fractional clearance rate was from 0.032/min

to 0.039/min in dogs, and 0.259 $\pm$ 0.014/min to 0.159 $\pm$ 0.037/min in human subjects [6, 14, 17]. In the heifers disappearance of the dye was exponential for 2 to 8 min, and the average $T_{1/2}$ increased slightly and the average fractional clearance rate decreased according to an increased dose of indocyanine green dye. In the calvers disappearance was also exponential for 8 to 10 min. In our experimental cows the excretion of plasma indocyanine green concentration seemed to slow down after the interval of 10 min from the injection of starting of indocyanine green dye solution. Therefore, the sampling should be finished within 8 min after starting the injection of indocyanine green dye.

Cornelius *et al.* [2] and Shultz [12] reported that the fractional clearance rates and the $T_{1/2}$ values after injection of 1 gm bromsulphalein in the cows were 0.230 $\pm$ 0.04/min and 3.11 $\pm$ 0.64 min, and 0.1616/min and 4.32 min, respectively. These fractional clearance rates were larger, and the $T_{1/2}$ values were smaller than those of our data for indocyanine green dye test. This might be attributable to the fact that the distribution of indocyanine green dye is more strictly limited to the intravascular volume than that of

bromsulphalein [7].

The indocyanine green excretion is slower in the pregnant calvers and in after parturition than in the heifers or in during the dry period. These results suggest that the better functioning of the liver might be seen in the heifers as well as in the stage of the drying time of the calvers.

Ueda *et al.* [14] stated that the average concentrations of indocyanine green dye at time "0" was 0.48 ± 0.01 mg/dl with a coefficient of variation of 2.0% in human subjects. However, the average concentration at time "0" has such a large coefficient of variation of 20.5% in cows that the per cent of retention is largely changeable depending upon the initial values of indocyanine green concentrations. From the fact that was mentioned above, the per cent of retention could not be used as a reliable indicator at present, and needs a further investigation concerning how to obtain the precise initial value. This inaccuracy may be attributable to the varying volume of blood circulation with age or to an inaccurate body weight measurement.

As was discussed above, the per cent of retention seems to be an unreliable indicator because of greatly varying initial values. However, the fractional clearance rates and the half-time values within 8 to 10 min after injection of indocyanine green dye solution seems to be greatly facilitate to a diagnose of bovine liver function.

Witte [16] had reported no side effects of bromsulphalein dye solution injected within 10 sec in dairy cows. In this study the indocyanine green dye solution was injected in 5 to 7 sec, but no side effects were observed during and after injection. It can be, therefore, concluded that indocyanine green dye might be used safely in dairy cows.

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REFERENCES

1. Cornelius, C. E. and Wheat, J. W. 1957. Bromsulphalein clearance in the horse-A quantitative liver function test. *Am. J. Vet. Res.* 18: 369-374.
2. Cornelius, C. E., Theilen, G. S., and Rhode, E. A. 1958. Quantitative assessment of bovine liver function, using the sulfobromophthalein sodium clearance technique. *Am. J. Vet. Res.* 19: 560-566.
3. Cherrick, G. R., Stein, S. W., Levy, C. M., and Davidson, C. S. 1960. Indocyanine green: Observations on its physical properties, plasma decay, and hepatic extraction. *J. Clin. Invest.* 39: 592-600.
4. Fox, I. I., Brooker, L. G. S., Heseltine, D. W., Essex, H. E., and Wood, E. H. 1957. A tricarbo-cyanine dye for continuous recording of dilution curve in whole blood independent of variation in blood oxygen saturation. *Proc. Mayo Clin.* 32: 478-484.
5. Futamata, H., Yoshida, S., Shimizu, N., Kometani, M., Nishibu, M., and Matsubara, F. 1974. A study on stability of indocyanine green in blood. *J. Med. Technol.* 18: 320-322 (In Japanese)
6. Hunton, D. B., and Clifford, Z. 1960. Studies of hepatic function with indocyanine green. *Gastroenterol.* 39: 713-723.
7. Kaneko, J. J., and Cornelius, C. E. 1970. *Clinical Biochemistry of Domestic Animals*, 2nd ed., Academic Press, New York.
8. Detterer, S. G., Wiegand, B. D., and Rapaport, E. 1960. Hepatic uptake and biliary excretion of indocyanine green and its use in estimation of hepatic blood flow in dogs. *Am. J. Physiol.* 199: 481-484.
9. Morgan, H. C. 1959. A qualitative sulfobromophthalein sodium retention test of liver function in the horse. *J. Am. Vet. Med. Assoc.* 135: 412-416.
10. Namihisa, T., Nanbu, K., Iizima, K., Ide, Y., Hisauchi, N., Sakurada, T., Rin, K., Kobayashi, Y., Morishita, H., Kasakura, E., and Watanabe, K. 1963. Liver function test with indocyanine green. *Acta Hepatol. Japaonica* 5: (No. 2), 114-116 (In Japanese)
11. Probst, P. G. 1970. Studies on clearance and placental transfer of indocyanine green during labor. *Clin. Chim. Acta* 29: 157-160.
12. Shulz, J. A., Rossow, N., und Urbaneck, D. 1960. Ein Beitrag zur Diagnose der Leberkrankungen des Rindes durch Clearance-Untersuchungen mit

- Bromsulphalein und gleichzeitig vorgenommene Leverbiopsie. *ümh. Mh. Vet. Med.* 15: 257-267.
13. Tsuneoka, H., Yamaguchi, E., Taniguchi, B., Yamamoto, K., Hattori, Y., and Yoshinaka, H. 1979. Studies on the indocyanine green binding serum protein. *Jap. J. Clin. Path.* 27: 533-536 (In Japanese)
 14. Ueda, H., Kameda, T., Migita, T., Yamada, H., Iwase, T., Sugishita, H., and Saito, S. 1962. Hepatic function test with indocyanine green. *Jpn. Med. J.* No. 2015, 7-12 (In Japanese)
 15. 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.* (N.Y.) 99: 11-14.
 16. Witte, D. M. 1970. Untersuchungen zur Methodik der Bromsulphaleinprobe beim Rind. *Vet. Med. Diss.*, Hochschule Hannover.
 17. Yokoyama, Y., Takeshige, K., Kasuya, M., Fukuhara, Y., Sawada, M., Kato, Y., Kurokawa, K., Takagi, H., Takimoto, H., Sasanabe, J., and Yamamoto, S. 1966. Hepatic function test with indocyanine green. *Acta. Hepatol. Japonica* 8(Suppl), 1-7.
 18. Yamane, O., Eguchi, H., and Sako, S. 1957. Normal values of bromsulphalein test in cattle. *J. Jpn. Vet. Med. Assoc.* 10: 505-506 (In Japanese)

要 約

乳牛におけるインドシアニングリーン異物排泄試験の応用: 佐藤輝夫(北海道農業共済組合連合会講習所)——乳牛でインドシアニングリーン異物排泄試験を実施し, その有用性を証明する次の結果が得られた。1) インドシアニングリーン色素の最大吸収は約 805 nm に見られた。2) 未経産牛で体重 1 kg 当り 0.2~0.75 mg のインドシアニングリーン色素を投与したが血漿クリアランスに有意差はみられなかった。3) 未経産牛および泌乳牛ではインドシアニングリーンは静脈注射後 2~8 分間指数関数的に減少した。4) インドシアニングリーン注射による副作用は観察されなかった。