A Method for Measurement of Total Circulating Blood Volume Using Indocyanine Green

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HANEDA, K. and HORIUCHI, T. *A Method for Measurement of Total Circulating Blood Volume Using Indocyanine Green.* Tohoku J. exp. Med., 1986, **148** (1), 49-56 — A method of measuring total circulating blood volume (TBV) using indocyanine green (ICG) dye was investigated in 7 dogs and 16 patients. Since concentration of injected ICG decreases exponentially from the circulating system after recirculation, concentration of the dye at the time of appearance (C_{ta}) can be extrapolated by extending the slope of the dye dilution curve transcribed to a semi-logarithmic graph. TBV was calculated using the following formula: $TBV = I/C_{ta}$, where I is the amount (mg) of ICG injected. Repeat measurements of TBV using ICG demonstrated good reproducibility (average coefficient of variation, 2.0%) and when compared to measurements made with Evans blue dye resulted in a correlation coefficient of 0.98 or more. We conclude that assessment of TBV using ICG is reliable and experimentally and clinically feasible. —————
total circulating blood volume; indocyanine green; dye dilution method

Total circulating blood volume (TBV) is one of the most important determinants in the understanding of circulatory physiology. At present, radio-isotopes such as ^{125}I , ^{131}I , ^{51}Cr , ^{99m}Tc and Evans blue dye (T-1824) are used for estimating blood volume. However, certain disadvantages are inherent to the use of these agents including: radiation contamination, long-term retention or temporary discoloration of the skin. Consequently, and although measurement of TBV is particularly important as a diagnostic tool, it is not routinely employed because of these methodological deficiencies. The development of a safe and reliable technique which could be easily performed is therefore needed.

In 1968, Bradley and Barr reported a technique to measure TBV using indocyanine green (ICG), but its application has not been widely accepted. We modified Bradley's method and compared experimental and clinical TBV measurements made with ICG to results obtained using Evans blue (EB) dye.

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MATERIALS AND METHODS

Experimental study

The reproducibility of repeat measurements of TBV using ICG and the correlation between ICG and EB methods were tested in 7 adult mongrel dogs ranging in weight between 8.2 and 21.0 kg (average 13.3 kg). All animals were anesthetized with sodium pentobarbital and ventilated with a volume constant respirator. Polyethylene catheters were inserted into the abdominal aorta and inferior vena cava (IVC) through a left femoral cut down.

(1) ICG method: ICG (2.5–5.0 mg) was injected into a catheter placed in the IVC as arterial blood was simultaneously withdrawn (SU-300 Erma Optics withdrawal pump) at a constant rate of 18 ml/min over a period of 2–3 minutes. A dye dilution curve was obtained and recorded on a dye densitograph (Erma Optics, EN-900, Tokyo) using the cuvette method (Fig. 1). Dye concentration was calibrated against a known concentration (0.05 mg ICG in 10 ml blood = 5.0 mg/liter) and the dye dilution curve was then transcribed

Fig. 1. Dye dilution curve (experimental study). Following intravenous injection of ICG, blood was withdrawn from the abdominal aorta and a dye dilution curve was obtained by the cuvette method. t_a , time of dye appearance; t_0 , time of injection.

Fig. 2. Dye dilution curve transcribed onto a semi-logarithmic graph. The dye dilution curve was transcribed onto a semi-logarithmic graph at 2 second intervals. The dilution curve falls exponentially after recirculation. From the declining slope, the concentration at t_a (C_{t_a}) can be extrapolated.

Fig. 3. Determination of blood concentration of Evans blue dye. The blood concentration of Evans blue at time zero was estimated by extrapolating from values obtained at 10, 20 and 30 minutes after injection.

onto a semi-logarithmic graph at 2 second interval. Dye concentration after recirculation decreased almost linearly on the semi-logarithmic graph after 60 seconds from the appearance of the dye (t_a). By extending the slope of the curve back to t_a , concentration at t_a (C_{t_a}) was extrapolated (Fig. 2) and assumed that concentration levels taken at C_{t_a} were evenly distributed throughout the body. TBV was then calculated using the following formula: $TBV = I/C_{t_a}$, where I is the amount (mg) of ICG injected.

TBV measurement was performed repeatedly at an interval greater than 10 min in order to test reproducibility of this method.

(2) EB method: Measurement of TBV using EB dye was performed immediately prior to TBV measurements made with ICG. EB (2.5–5.0 mg) was injected into a peripheral vein and arterial blood was sampled for dye concentration prior to injection, and at 10, 20 and 30 min following injection. Concentration was determined with a spectrophotometer (Erma Optics, Model LS-7). Concentration at the time of injection (C_{t_0}) was obtained by averaging data collected at 10, 20 and 30 min after injection or by extrapolating concentration onto a semi-logarithmic graph (Fig. 3). TBV was calculated using the following formula:

$$TBV = \frac{I}{C_{t_0}} \times \frac{100}{100 - 0.96 \times 0.91 \text{ Hct}}$$

I : Amount (mg) of EB injected, Hct : Hematocrit, 0.96: Correction factor for trapped plasma, 0.91: Body/venous hematocrit ratio (Chaplin et al. 1953)

Blood loss due to sampling was replaced with an equal volume of normal saline. Additional fluids were not given.

Clinical study

TBV was measured with ICG and EB 16 times in 15 patients with various heart diseases. Patient age ranged from 3 to 52 years. Six patients had congenital and 9 had acquired heart disease. TBV in 12 patients was measured in an Intensive Care Unit between 1 hr and 20 days postoperatively and in 3 patients during cardiac catheterization.

ICG (10.0–15.0 mg in adults; 5.0–10.0 mg in children) was injected into the IVC or right atrium as blood was simultaneously withdrawn from the femoral or radial artery. Arterial blood was withdrawn at a rate of 7.5 ml/min over a period of 3–4 min. TBV measurements using EB were performed immediately prior to measurements made with ICG. EB (12.0–15.0 mg in adults; 4.0–6.0 mg in children) was injected into a peripheral vein and arterial blood was collected prior to injection, and at 10, 20 and 30 min following injection.

TABLE 1. *TBV measurements in dogs*

Dog No.	Body weight (kg)	TBV (ICG)			TBV	Difference
		First	Second	CV	(FB)	(EB-ICG/EB)
1	8.2	570 ml	560 ml	1.2%	540 ml	-4.6%
		565 ml				
2	12.4	720	740	1.9	—	—
		730				
3	15.5	880	860	1.6	—	—
		870				
4	12.3	1190	1210	1.2	1040	-15.4
		1200				
5	21.0	1550	1500	2.3	1540	+1.0
		1525				
6	12.4	820	870	4.1	800	-5.6
		845				
7	11.0	830	850	1.7	850	+1.2
		840				
13.3±4.0 kg		71.2±13.7 ml/kg		2.0±1.0%	73.1±8.3 ml/kg	-4.7±6.7%

CV, coefficient of variation ; EB, Evans blue.

Values are mean±s.d.

RESULTS

Experimental

The average TBV determined by repeat measurements with ICG in 7 dogs was 71.2 ml/kg (range=56.1-97.6 ml/kg) (Table 1). Duplicate measurements of TBV gave a mean coefficient of variation of 2.0% (range=1.2-4.1%) with a range of 10-50 ml between the first and second measurements.

TBV in 5 dogs using EB averaged 73.1 ml/kg (range=64.5-84.6 ml/kg). The difference in TBV estimated with ICG versus measurements made with EB ranged from +40 to -160 ml. When normalized to values obtained with EB (100%), percent difference between the two methods ranged from -15.4 to +1.2% (average = -4.7%) giving a correlation coefficient of 0.98.

Clinical

The values of TBV ranged from 49-90 ml/kg (Table 2). This large variation in range was presumably due to differences in condition between individual patients. TBV in 8 patients (cases 1-8) performed within 2 days after open heart surgery in which extracorporeal circulation was used averaged 62.2 ml/kg, whereas

TABLE 2. *TBV measurements using ICG in patients with various heart diseases*

Cases	Age (year)	Diagnosis	TBV		
1*	13	TOF	71.7 ml/kg	(69.3 ml/kg)	62.2 ± 8.6 ml/kg (59.0 ± 9.0)
2	9	VSD, AR	49.3	(44.6)	
3	16	TOF	71.4	(68.1)	
4	24	AR	60.7	(57.0)	
5	34	MSR	52.5	(51.2)	
6	42	MS	70.4	(68.0)	
7	6	TOF	58.0	(53.7)	
8	44	IHD	63.3	(59.8)	
9	17	AR	71.4	(68.0)	75.6 ± 7.0 ml/kg (72.5 ± 7.4)
10	21	MS	71.1	(67.5)	
11	52	CP	79.6	(76.8)	
12	27	CP	90.0	(87.6)	
13*	13	TOF	69.8	(67.3)	
14	30	LA myxoma	69.3	(65.7)	
15	6	VSD, AR	77.4	(76.0)	
16	3	VSD, MR	76.4	(70.9)	

TBV in cases 1 through 8 were measured within 2 days of heart surgery in which extracorporeal circulation was used; in case 9 was obtained preoperatively; in cases 10, 11 and 12 within 3 hours following heart surgery wherein the use of extracorporeal circulation was not employed; and in cases 13, 14, 15 and 16 after more than 14 days following open heart surgery.

AR, aortic regurgitation; CP, constrictive pericarditis; IHD, ischemic heart disease; LA, left atrium; MR, mitral regurgitation; MS, mitral stenosis; MSR, mitral stenosis with regurgitation; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

* indicates the same case. (), TBV by Bradley's method. Values are mean $\pm$ S.D.

TBV in the second group of 8 patients averaged 75.6 ml/kg ($p < 0.005$ when compared with Student's *t*-test). Measurements taken from the latter group included: four measurements made after 2 weeks post-bypass, three measurements in which extracorporeal circulation was not employed, and one measurement that was made preoperatively.

TBV measured with EB ranged from 47–89 ml/kg. The percent difference between TBV measurements made with ICG and EB ranged from -5.5 to $+13.0\%$ (average = $+4.2\%$). The correlation coefficient between the two methods gave an *r* value of 0.99 (Fig. 4).

DISCUSSION

ICG is a dye of the tricarboyanine group with a chemical name of 3, 3', 3', 3'-tetramethyl-1, 1'-di (4-sulfobutyl)-4, 5, 4', 5'-dibenzoindotricarboyanine iodide

Fig. 4. Comparison of TBV measurements using ICG and Evans blue (clinical study). A high correlation value of +0.99 was obtained.

disodium salt with a molecular weight of 924.88. Its maximum optical density when bound in serum is at 805 nm. When injected in vivo it is excreted via the liver and blood concentration levels of ICG are known to decrease as a function of time. For this reason, it has been thought impossible to perform TBV measurements using ICG. However, ICG is removed from blood at an exponential rate (Wheeler et al. 1958; Leevy et al. 1959; Rapaport et al. 1959) and the slope of dilution curve following recirculation shows an almost linear decline when transcribed onto a semi-logarithmic graph. By extending the declining slope back to appearance time and assuming that blood ICG concentration is even throughout the body, TBV can be calculated. Therefore, if we assume that ICG is instantaneously distributed throughout the body, almost no decrease in dye concentration should occur until ICG has circulated through the heart and entered the liver. Upon reaching the hepatic circulation it is thought to be removed exponentially. Actually, the time at which ICG reaches the liver is not known but is thought to be approximately the same time as its appearance in the peripheral arteries (t_a). Consequently, removal of the injected ICG from the blood begins at t_a . The value of t_a (Ct_a) was arrived at by extrapolating from the exponential decay of the dilution curve and TBV was calculated from it.

In 1968, Bradley and Barr reported a technique designed to measure TBV using ICG. They calculated TBV by extending the declining slope of the dye dilution curve back to the time of injection (t_o) and assuming that dye concentration at t_o (Ct_o) was evenly distributed throughout the body immediately after injection. Because Ct_o is larger than Ct_a when the decay slope of an ICG curve is extrapolated onto a semi-logarithmic graph, estimated TBV using Bradley's method was 1.9 to 7.5% (average 4.8%) less than clinical results we obtained with our method ($p < 0.001$ with Student's *t*-test; Table 2). Since ICG is excreted

from blood primarily via the liver and because rapid extravasation of ICG is quite small immediately after injection, we believe that there is almost no ICG removed from the systemic circulation until it enters the hepatic system and ICG is not excreted from blood between t_o and t_a . Furthermore, TBV measurements that assume uniform distribution at Ct_o would result in values consistently less than calculations in which Ct_a was used as the denominator. In addition, our method did not require calculation of transit time in the withdrawal circuit in order to determine when t_o occurred. Therefore, we believe that our method offers both theoretical and practical advantages over Bradley's technique.

Normal TBV in humans is considered to be within the range of 68–76 ml/kg (Albert et al. 1968 ; Spears et al. 1974 ; Bauer et al. 1975 ; Myhre and Robinson 1977). TBV following open heart surgery falls about 20% and is reported to return to preoperative levels within one week (Kloster et al. 1966 ; McClenahan et al. 1966 ; Yashar et al. 1971). Alterations in TBV following open heart surgery have been attributed to phenomena associated with intravascular sequestration or extravasation processes (Kloster et al. 1966 ; Yashar et al. 1971). The present study gave results consistent with those reported by others.

From this study, we conclude that ICG can be used to measure TBV safely and with good reproducibility. Cardiac output can be simultaneously measured from the dye dilution curve and hepatic excretory function can be assessed from the declining slope of the ICG curve. Therefore, we feel that this method not only offers certain quantitative advantages in the clinical evaluation of preoperative and postoperative hemodynamics, but also permits the clinician to easily measure blood volume status without incurring the risks and disadvantages of other currently available techniques.

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