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ORIGINAL ARTICLE

Identifying a long standing error in single-bolus determination of the hepatic extraction ratio for indocyanine green

Eugene H. Wissler

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Abstract For approximately 50 years, hepatic clearance of indocyanine green (ICG) has been used to assess liver function. Steady-state infusion of ICG with simultaneous measurement of arterial and hepatic venous ICG concentrations provides unambiguous measures of the extraction ratio for ICG and the hepatic blood flow rate, but also requires cannulation of a hepatic vein. Transient clearance following injection of a single bolus of ICG, which typically involves only measurement of arterial ICG concentration, is a more commonly used procedure. Since drawing blood from a hepatic vein is often impossible, and, in any event can be difficult, there has been considerable interest in the claim by Grainger et al. (Clin Sci 64:207–212, 1983) that a single-bolus, two-compartment model "enabled the hepatic extraction ratio (ER_{ss}) of dye to be determined solely from the plasma disappearance curve". The principal purpose of this paper is to show that the claim by Grainger et al. is not valid because it ignores the fact that a finite fraction of ICG entering the liver passes directly into hepatic veins without being sequestered in the liver. A valid relationship between ER_{ss} and parameters determined from single-bolus clearance data is derived in this paper. For individuals with normally functioning livers, the single-bolus method of Grainger et al. yields an extraction ratio approximately 20% too large, but in cirrhotic patients with extensive intrahepatic shunting, the extraction ratio evaluated using the single-bolus method of Grainger et al. may be too large by a factor of two.

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E. H. Wissler (✉)
The University of Texas at Austin, Austin, TX, USA
e-mail: ehwissler@mail.utexas.edu

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Introduction

Transient removal of the tricarbocyanine dye, indocyanine green (ICG) from blood has been used for a half century as an indicator of liver function. In some applications, especially recently with the development of near-infrared spectroscopy, a gross measure of transient clearance following injection of a single bolus of ICG is correlated with a particular aspect of liver disease. Those applications are not particularly dependent on details of the theoretical basis for ICG clearance. In other applications when the value of a specific physiological quantity, such as hepatic blood flow rate, is derived from clearance data, it is essential that the procedure employed and the computations performed properly employ the underlying theory. This paper is primarily concerned with applications of the second kind.

The experimentally determined, time-dependent concentration of ICG in systemic plasma, $[ICG]_p$, following injection of a single bolus of dye generally decreases exponentially defined by a four-parameter function of the form [see Wiegand et al. (1960)],

$$[ICG]_p = a_p e^{-\alpha t} + b_p e^{-\beta t} \quad (1)$$

Connection between physiological quantities and the four parameters in Eq. 1 is provided by a linear physical model such as the two-compartment model developed in 1959 by Richards et al. Although they investigated the clearance of bromsulphthalein from dogs, their model is conceptually the same as a two-compartment model often used to analyze ICG clearance.

In 1983, Grainger et al. published a relationship between the four empirically derived parameters, a_p, α, b_p, and β, and a "steady-state extraction ratio (ER_{ss})" defined as follows:

$$ER_{ss} = \frac{[ICG]_p - [ICG]_{hv}}{[ICG]_p}, \quad (2)$$

in which $[ICG]_{hv}$ is the concentration of ICG in hepatic venous blood, and ER_{ss} is the fraction of ICG extracted whenever the amount of ICG in liver is not changing with respect to time, which can be either at the moment when ICG in the liver reaches its maximum value following injection of a single bolus of ICG, or during steady-state, continuous injection of ICG. Grainger et al. claimed that "This analysis enabled the hepatic extraction ratio (ER_{ss}) of dye to be determined solely from the plasma disappearance curve". Unfortunately, their claim is not supported either by their own experimental data or by the results of subsequent experimental studies (Clements et al. 1987; Burns et al. 1990).

In this paper, we show that when one derives from first principles the equations employed by Grainger et al. and compares computed $[ICG]_{hv}$ values with measured values reported in the literature, it is apparent that something is wrong with the model. The analysis presented in this paper identifies the source of difficulty as the assumption by Grainger et al. that ICG is totally extracted from blood and is only released into hepatic veins after sequestration in the liver although, in fact, a fraction of ICG always passes directly through the liver (Leevy et al. 1962).

Theoretical analysis

The two-compartment model used in this analysis and by others in previous analyses is represented schematically in Fig. 1. Even though certain details of ICG sequestration in the liver remain unclear, studies by Meijer et al. (1984, 1988) confirmed for ICG clearance in humans what Richards et al. and others (for example, Brauer and Pessotti 1950) had previously demonstrated for bromsulphthalein clearance in dogs. The studies by Meijer et al. clearly establish that ICG sequestered in the liver is removed in bile at a rate proportional to the amount of dye in the liver. A more recent study supporting the two-compartment model was conducted by Shinohara et al. (1995) who used near-infrared spectroscopy to measure the ICG concentration in livers of rabbits following injection of a bolus of dye. As predicted by the model, they observed that ICG in the liver increases to a maximum before decreasing exponentially.

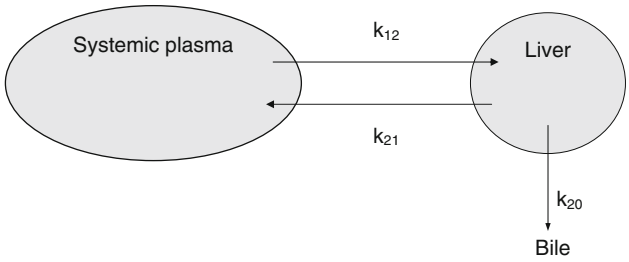

Fig. 1 Two-compartment model for dye clearance

Basic assumptions

The two-compartment model is based on the following assumptions:

1. ICG in blood binds almost exclusively to plasma proteins, principally albumin.
2. The concentration of ICG in peripheral veins is the same as the concentration in the hepatic artery and portal vein. In other words, systemic blood can be treated as a well-mixed pool in which the dye concentration is uniform. That assumption is only valid several minutes after injection of the bolus.
3. The healthy liver has considerable capacity for sequestering ICG at sites that are not well defined.
4. ICG is removed from the body exclusively through bile at a rate proportional to the ICG content of the liver.
5. The concentration of ICG in hepatic venous blood is a linear function of the ICG content of the liver and that of arterial blood.

Definition of relevant variables in a consistent set of units

$X_p = V_p\,[ICG]_p$ = amount of ICG in circulating plasma, mg
X_l = amount of ICG sequestered in the liver, mg
V_p = systemic plasma volume, ml
$[ICG]_p$ = concentration of ICG in circulating plasma, mg/ml of plasma
$[ICG]_{hv}$ = ICG concentration in hepatic venous blood, mg/ml plasma
ER = extraction ratio of ICG by the liver = $\frac{[ICG]_p - [ICG]_{hv}}{[ICG]_p}$, dimensionless
PF_h = rate of plasma flow through the liver, ml/min
$Q_{bile} = k_{20}X_l$ = rate of removal of ICG from the liver in bile, mg/min
$k_{20}X_l$ = rate at which ICG sequestered in the liver is released into bile, mg/min
$k_{21}X_l$ = rate at which ICG sequestered in the liver is released into venous blood, mg/min

η = fraction of ICG that passes directly through the liver, dimensionless
I = rate of infusion of ICG into an artery or vein, mg/min
D = ICG dose injected in a bolus = $\int_0^{\Delta t} I\mathrm{d}t$, mg
Δt = time required to inject the bolus, min

Basic equations

Material balances for ICG in the two compartments are expressed as follows: for ICG in systemic blood,

$$\frac{\mathrm{d}X_\mathrm{p}}{\mathrm{d}t} = -k_{12}X_\mathrm{p} + k_{21}X_\mathrm{l} + I \tag{3}$$

and for ICG sequestered in liver,

$$\frac{\mathrm{d}X_l}{\mathrm{d}t} = k_{12}X_\mathrm{p} - k_{21}X_\mathrm{l} - k_{20}X_\mathrm{l}, \tag{4}$$

where

$$k_{12} = \frac{(1-\eta)\mathrm{PF_h}}{V_\mathrm{p}}. \tag{5}$$

In formulating Eqs. 3 and 4, we have assumed that $[\mathrm{ICG}]_\mathrm{hv}$ and Q_bile increase linearly as X_l increases. Although the form of the assumed relationships cannot be established directly, three studies indicate that the system is overall linear, which implies that the relationship between $[\mathrm{ICG}]_\mathrm{hv}$, $[\mathrm{ICG}]_\mathrm{p}$ and $[\mathrm{ICG}]_\mathrm{l}$ is also linear. For example, Leevy et al. (1962) observed no significant difference in the extraction ratio or percentage ICG clearance rate when three different bolus sizes (0.15, 0.25, and 0.5 mg of ICG per kg body weight) were injected into two normal subjects. Similarly, Meijer et al. (1988) measured $[\mathrm{ICG}]_\mathrm{p}$ and the bilary excretion rate in post-cholecysystectomy patients following injection of 0.5, 1.0, and 2.0 mg of ICG/kg body weight. Data for the two smaller doses were in all respects consistent with the assumption that $[\mathrm{ICG}]_\mathrm{hv}$ and Q_bile are linear functions of X_p and X_l. A subsequent study by Soons et al. (1991) also supports that assumption. Their study tested linearity in two different ways. They observed that steady-state values of $[\mathrm{ICG}]_\mathrm{p}$ were proportional to the rate of infusion for three different infusion rates, 0.5, 1.0, and 2.0 mg/min. They also measured the incremental change in $[\mathrm{ICG}]_\mathrm{p}$ following the injection of 0.5 mg/kg of dye while dye was continuously infused at 1.0 mg/min. Fifty minutes separated injection of successive boluses. An analysis of their data, which is not included in this paper, indicates that the transient responses were consistent with an assumption that $[\mathrm{ICG}]_\mathrm{hv}$ and Q_bile are linearly related to X_l. Therefore, it is reasonable to assume that

$$[\mathrm{ICG}]_\mathrm{hv} = \eta[\mathrm{ICG}]_\mathrm{p} + \frac{k_{21}X_\mathrm{l}}{\mathrm{PF_h}} \tag{6}$$

and

$$Q_\mathrm{bile} = k_{20}X_\mathrm{l}, \tag{7}$$

where η, k_{20}, and k_{21} are constants. Since η and $\mathrm{PF_h}$ occur as products in Eqs. 4 and 5, neither factor can be determined from experimental data involving only $[\mathrm{ICG}]_\mathrm{p}$.

Equations 3 and 4 have the same form as equations used by Richards et al. (1959) and Clarkson and Richards (1967) in analyses that provided the theoretical basis for the paper by Grainger et al. (1983). The difference between the current paper and previous papers lies in the physical interpretation of the parameter, k_{12}. In previous papers, either k_{12} was not explicitly defined, or it was assumed that $k_{12} = \mathrm{PF_h}/V_\mathrm{p}$. Since Richards, and Clarkson and Richards employed a definition not specifically related to $\mathrm{PF_h}$, their definition of a parameter corresponding to k_{12} could include the definition in Eq. 5. On the other hand, the definition of k_{12} employed by Grainger et al. (1983) specifically assumes that $\eta = 0$.

Equations 3 and 4 must be solved subject to appropriate initial conditions. If dye concentrations in blood and the liver are both zero at time = 0 and dye is injected sufficiently rapidly, we have

$$X_\mathrm{p}(0) \approx \int_0^{\Delta t} I\mathrm{d}t = D \tag{8}$$

and

$$X_\mathrm{l}(0) \approx 0. \tag{9}$$

One can establish by substitution that when $I = 0$, Eqs. 3 and 4 have solutions of the form

$$X_\mathrm{p} = V_\mathrm{p}\left(a_\mathrm{p}\mathrm{e}^{-\alpha t} + b_\mathrm{p}\mathrm{e}^{-\beta t}\right) \tag{10}$$

and

$$X_\mathrm{l} = A_\mathrm{l}\mathrm{e}^{-\alpha t} + B_l\mathrm{e}^{-\beta t}, \tag{11}$$

in which

$$\alpha = \frac{k_{12}+k_{20}+k_{21}+\sqrt{(k_{12}+k_{20}+k_{21})^2 - 4k_{12}k_{20}}}{2}, \tag{12}$$

$$\beta = \frac{k_{12}+k_{20}+k_{21}-\sqrt{(k_{12}+k_{20}+k_{21})^2 - 4k_{12}k_{20}}}{2}, \tag{13}$$

$$A_\mathrm{l} = \frac{k_{12}-\alpha}{k_{21}}V_\mathrm{p}a_\mathrm{p}, \tag{14}$$

and

$$B_\mathrm{l} = \frac{k_{12}-\beta}{k_{21}}V_\mathrm{p}b_\mathrm{p} = -A_\mathrm{l}. \tag{15}$$

Evaluation of the extraction ratio

To determine ER, one needs the values of k_{12}, k_{20}, and k_{21}, which can be computed from values of α, a_p, β, and a_p. We have from Eq. 3 in the limit as $t \to 0$

$$k_{12} = \frac{\alpha A_p + \beta B_p}{A_p + B_p} = \frac{\alpha a_p + \beta b_p}{a_p + b_p}. \quad (16)$$

In addition, straightforward calculation using Eqs. 12 and 13 establishes that

$$k_{20} = \frac{\alpha\beta}{k_{12}} \quad (17)$$

and

$$k_{21} = \alpha + \beta - k_{12} - k_{20}. \quad (18)$$

The extraction ratio for the liver is defined in terms of ICG concentrations as follows:

$$ER = \frac{[ICG]_p - [ICG]_{hv}}{[ICG]_p} = (1-\eta)\left[1 - \frac{k_{21}X_l}{k_{12}X_p}\right]. \quad (19)$$

Since X_l/X_p increases with increasing time, ER decreases as ICG is transferred from circulating blood to the liver.

Even though ER is a function of time, investigators tend to think in terms of a single extraction ratio that presumably exists during their experimental procedure. A unique value is the extraction ratio during steady infusion of dye. Clarkson and Richards (1967) noted that when X_l is unchanging, the two-compartment model yields a simple relationship between ER and the parameters, k_{20} and k_{21}. In that case, it follows from Eq. 4 that

$$\frac{X_l}{X_p} = \frac{k_{12}}{k_{20} + k_{21}}. \quad (20)$$

Combining Eqs. 19 and 20 yields the result

$$ER_{ss} = (1-\eta)\left[\frac{k_{20}}{(k_{20} + k_{21})}\right]. \quad (21)$$

Note that Eq. 21 contains the factor $(1 - \eta)$, which does not appear in the ER_{ss} equation of Grainger et al.

Experimental observations

Transient concentration of ICG in hepatic venous blood following injection of a single bolus of dye

The cardinal assumption in this analysis is that ICG appears in hepatic venous blood both by passing freely through the liver and by being released into venous blood after sequestration in the liver. The parameter, η, accounts for the fact that extraction of ICG from blood passing through the liver is not complete even when no ICG is sequestered in the liver. That behavior is obvious in experimental data reported by all investigators who measured $[ICG]_{hv}$ during a single-bolus procedure (Wiegand et al. 1960; Leevy et al. 1962; Rowell et al. 1964, 1965, 1968, Teranaka et al. 1977; Grainger et al. 1983). Those investigators observed that ICG appears in hepatic venous blood soon after injection of the bolus and decreases with increasing time. If extraction by the liver were perfect and ICG appeared in hepatic venous blood only after being sequestered and then released back into the blood stream (i. e., if $\eta = 0$), $[ICG]_{hv}$ would be zero initially and would increase to a maximum value in proportion to X_l. However, experimental data plotted in Fig. 2 and similar data from every single-bolus study in which $[ICG]_{hv}$ was measured clearly show that $[ICG]_{hv}$ increases very quickly following injection of ICG, and then decreases roughly parallel to $[ICG]_p$ on a semi-log plot. Those data clearly establish that a fraction of ICG flowing into the liver passes directly into a venous stream. To quote Leevy, "At no time after its injection (0.5 mg of ICG per kg of body weight) was there 100 percent hepatic extraction of ICG; during the decelerated phase of removal of dye from plasma, the extraction ratio exhibited a further decrease".

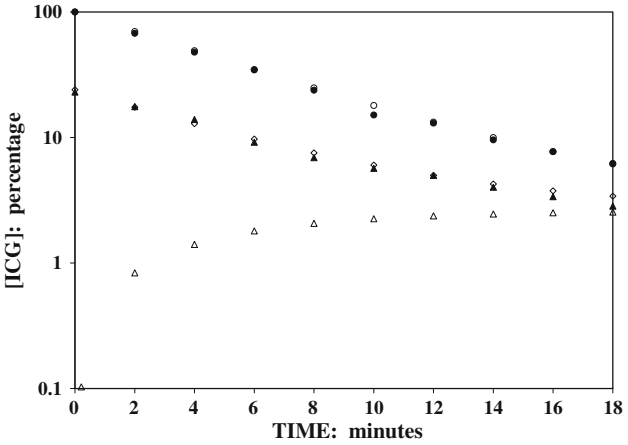

Fig. 2 ICG concentrations in arterial and hepatic venous blood plotted as functions of time following injection of a single bolus of dye at time = 0. Five curves are identified as follows: *filled circles* measured $[ICG]_p$; *filled triangles* measured $[ICG]_{hv}$; *open circles* and *open triangles* $[ICG]_p$ and $[ICG]_{hv}$, respectively, computed using the two-compartment model with $\eta = 0$; and *diamonds* $[ICG]_{hv}$ computed with $\eta = 0.24$. Values of $[ICG]_p$ computed with $\eta = 0.24$ are essentially identical to values computed with $\eta = 0$. Measured values were reported by Leevy et al. (1962)

Extraction ratio for ICG

Grainger et al. (1983) determined directly the extraction ratio in 11 baboons by simultaneously withdrawing blood samples from a peripheral vein and a right hepatic vein. Results shown in Fig. 3 of their paper unambiguously

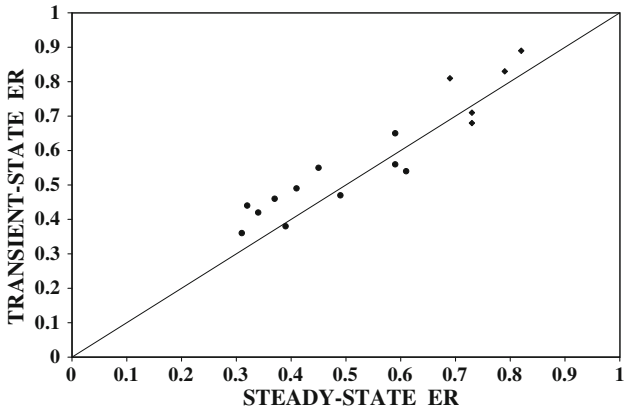

Fig. 3 Comparison of ICG extraction ratios measured by Grainger et al. (1983) using continuous infusion of dye and injection of a single bolus of dye. Subjects were 11 baboons and 5 normal men

indicate that ER measured 8 min after injection of the dye was lower than ER measured 4 min after injection, as predicted by Eq. 19.

Grainger et al. also compared values of ER_{ss} determined in two ways: using the first relationship in Eq. 19 with values of $[ICG]_p$ and $[ICG]_{hv}$ measured at the moment when X_1 has its maximum value, and computed using the second relationship in Eq. 19 with $\eta = 0$. As shown in Fig. 3, values determined using the single-bolus method tend to be larger than corresponding values determined from measurement of $[ICG]_p$ and $[ICG]_{hv}$. The mean value of the ratio, $ER_{ss,\ \text{single bolus}}/ER_{ss,\ \text{direct measurement}}$, is 0.87, which corresponds to a mean value of $\eta = 0.13$.

Clements et al. (1987) and Burns et al. (1990) compared values of ER_{ss} determined by continuous infusion with values determined by injection of a single bolus, and concluded that the single-bolus method is definitely not applicable to subjects with cirrhotic livers. As shown in Table 1, extraction ratios determined by the single-bolus method were several times larger than ratios determined by continuous infusion, and hepatic flow rates were correspondingly smaller. A reasonable explanation for the disparity between corresponding extraction ratios is the failure

Table 1 Comparison of ICG extraction ratios determined in two ways: continuous infusion and single-bolus injection (Burns et al. 1990)

Cause of cirrhosis	Constant infusion	Single bolus
Chronic active	0.41	0.77
Alcoholic	0.23	0.77
Haemochromatosis	0.25	0.61
Alcoholic	0.23	0.86
Primary biliary	0.34	0.82
Wilson's disease	0.61	0.84

to include the factor $(1 - \eta)$ in the computation of ER_{ss} from single-bolus data. It is well known that intrahepatic shunting accompanies liver cirrhosis, with the value of η increasing as the degree of shunting increases. Since the error in the value of ER_{ss} from assuming that $\eta = 0$ increases as η increases, it is not surprising that attempts to apply the method of Grainger et al. to patients with liver disease were unsuccessful.

Plasma volume and hepatic perfusion rate

Of the three parameters, k_{12}, k_{20}, and k_{21}, derivable from transient $[ICG]_p$ data, only k_{12} is directly related to PF_h. We have

$$k_{12} = \frac{(1-\eta)PF_h}{V_p} = \frac{\alpha a_p + \beta b_p}{a_p + b_p} = \lim_{t\to 0}\left(\frac{d\ln\left([ICG]_p\right)}{dt}\right). \tag{22}$$

Using Eq. 22 to evaluate PF_h requires values of V_p and η. It is possible to evaluate V_p from ICG clearance data as follows:

$$V_p = \frac{D}{a_p + b_p} = \lim_{t\to 0}\left(\frac{D}{[ICG]_p}\right). \tag{23}$$

Unfortunately, it is not possible to evaluate η without measuring $[ICG]_{hv}$. It follows from Eq. 6 that

$$\eta = \lim_{t\to 0}\left(\frac{[ICG]_{hv}}{[ICG]_p}\right) \tag{24}$$

because $X_1 = 0$ at $t = 0$.

The single-bolus method was used correctly by several early investigators who determined the hepatic extraction ratio and blood flow rate by measuring the concentration of ICG in arterial and hepatic venous blood (Wiegand et al. 1960; Rowell et al. 1964, 1965, 1968). However, it has also been used incorrectly by recent investigators who measured only $[ICG]_p$ and assumed that the relationship proposed by Grainger et al. (1983) to evaluate ER_{ss} is valid (Kenney and Ho 1995; Ho et al. 1997; Minson et al. 1998; Proctor et al. 2001). In some of the cited papers, only a relative value of PF_h was reported, such as the ratio of hepatic blood flows during exercise and rest. In that case, the extraction ratio cancels out and it doesn't matter what value is used.

Conclusions

The two-compartment model for hepatic clearance of a dye such as bromsulphthalein or indocyanine green following injection of a single bolus was introduced more than

50 years ago and has been used since then in various ways. While early applications that employed simultaneous measurement of arterial and hepatic venous concentrations of dye yielded valid results, more recent applications that did not measure the hepatic venous concentration yielded incorrect values for the extraction rate and hepatic blood flow rate. The analysis presented in this paper establishes that hepatic extraction ratios computed using the relationship proposed by Grainger et al. (1983) are too large because they fail to account for direct passage of dye through the liver. That omission provides a logical explanation for the large difference between extraction ratios measured by continuous infusion and single-bolus injection in patients with liver disease.

Contrary to the claim of Grainger et al., it is not possible to determine the extraction ratio from single-bolus data without measuring the hepatic venous concentration of dye, and the hepatic blood flow rate cannot be determined from ICG clearance data if the extraction is unknown.

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