Pulse Dye Densitometry and Indocyanine Green Plasma Disappearance in ASA Physical Status I-II patients

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Introduction

Indocyanine Green dye (ICG) is extracted from the blood by hepatic parenchymal cells without undergoing enterohepatic circulation.\(^1\) The hepatic clearance of ICG is used for the assessment of hepatic (residual) function.\(^2-5\) The elimination of ICG can be reported as ICG plasma disappearance rate (ICG-PDR), which is the decline in the log-linear elimination curve after the iv administration of a bolus of ICG, expressed as percentage per min. Various reports have been published on the measurement of ICG-PDR for the monitoring of hepatic function during anesthesia and after liver transplantation.\(^6-9\) In addition, ICG-PDR is reported as a prognostic factor in the critically ill.\(^10-13\)

With Pulse Dye Densitometry (PDD), an adaptation of pulse spectrophotometry, it is now possible to measure ICG non-invasively by means of transcutaneous pulse spectrophotometry using a finger or nose probe. Transcutaneously measured ICG concentrations have been shown to correlate very closely to arterial blood ICG concentration measurements in man\(^14,15\) and in a porcine model.\(^16\)

Although the measurement of ICG has been validated properly, the actual mean value and range of ICG disappearance in the healthy population and the cutoff value that discriminates between a normal and impaired hepatic function has not yet been described clearly. Often one refers to a single publication, which is a book chapter that is not readily available.\(^17\) Other publications on the subject date from almost 50 years back and are determined by invasive blood sampling. Nonetheless, information on the range and variability of ICG-PDR in healthy persons is prudent for the interpretation of single or repeated ICG disappearance measurements in the evaluation of liver graft function, hepatic blood flow, or assessment of liver failure in septic shock.

To increase the knowledge of ICG disappearance in a healthy population we evaluated the ICG disappearance rate (ICG-PDR values) in ASA physical status I-II patients not known with hepatic or cardiovascular disease. Measurement of ICG-PDR was performed transcutaneously by Pulse Dye Densitometry using either a finger probe or nose probe. In addition, we compared the transcutaneous measurement of ICG-PDR using PDD to invasive measurement of ICG-PDR in arterial blood.
Methods

Patients
For the determination of the ICG plasma disappearance in an otherwise healthy surgical population, 41 patients were evaluated as they participated in one of two sequential pharmacological studies. In 33 patients, involved in a study on the cardiovascular effects of lumbar epidural anaesthesia with ropivacaine 0.75%, ICG concentrations were measured using the PDD finger probe. Measurements were performed before introduction of epidural anaesthesia. In 8 other patients, involved in a study on pharmacokinetic-pharmacodynamic modeling of propofol, ICG concentrations were measured using the PDD nose probe. To compare transcutaneous measurement of ICG-PDR with invasive measurements, multiple simultaneous ICG measurements in arterial blood were collected in a subset of 10 patients from the first group and all 8 patients from the second group. The studies were performed after obtaining approval from the Medical Ethics Committee of the Leiden University Medical Centre and the patients’ written informed consent. The patients were ASA physical status I or II scheduled for elective surgery. Exclusion criteria included a medical history of severe cardiovascular, respiratory, renal, hepatic, neurological or psychiatric disease, use of anti-hypertensive or anti-arrhythmic medication, pregnancy or lactation and a history of hypersensitivity to amide local anesthetics or indocyanine green. In 9 of the 41 patients (22%) chemical testing of liver function had been performed previously by our hospital laboratory and the results were within normal limits.

Procedures
Prior to the ICG clearance measurements a cannula was inserted in a large vein in the fossa cubiti. In 18 patients the radial artery was also cannulated for the collection of arterial blood samples. Pulse Dye Densitometry was performed using the DDG-2001 A/K (Nihon Kohden, Tokyo, Japan). Each session started with the intravenous administration of 10 mg ICG (Infracyanine®), followed by a rapid bolus of 20 ml of saline. In the group of patients in whom arterial blood samples were taken, a computer-controlled syringe pump was programmed to draw up to 33 arterial blood samples of 1.5 ml in the first 2 min after ICG administration. Starting from the second minute a waste sample was drawn first, to avoid mixing within the sampling line. The blood samples were collected in heparinized glass tubes and processed immediately. In the finger probe group, arterial sampling continued up to 15 min following ICG bolus administration. In the nose probe group, arterial blood sampling continued
6-10 min after bolus administration, depending on the return of consciousness, when measurements were terminated. Once complete mixing of the dye was observed, a median number of 7 arterial ICG concentration data points were available for fitting the log-linear elimination curve.

**Measurements of ICG in blood**

The concentrations of ICG were determined in whole blood using High Performance Liquid Chromatography (Separations analytical instruments, Hendrik-Ido-Ambacht, The Netherlands; column: Ultrasphere ODS 4.6 x 7.5 cm 244254, Beckman Coulter, Mijdrecht, The Netherlands) with ultraviolet and fluorescence detection. The fluorescence settings were as follows: excitation at a wavelength of 780 nm and emission wavelength at 810 nm with a gain of 100. ICG was also measured at 777 nm, its peak in the spectrum. Both ICG and its degradation product were identified by a diode array (Photodiode detector PDA 100, Dionex, Amsterdam, The Netherlands). The detection limit of the whole blood assay for ICG was at 0.15 or 0.2 mg.L⁻¹. The coefficient of variation was less than 6 % over the range from 0.5 to 10.45 mg.L⁻¹.

**Data collection and processing**

ICG-blood concentration data as determined by PDD and from the arterial blood were imported in a spreadsheet program (Excel 2000, Microsoft Corporation, Seattle, U.S.A.). K_{ICG}, the rate constant characterizing the ICG decay curve, was calculated by fitting a semi logarithmic regression curve through the declining part of the ICG concentration curve in the interval of 2-5 min after administration of the bolus ICG. The concentration curve is a monoexponential decay curve,

\[ C = C_0 \times e^{(-K_{ICG}t)} \]

where C is the ICG concentration, K (min⁻¹) is the elimination constant and t is time in min after administration of the bolus. ICG-PDR (%.min⁻¹) equals Kx100.

**Statistical analysis**

Comparison of the two methods (non invasive and invasive measurement of ICG) in both groups (finger and nose probe) was done by Bland-Altman analysis, reporting mean difference (bias) and Limits of Agreement (LOA, bias ± 2 SD). Descriptive statistics were generated by the SPSS statistical package (SPSS, version 14.0).
Results

Patients

The characteristics of the 41 patients in which the PDD probes were used for ICG determination, are presented in table 1. In a subset of 10 patients, 4 males and 6 females, 22 dual ICG arterial measurements were taken.

Table 1 Patient characteristics (data presented as median ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Finger probe group</th>
<th>Subset for arterial sampling</th>
<th>Nose probe group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>33</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>11 / 22</td>
<td>4 / 6</td>
<td>1 / 7</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>56.3 ± 18.8</td>
<td>54.2 ± 22.5</td>
<td>42.8 ± 9.6</td>
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<tr>
<td>Weight (kg)</td>
<td>72.8 ± 12.2</td>
<td>70.8 ± 9.2</td>
<td>65.1 ± 6.5</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.71 ± 0.09</td>
<td>1.70 ± 0.10</td>
<td>1.70 ± 0.10</td>
</tr>
<tr>
<td>Body mass index (kg m(^{-2}))</td>
<td>24.9 ± 3.7</td>
<td>24.0 ± 2.5</td>
<td>22.5 ± 2.3</td>
</tr>
</tbody>
</table>

ICG-PDR values in ASA physical status I-II patients

ICG-PDR measured transcutaneously in 41 healthy patients was 23.1 ± 7.9 % min\(^{-1}\) (n=41; 95% confidence interval 7.4 – 38.8). The range was 9.7 – 43.2 % min\(^{-1}\). The cumulative frequency distribution and the cumulative normal distribution calculated from the mean and standard deviation of the datasets measured by both PDD probes are presented in figure 1.

Comparison of transcutaneous and invasive ICG-PDR measurements

Bland-Altman analysis showed that with the finger probe, PDD overestimated ICG-PDR by 1.6 % min\(^{-1}\). The limits of agreement (LOA) were -5.2 to 8.3 % min\(^{-1}\) (Figure 2, panel A). The relative bias and LOA were 11.6% and -31.5 to 54.7% (Figure 2, panel B). Using the nose probe, PDD underestimated ICG-PDR by -6.0 % min\(^{-1}\). The limits of agreement were -15.5 to 3.4 % min\(^{-1}\) (Figure 3, panel A). The relative bias and LOA were -23.1% and -57.2 to 11.0% (Figure 3, panel B).
Discussion

The most important finding of this study is that in our healthy patient population, combining 41 non-invasive measurements by finger and by nose probes, the mean ICG-PDR was 23.1 % min⁻¹, with an SD of 7.9 (n=41; 95% confidence interval 7.4 – 38.8). The PDD finger and nose probe both performed adequately in the noninvasive determination of the ICG concentration.

Study limitations

We included otherwise healthy patients (ASA physical status I or II) who were scheduled for elective surgery. Patients with liver disease were excluded from the study based on patient history and physical examination. In 22% of the patients liver function tests (aminotransferases) were available and found to be within normal limits as set by the hospital laboratory.
Figure 2 Panel A represents the absolute results (bias and limits of agreement (± 2 SD) from the Bland-Altman analysis of indocyanine green plasma disappearance rate (ICG-PDR) measured by the pulse dye densitometry (PDD) finger probe or in an arterial blood sample. Panel B represents the relative difference in ICG-PDR measurement between the PDD finger probe and from arterial blood.
Figure 3 Panel A represents the absolute results (bias and limits of agreement (= ± 2 SD) from the Bland-Altman analysis of indocyanine green plasma disappearance rate (ICG-PDR) measured by the pulse dye densitometry (PDD) nose probe or in arterial blood. Panel B represents the relative difference in ICG-PDR measurement between the PDD nose probe and from arterial blood.
One may argue that ideally hepatic function testing should have been available in all patients. However, none of our patients had a history of hepatic dysfunction, showed any signs of hepatic dysfunction during physical examination or were scheduled for liver related surgery. Furthermore, patients suspected of malnutrition as associated with abnormal body mass index or substance abuse including alcohol abuse or any sign of bleeding disorder were excluded.

It is known that it is not possible to use ICG-disappearance to discriminate between a slight impairment in hepatic cellular function and a change in the hepatic blood flow.\textsuperscript{19} For practical purposes, one of these factors is therefore assumed to be constant, in order to interpret changes in ICG-PDR. Changes in the ICG-PDR can be caused by alterations in the excretory function of the liver, even before changes in serum bilirubin levels are observed.\textsuperscript{20} A decrease in ICG-PDR appears more sensitive in the detection of early liver failure than an increase in serum bilirubin level\textsuperscript{21,22} or critical illness scores like APACHE II.\textsuperscript{11} However, generally, changes in ICG-PDR are caused by changes in the splanchnic blood flow and thus result from alterations in hepatic blood flow.\textsuperscript{23} In the patients where the nose probe was used, measurements were performed during induction with a bolus dose of propofol. During induction changes in cardiac output, splanchnic blood flow and mean arterial pressure may occur. The results from the nose probe group were evenly distributed among the results from the finger probe group, in which measurements were performed in patients who were awake and under baseline conditions. This might be explained by the fact that the population studied with the nose probe was younger and more homogenous than the finger-probe population. The younger patients may have a more vigorous compensating mechanism to respond to changes in hemodynamics. Since the values found during induction did not compose a separate group, we chose to include the data in the representation of the ICG-PDR values found in healthy patients.

Ideally, repeated measurements would be performed using both the finger and nose probe in the same patient under stable conditions to gather more information on the variability of the measurement. Also, changing of physiological conditions like volume expansion would be nice to investigate further. However, due to the pre-elective surgery setting of the experiments and technical limitations, this was not possible.
Interpretation of the results

We concluded, in agreement with previously published data\textsuperscript{16,24,25}, that the PDD finger and nose probe noninvasively determine the ICG decay with a relative bias of 12% for the finger probe and -23% for the nose probe. The availability of this noninvasive transcutaneous measurement of ICG by pulse dye densitometry (PDD) makes ICG-PDR a favorable parameter in the evaluation of hepatic flow and/or function. A proper judgment between normal and impaired hepatic function or flow then only is realistic in the presence of a valid description of ICG-PDR in the healthy population. Up until now the normal range of transcutaneously measured ICG-PDR is poorly described\textsuperscript{17} as $>18 \text{ %\,min}^{-1}$.

We report in our study population of 41 patients without liver disease an ICG-PDR of 23.1 (7.9) %\,min$^{-1}$, using noninvasive PDD measurements. Fifteen of our 41 patients (37%) had an ICG-PDR of $<18 \text{ %\,min}^{-1}$. Ten patients (24%) had an ICG-PDR below 16 %\,min$^{-1}$. According to the study of Sakka and colleagues\textsuperscript{11} these persons may have higher mortality. In different studies in critically ill patients, or patient after hepatectomy in whom liver failure may be expected, adjustment of treatment and fluid administration based on a ICG-PDR of $<7 \text{ %\,min}^{-1}$ or $<16 \text{ %\,min}^{-1}$ is recommended.\textsuperscript{2,11} In case of liver transplantation, 24 h post-transplantation ICG-PDR values of $<18 \text{ %\,min}^{-1}$ may predict hepatic failure.\textsuperscript{7}

All the above mentioned studies illustrate the search for a clinical implementation of ICG-PDR, yet emphasize the difficulties in interpretation. Figure 4 illustrates these interpretation problems. Depicted are the cumulative normal distribution curves calculated from the means and standard deviations of the present surgical study population, the values of a group of patients who underwent major hepatectomy without and with postoperative liver failure as studied by Sugimoto\textsuperscript{2} and preoperative patients undergoing liver transplantation studied by Faybik.\textsuperscript{9} In all studies PDD was used to measure ICG-PDR. We have chosen these studies as they offer ICG-PDR data from patients with normal hepatic function, and patients with reduced and severely reduced functional liver tissue, respectively. The results in these studies are represented as mean and standard deviation; this is only valid if the results are normally distributed. We assumed the authors tested their data for normal distribution and simulated the data based on the reported mean and standard deviation. As can be observed from figure 4 a decrease in functional liver tissue not only decreases the mean ICG-PDR but also concurs with a decrease in variation around the mean, indicated by an increased steepness of the cumulative distribution curve. As the liver function of the group becomes better,
the distribution curve stretches to the right, but maintains its origin at a value below 10 %.min\(^{-1}\). This has implications for the ability of ICG-PDR to detect differences between patient groups. At any chosen threshold, ICG-PDR value groups will be easier distinguished if the distance between their curves at that value of ICG-PDR is larger. Obviously patients with terminal liver failure or with a decreased but survivable liver function after hepatectomy can be best discriminated from healthy patients around an ICG-PDR of 7.5 %.min\(^{-1}\). Similarly, the reduction in ICG-PDR of surviving post-hepatectomy patients in comparison to healthy patients is best observed around an ICG-PDR of about 20 %.min\(^{-1}\), as the distance between their curves is largest. Since the distance between these curves is diminished their distinction will be hindered by a decreased sensitivity and specificity.

![Cumulative frequency curves](image)

**Figure 4** Cumulative frequency curves calculated from the mean and average of the present study (small dash), patient without (dash-dot-dash) and with liver failure (long dash) developing on the first day after major hepatectomy studied by Sugimoto\(^{2}\) and preoperative patients undergoing liver transplantation surgery (solid line) studied by Faybik\(^{9}\).

We used receiver operating characteristics (ROCs) to further explore this subject, and this is represented in figure 5. The simulated patient populations from the 2 studies were compared to our healthy population to determine the ICG-PDR cutoff value with the highest sensitivity and specificity to identify the
affected patients. Taking into consideration all noninvasive measurements of ICG-PDR, the patients with liver failure in the study of Faybik would be best identified from our healthy patients, if a cutoff value for ICG-PDR of < 9 %.min\(^{-1}\) would be used, with a sensitivity of 98.5%, a specificity of 96.3%, and an AUC under ROC curve of 0.99 (Figure 5, panel A). Similarly, the patients with terminal liver failure after hepapectomy would be detected with a sensitivity of 98.7% and a specificity of 93.7% if an ICG-PDR value < 11 %.min\(^{-1}\) would be used. The AUC under the ROC curve is 0.99, indicating good accuracy (Figure 5, panel B). To detect decreased liver function of the patient surviving hepaectomy in the study of Sugimoto compared to our healthy patients the detection level would have to be set to ICG-PDR < 19 %.min\(^{-1}\), but the sensitivity would only be 69.8% and the specificity 82.4%. Similarly, the AUC under the ROC curve indicates only moderate accuracy with a value of 0.82 (Figure 5, panel C). Our exploration suggests that terminal liver failure can adequately be detected with ICG-PDR, but non-terminal decrease of liver function is harder to detect. This is supported by the finding of Sugimoto that they were unable to discern patients that would experience liver failure after major hepaectomy using preoperative ICG-PDR values. On the other hand, Hori and co-workers\(^7\) were able to detect differences in a homogeneous group of post liver transplant patients as soon as 24 h after transplantation between patients with good outcome (ICG-PDR of 21.0 ± 2.4 %.min\(^{-1}\), (mean ± SD) and bad outcome (ICG-PDR of 16.3 ± 2.1 %.min\(^{-1}\), (mean ± SD). We suggest that this finding is the result of the relative homogeneity of their study group and the accompanying diminished variability of their ICG values.

In conclusion, we evaluated the transcutaneous measurement and defined the absolute value of the ICG-disappearance rate by PDD in an otherwise healthy group of patients scheduled for general non-hepatic surgery. The PDD finger and nose probe showed a relative bias of 12% and -23% respectively, compared to the measurement of ICG-PDR in arterial blood. Within this healthy population the mean ICG-PDR was 23.1 %.min\(^{-1}\) (95% confidence interval 7.4 – 38.8), which is more variable than the value of >18%.min\(^{-1}\) which is usually referred to as normal value. Variability in transcutaneous measurement is probably larger than by intravascular fiberoptic measurement. We suggest that a broader evaluation of noninvasive measurement of ICG-PDR in the healthy population is warranted, before this parameter is used as a discriminating tool in the judgment on hepatic function or flow.
Figure 5 Representation of ROC curves (solid line) and the area under the curve versus the ICG-PDR value (dashed line) of three study populations. In panel A test results for the detection of liver failure in a population undergoing liver transplantation from the study of Faybik\textsuperscript{9} are used. An ICG-PDR cut-off value of 9\%.min\textsuperscript{-1} generates the optimum sensitivity and specificity in this population. In panel B test results for the detection of liver failure in a population undergoing major hepatectomy from the study of Sugimoto\textsuperscript{2} are used. An ICG-PDR cut-off value of 11\%.min\textsuperscript{-1} generates the optimum sensitivity and specificity in this population. Panel C represents the results from the population experiencing only decreased liver function after major hepatectomy, also from the study by Sugimoto\textsuperscript{2}. The cut off value of 19 \%.min\textsuperscript{-1} is much less accurate.
References


