Quality Improvement Study

Paradoxical alteration of indocyanine green concentration in bile and the visibility of the intra-operative fluorescence cholangiography in pigs

Shohei Yoshida\textsuperscript{a,}\textsuperscript{*}, Masashi Yoshida\textsuperscript{a,}\textsuperscript{b}, György Wéber\textsuperscript{a}, Domokos Csukás\textsuperscript{a}, Anna Blázovics\textsuperscript{c}, Györgyi Szabó\textsuperscript{a}, József Sándor\textsuperscript{a}, Hironori Ohdaira\textsuperscript{b}, Yutaka Suzuki\textsuperscript{b}, Andrea Ferencz\textsuperscript{a}

\textsuperscript{a} Department of Surgical Research and Techniques, Semmelweis University, Budapest, Hungary
\textsuperscript{b} Department of Surgery, International University of Health and Welfare Hospital, Tochigi, Japan
\textsuperscript{c} Department of Pharmacognosy, Semmelweis University, Budapest, Hungary

\textbf{ARTICLE INFO}

\textbf{Keywords:}
Indocyanine green
Fluorescence cholangiography
Fluorescence intensity ratio

\textbf{ABSTRACT}

\textbf{Background:} A significant difference exists between the reported optimal timing of indocyanine green (ICG) injection during fluorescence cholangiography and ICG dissipation time from the serum. There are no reports on alterations in ICG concentration in biliary fluid over time. Herein, we measured the concentration of ICG and the fluorescence intensity ratio between the common bile duct (CBD) and liver, which was recognized as a parameter of the visibility of the CBD.

\textbf{Materials and methods:} ICG (0.05 mg/kg) was injected intravenously into female pigs (n = 7). Afterwards, the fluorescence of the CBD and liver was detected at 30 min, 2 h, and 4 h. Biliary fluid was collected from cycled CBD tubes. The fluorescence intensity was measured using captured images and calculated using the ImageJ image-processing program. ICG concentration was measured using spectrophotometry and compared using an analysis of variance test.

\textbf{Results:} Biliary ICG concentrations at 30 min, 2 h, and 4 h were 92.07 ± 27.72 \(\mu\)g/mL, 37.14 ± 9.76 \(\mu\)g/mL (p < 0.05 vs. 30 min), and 13.91 ± 5.71 \(\mu\)g/mL (p < 0.05 vs. 30 min), respectively. The CBD/liver fluorescence intensity ratio at 30 min, 2 h, and 4 h were 1.25 ± 0.72, 2.39 ± 1.28 (p < 0.05 vs. 30 min and 4 h), and 3.38 ± 1.73 (p < 0.05 vs. 30 min and 2 h), respectively.

\textbf{Conclusions:} The ICG biliary concentration was highest at 30 min, whereas the CBD/liver fluorescence intensity ratio was highest at 4 h. Decreasing the fluorescence intensity of the liver may be an important approach for improving the visualization of the CBD during fluorescence cholangiography.

\textbf{Institutional protocol number:} PE/EA/491-5/2020.

1. Introduction

Fluorescence-guided surgery is increasingly being used as safer and less invasive surgical techniques are developed. This type of guided surgery is being used in hepatobiliary surgery. Ishizawa et al. first reported indocyanine green (ICG) fluorescence cholangiography (FC) in 2008 and showed its usefulness in displaying real-time intraoperative imaging of the hepatobiliary anatomy [1]. This technique involves the preoperative administration of intravenous ICG. Optimal timing of ICG (25 mg) injection for FC is reported to be 15–18 h preoperatively [2]. Matsumura et al. also reported that the administration of ICG (0.25 mg/kg) one day preoperatively may increase bile duct detectability on FC during laparoscopic cholecystectomy [3]. However, no report has shown alterations in ICG concentration in biliary fluid over time. More than 90% of intravenously injected ICG disappears from the serum within 15 min; therefore, there is a significant time gap between the ICG dissipation time and the optimal timing of ICG injection for FC [4–6]. To elucidate this gap, time-based measurements of alterations in ICG concentrations in the common bile duct (CBD) and biliary fluid are required. Furthermore, measurements of the fluorescence intensity of the liver and CBD are needed to compare the difference of the peaks between the ICG concentration and the fluorescence intensity over time.

\textbf{Abbreviation:} CBD, common bile duct; FC, fluorescence cholangiography; ICG, indocyanine green; SD, standard deviation; HEMS+, HyperEye Medical System Plus\textsuperscript{TM}.

\textsuperscript{*} Corresponding author. Keio University Hospital, 35 Shinanomachi, Shinjuku-ku, Tokyo, 160-8582, Japan.
E-mail address: shohei_yoshida@keio.jp (S. Yoshida).

https://doi.org/10.1016/j.amsu.2022.104923
Received 8 September 2022; Received in revised form 6 November 2022; Accepted 13 November 2022
Available online 15 November 2022
2049-0801/© 2022 The Authors. Published by Elsevier Ltd on behalf of JIS Publishing Group Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
The history of fluorescence-guided techniques in the field of surgery began in the 1950s. ICG has been used in the general field of medicine since its safety was approved by the FDA in 1954. Traditionally, ICG has been used for conducting hepatic function tests and assessing cardiac output in patients with congenital heart diseases [7,8]. In the 1970s, ICG was found to have a fluorescence characteristic, which was first applied in ophthalmic fundus angiography [9,10]. In the 21st century, the ICG fluorescence imaging method has been widely applied in several surgical fields, including FC, as an intraoperative navigation tool [4,11].

Due to the anatomical relationship between the liver and the CBD, the visibility of the CBD during FC largely depends on the contrast intensity between the bile duct itself and the structure behind it, most commonly the liver [2]. No reports have been published on alterations in ICG concentrations in the biliary fluid over time; therefore, this study aimed to measure the concentration of ICG in the biliary fluid. The ratio of the fluorescence intensity between the CBD and the liver was also measured over time. We used animals, in particular pigs, for this study in order to repeatedly sample the biliary fluid and measure the biliary concentration of ICG.

2. Materials and methods

2.1. Animal experiments

This study is fully compliant with the ARRIVE criteria [12]. All procedures were performed at a tertiary institution in accordance with the ethical guidelines of the National Scientific Ethical Committee on Animal Experimentation. This study was approved by the relevant country government office (approval number PE/EA/491-5/2020). Seven healthy, 5-month-old (body mass 40 ± 5 kg), domestic landrace, female pigs from a local conventional breeding facility were prepared in the Surgical Training and Research Laboratory of our tertiary institution. Each animal was administered isoflurane-maintained general anesthesia, and its vital parameters were constantly monitored during the procedure. The abdomen of each pig was shaved and opened using an open surgical approach. To collect the biliary fluid, the cystic duct was incised and a 6 Fr cannulation tube was inserted into the incision site. The tip of the cannulation tube was then brought to the CBD to collect the biliary fluid samples (2 mL). After the cannulation, ICG (0.05 mg/kg) was administered intravenously, and the fluorescence of the ICG emitted from the CBD was detected using a fluorescence camera, with the HyperEye Medical System Plus™ (HEMS+; MIZUHO Co., Tokyo, Japan) at 30 min, 2 h, and 4 h after injecting the ICG. Biliary fluid samples were simultaneously collected using cannulation tubes. After completing the procedure, each animal was euthanized.

2.2. Measurement of biliary concentration of ICG

Biliary fluids collected from the pigs were used for concentration measurements. Biliary ICG concentration was measured by absorption spectrophotometry using a Jasco V-550 spectrophotometer (JASCO Co., Tokyo, Japan).

2.3. Measurement of the bile duct-liver intensity ratio

The contrast of ICG fluorescence between the CBD and liver was evaluated by calculating the CBD/liver ratio. Still images were captured during the HEMS+ detection. Fluorescence intensity was measured using the ImageJ image-processing program (https://imagej.nih.gov/ij/; National Institutes of Health, Bethesda, USA).

2.4. Statistical analysis

Data analysis was performed using ANOVA4 on the Web (Copyright 2002 Kiriki Kenshi: https://www.hju.ac.jp/~kiriki/anova4/about.html). Differences between 30 min, 2 h, and 4 h groups were analyzed with the two-way analysis of variance and Holm post-hoc test, and data are presented as mean (m) ± standard deviation (SD).

3. Results

3.1. Biliary concentration of ICG

The changes in the concentration of ICG in biliary fluid over time are shown in Fig. 1. The biliary concentration of ICG was highest at 30 min after the administration of ICG. The concentration then decreased gradually and reached its lowest value at 4 h. The mean values of the biliary ICG concentration at 30 min, 2 h, and 4 h were 92.07 ± 27.72 μg/mL (n = 7), 37.14 ± 9.76 μg/mL (n = 7), and 13.91 ± 5.71 μg/mL (n = 7), respectively; p < 0.01 (analysis of variance). Comparisons of the measurements at 30 min and 2 h, as well as those at 30 min and 4 h, showed a significant difference (p < 0.05), whereas the difference between the 2 h and 4 h measurements was not significant.

3.2. CBD/liver intensity ratio

Representative images of the degree of visibility of the CBD are shown in Fig. 2. At 30 min, the fluorescence intensity of the liver was high. The abdominal cavity and CBD appeared dark. At 4 h, the fluorescence intensity of the liver was relatively low. The abdominal cavity and the CBD were brightly displayed.

The CBD/liver intensity ratio increased with time as shown in Fig. 3. The mean values of the CBD/liver fluorescence intensity ratio at 30 min, 2 h, and 4 h were 1.25 ± 0.72 (n = 7), 2.39 ± 1.28 (n = 7) (p < 0.05 vs. 30 min and 4 h), and 3.38 ± 1.73 (n = 7) (p < 0.05 vs. 2 h and 4 h), respectively. Comparisons between each time group showed significant differences.

4. Discussion

In this study, time-related changes in the ICG concentration in the biliary fluid were simultaneously measured and compared with the intensity ratio of the fluorescence of the CBD and the liver. According to our evaluations, the biliary concentration of ICG was highest 30 min after injection, whereas the intensity ratio of the CBD/liver fluorescence was highest 4 h after injection. Hence, the visibility of the CBD during intraoperative FC was the best at 4 h whereas the concentration of ICG in the bile was paradoxically the lowest.

FC has potential advantages over conventional radiographic cholangiography as it is less time consuming, easy to access intraoperatively, and does not emit radiation [4,11]. In a randomized controlled trial, FC was statistically superior to white light alone in visualizing extraperitoneal biliary structures during laparoscopic cholecystectomy [13]. Moreover, it has been reported that FC may reduce the risk of bile duct injury associated with catheterization required for the injection of contrast materials [11]. However, in FC, the ICG fluorescence intensity is likely to be stronger in the liver than in the bile duct [2].

Van den Bos et al. conducted ex vivo experiments and reported that the optimum ICG concentration was between 0.0039 and 0.025 mg/mL [14]. These experiments were performed with ICG diluted in 35 mg/mL albumin in a 0.9% NaCl solution. In our present study, the ICG concentration of the 4 h group (13.91 μg/mL: 0.01391 mg/mL) was within the optimum range, which could be one of the reasons the intensity ratio of the CBD/liver fluorescence was highest at 4 h.

However, in vivo observations are more complex than ex vivo experiments. It was believed that most of the ICG injected into the serum was bound to albumin [8,14]. Baker et al. pointed out that ICG binds to alpha-1 lipoprotein instead of albumin [15]. ICG also binds intimately to high-density lipoproteins but moderately to low-density lipoproteins [16]. Lipoproteins in the biliary fluid are derived from plasma lipoproteins. As components of biliary fluid are thus completely different from components found in ex vivo studies [16], the findings of ex vivo
Experiments are not entirely comparable to those of in vivo ones. Furthermore, in our study, the concentration of ICG at 2 h was close to the optimum concentration indicated by the ex vivo experiment of van den Bos et al. The difference in ICG concentration between 2 h and 4 h was not as evident as the difference in intensity ratio of the CBD/liver fluorescence at those times. Hence, it has been suggested that the biliary concentration of ICG is not a major factor in the visibility of the CBD during intraoperative FC.

Tsutsui et al. showed that the actual visibility of the CBD during FC was consistent with the intensity ratio of the CBD/liver [2]. A modern camera that detects ICG fluorescence, such as the HEMS+, can automatically adjust the brightness of the screen with the setpoint on the brighter area. The visibility of the CBD is expected to be lower when the intensity of the liver fluorescence is higher. Therefore, the actual visibility of biliary anatomy should not be measured using absolute values of individual fluorescence intensity, but rather by the intensity ratio of CBD/liver fluorescence, as was done in this study.

Accordingly, to increase the intensity ratio of the CBD/liver, it is important to decrease the intensity of the liver fluorescence rather than increase the intensity of the CBD fluorescence alone. Therefore, the time required to decrease the fluorescence intensity of the liver has been considered as one of the important reasons for the optimal timing of ICG.
administration. The present study shows that administration of ICG is required at least 4 h in order to attenuate the fluorescence of the liver and better visualize the CBD. This could also explain why Tsutsui et al. found that the optimal timing of ICG administration is 15–18 h prior to surgery [2].

One limitation of the present study is that changes could only be observed up to 4 h after ICG administration as it was not clear whether pigs could tolerate 18 h of continuous experimentation. Different experimental designs are necessary to study the changes in parameters and optimum conditions. However, the 4-h results of the present study provide a better understanding of the mechanisms underlying the visibility of the common bile duct.

To conclude, our findings suggest that decreasing the fluorescence intensity of the liver improves the visibility of the CBD during FC. However, the concentration of ICG in biliary fluid is not the most important determinant of the visibility of the CBD.

Ethical approval

This study was approved by Pest County Government Office (PE/EA/491–5/2020).

Funding support

This study did not receive any specific funding.

Author contribution

Before the experiments we had conference and all authors contributed to the study concept and design. Experiments were performed by Shohei Yoshida, Masashi Yoshida, György Wéber, Domokos Csukás, Györgyi Szabó and Andrea Ferencz. Data collection, data analysis and interpretation were performed by Shohei Yoshida, Masashi Yoshida and Anna Blázovics. Critical revision of the manuscript was done by Masashi Yoshida, József Sándor, Hironori Ohdaira and Yutaka Suzuki. The review and revision of the manuscript was performed by all of authors.

Registration of research studies

This study is animal experiment performed in accordance with the ethical guidelines of the National Scientific Ethical Committee on Animal Experimentation.

Guarantor

Masashi Yoshida accept full responsibility for the work and the conduct of the study, access to the data and controlled the decision to publish.

Consent

This study is animal experiment performed in accordance with the ethical guidelines of the National Scientific Ethical Committee on Animal Experimentation.

Provenance and peer review

Not commissioned, externally peer reviewed.

Statement of human and animal rights

All procedures were performed at Semmelweis University in accordance with the ethical guidelines of the National Scientific Ethical Committee on Animal Experimentation and this study was approved by Pest County Government Office (PE/EA/491–5/2020).

Declaration of competing interest

The authors have no conflicts of interest to declare.

Acknowledgements

The authors dedicate this paper to late Prof. Masaki Kitajima, who was a founding co-president of the Japan-Hungary-Poland Surgical Society. This paper was written in the memory of Prof. Masaki Kitajima, without whose leadership this international study would not exist.

References

[1] T. Ishizawa, S. Tamura, K. Masuda, T. Aoki, K. Hasegawa, H. Imamura, Y. Beck, N. Kokudo, Intraoperative fluorescent cholangiography using indocyanine green: a biliary road map for safe surgery, J. Am. Coll. Surg. 208 (2009) e1–e4, https://doi.org/10.1016/j.jamcollsurg.2008.09.024.
[2] N. Tsutsui, M. Yoshida, H. Nakagawa, E. Ito, R. Iwase, N. Suzuki, T. Imakita, H. Ohdaira, M. Kitajima, Y. Yanaga, Y. Suzuki, Optimal timing of preoperative indocyanine green administration for fluorescent cholangiography during laparoscopic cholecystectomy using the PINPOINT(R) Endoscopic Fluorescence Imaging System, Asian J. Endosc. Surg. 11 (2018) 199–205, https://doi.org/10.1111/res.12440.
[3] M. Matsumura, Y. Kawaguchi, Y. Kobayashi, K. Kobayashi, T. Ishizawa, N. Akamatsu, J. Kaneko, J. Arita, N. Kokudo, K. Hasegawa, Indocyanine green administration a day before surgery may increase bile duct detectability on fluorescence cholangiography during laparoscopic cholecystectomy, J. Hepatobiliary Pancreat. Sci. 28 (2021) 202–210, https://doi.org/10.1002/jhbp.855.
[4] B.J. Sandler, D. Sherwinter, L. Panait, R. Parent, J. Schwartz, D. Renton, SAGES Technology and Value Assessment Committee safety and effectiveness analysis on immunofluorescence in the operating room for biliary visualization and perfusion assessment, Surg. Endosc. 31 (2017) 3801–3810, https://doi.org/10.1007/s00464-017-6388-2.
[5] A. De Gasperi, E. Mazza, M. Prosperi, Indocyanine green kinetics to assess liver function: ready for a clinical dynamic assessment in major liver surgery? World J. Hepatol. 8 (2016) 355–367, https://doi.org/10.4245/wjh.v8.i7.355.
[6] A.H. Abdelhamid, L.A. Yannuzzi, K.B. Freund, J.J. Jueng, Differential response to glucocorticoid immunosuppression of two distinct inflammatory signs associated with punctate inner choroidopathy, Retina 41 (2021) 812–821, https://doi.org/10.1097/IAE.0000000000002950.
[7] E.H. Wood, Diagnostic applications of indicator-dilution technics in congenital heart disease, Circ. Res. 19 (1966) 531–568, https://doi.org/10.1161/01.res.10.3.3531.
[8] G.R. Cherrick, S.W. Stein, C.M. Leevy, C.S. Davidson, Indocyanine green: observations on its physical properties, plasma decay, and hepatic extraction, J. Clin. Invest. 39 (1960) 592–600, https://doi.org/10.1172/JCI94072.
[9] K. Kogure, E. Choromokos, Infrared absorption angiography, J. Appl. Physiol. 26 (1969) 154–157, https://doi.org/10.1152/jappl.1969.26.1.154.
[10] L.A. Yannuzzi, Indocyanine green angiography: a perspective on use in the clinical setting, Am. J. Ophthalmol. 151 (2011) 745–751, https://doi.org/10.1016/j.ajo.2011.01.043, e1.
[11] T. Ishizawa, S. Satura, N. Kokudo, Clinical application of indocyanine green-fluorescence imaging during hepatectomy, Hepatobiliary Surg. Nutr. 5 (2016) 322–328, https://doi.org/10.21037/hbn.2015.10.01.
[12] C. Kilkenney, W.J. Browne, I.C. Cuthill, M. Emerson, D.G. Altman, Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research, PLoS Biol. 8 (2010), e1000412, https://doi.org/10.1371/journal.pbio.1000412.
[13] F. Dip, E. LoMenzo, L. Sarotto, E. Phillips, H. Todeschini, M. Nahmod, L. Alle, S. Schneider, L. Kaja, L. Roni, P. Ferraina, T. Carus, N. Kokudo, T. Ishizawa, M. Walsh, C. Simpfendorfer, R. Mayank, K. White, R.J. Rosenthal, Randomized trial of near-infrared incisioonless fluorescent cholangiography, Ann. Surg. 270 (2019) 992–999, https://doi.org/10.1097/SLA.0000000000003178.
[14] J. van den Bos, F.P. Wieringa, N.D. Bossuyt, L.P. Stassen, Optimizing the image of fluorescence cholangiography using ICG: a systematic review and ex vivo experiments, Surg. Endosc. 32 (2018) 4820–4832, https://doi.org/10.1007/s00464-018-6233-x.
[15] K.J. Baker, Binding of sulfobromophthalein (BSP) sodium and indocyanine green (ICG) by plasma alpha-1 lipoproteins, Proc. Soc. Exp. Biol. Med. 122 (1966) 957–963, https://doi.org/10.3181/00379727-122-31299.
[16] S. Yoneya, T. Saito, Y. Komatsu, I. Koyama, K. Takahashi, J. Duvall-Young, Binding properties of indocyanine green in human blood, Invest. Ophthalmol. Vis. Sci. 39 (1998) 1286–1290.