Editorial Commentary

Transforming a clinical fluorescent dye to sense and treat iron overload disorders: a new reverse translational approach in precision medicine

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Iron overload disorder (IOD) is a group of diseases characterized by a gradual build-up of excess iron in organs, especially in the liver, heart, and pancreas. IOD consequently induces accumulative oxidative stress and tissue injury before clinical symptoms appear (1). The common form of IOD includes hereditary hemochromatosis (genetic IOD) and ineffective erythropoiesis (acquired IOD) (1-3). Iron overload in the liver is considered a co-factor for the progression of liver diseases, such as in patients with chronic hepatitis (4), non-alcoholic fatty liver disease (5), chronic viral hepatitis, and hepatocellular carcinoma (6). Maintaining a high vigilance for IOD is essential to minimize the incidence of organ damage from iron overload. A number of diagnostic techniques can quantify iron overload *in vivo*, including MRI (7-10). Although the quantification of liver iron concentration (LIC) through the MRI approach may guide the further diagnostic and therapeutic initiation, accurately distinguishing between normal iron stores and iron overload can be a challenge (11,12).

Recent advances in the understanding of metal-organic supramolecular assembly in biological systems provide new insights into the diagnosis and treatment of diseases. Reports suggest that several stimuli-responsive self-assembled or disassembled metal-organic dye nanocomplexes, including metal-organic nanodrugs (13,14), membrane-coated nanosensitizers (15,16), and nanosonosensitizers (17,18), can control the metal-bio interactions and activate spontaneously in a physiological milieu with diagnostic/therapeutic components, which draws increasing attention in precision medicine.

Among the existing small molecule dyes, indocyanine green (ICG) was the first fluorescent dye approved by the United States Food and Drug Administration (FDA) for diagnostic imaging, including lymphangiography, intra-operative lymph node identification, identification of tumor margins, and superficial vascular imaging. The well-studied safety profile and functional diversity of ICG define the high potential for reverse translation in medical research and clinical settings (19). Notably, growing number of studies have reported that ICG dye can strongly coordinate to iron ions with selective and stimulated switch-on therapeutic effect/signal readout in the living system (16,20). Recently, Lin *et al.* reported a novel strategy that repurposes ICG dye for high-accuracy evaluation and quantification of LIC *in vivo* (21).

ICG has a complex molecular structure that consists of two polycyclic (benzoindotricarbocyanine) lipophilic moieties with conjugated backbone, which contribute to its near-infrared (NIR) fluorescence property, and two extended sulfonyl groups impart the hydrophilicity and chelating activity. The new study of Lin *et al.* (21) builds

on previous works in supramolecular assembly to confirm the formation of multiple coordination bonds between ICG and iron ions which leads to stimulated theragnostic applications (13,14,16-19,22). Lin *et al.* proposed and validated that a self-assembly ICG-Lecithin (ICG-Leci) system can effectively coordinate with focal iron to form stable bivalent Fe(III)-ICG_2 complexes. This ICG activated Fe(III) chelation framework creates compact architecture that shields iron ions from water molecules and lowers water exchange rates, producing an augmented T_1 contrast effect. Such stable Fe(III) chelate of ICG promotes iron excretion and reduces iron toxicity. To validate the sensitivity and specificity of ICG-iron chelation for MR liver iron quantification, Lin *et al.* initially identified the correlation between r_1 values and Fe(III) ions with and without the presence of ICG molecules in different main magnetic fields from 1.5, 3 to 9.4 T. The results showed that the addition of ICG remarkably reduced the r_1 value of Fe Fe(III) ions by approximately 50-fold (21). A comparable reduction of the r_1 value was also observed in Fe(III)/aggregated-ferritin mixture. In comparison, no observable r_1 value change was noticed when ICG was added to hemosiderin-like aggregated ferritin phantoms. The T_2* values showed no correlation with iron concentration (>0.1 mM). Lin *et al.* speculated that the significantly low r_1 value of Fe(III) ions was achieved primarily by forming a stable hydrophobic Fe(III)-ICG coordination network, which reduces the local water exchange rate and leads to a shorter spin-lattice relaxation time and a lower r_1 value.

Lin *et al.* (21) next explored the optical and photoacoustic (PA) characteristics of ICG-Leci and described the *in vitro* results, and indicated that ICG can sensitively respond to cellular iron concentration and produce an amplified PA signal. It was demonstrated that the "bi-augmentation" of MRI and PA contrast effects is in consequence of photophysical property changes of ICG. Leci facilitates ordered aggregation and promotes the formation of J-aggregation of ICG upon exposure to Fe(III) in the biological milieu, which results in increased PA intensity in the NIR window and a significant decrease in the value of r_1 of Fe(III) ions. These results suggested that ICG/Leci may enable high specificity and sensitivity for the detection of iron deposition. Furthermore, an *in vivo* study was carried out in two different iron-overload phenotype knockout mice ($Hfe^{-/-}$ mice and $Hjv^{-/-}$ mice) to evaluate the performance of ICG-mediated iron-overload quantification (21). It was found that the signal changes in the liver of $Hfe^{-/-}$ and $Hjv^{-/-}$ mice were negatively correlated with iron concentration, which showed apparent contrast changes of 19.19% and 29.97% at 4 hours, respectively (21). To further explore the iron concentration detection ability of ICG in chronic viral hepatitis, Lin *et al.* examined the T_1 signal response in hepatitis B virus transgenic (HBV-Tg) mice with hepatic iron overload. T_1 signal intensity of the muscle/T_1 signal intensity of the liver (T_1SI_M/T_1SI_L) of the liver tissues demonstrated a 14.91% change in the relative T_1 signal intensity at 4 hours post-injection (21). The ICG contrast effect was in agreement with the LIC measurement. Furthermore, Lin *et al.* performed a pilot clinical assessment of patients with chronic viral hepatitis-related hepatocellular carcinoma to study the MRI performance of ICG in iron-overload conditions prior to the surgical removal of the tumor. It was observed that the health volunteer detected a –3.20% change of T_1 signal intensity of liver tissues, while patient case one showed a –0.5% change at 1 versus 0 hour, and 14.71% change in patien case two. The LIC measurement confirmed the MRI results and further support the rationale for developing ICG as a contrast agent in iron-overload quantification.

By virtue of the coordination feature of ICG, Lin *et al.* explored binding affinities of ICG toward Fe(III) for potential chelation therapy (21). A significant improvement in iron chelation effects of ICG was observed compared to deferoxamine (DFO, an iron-chelating agent for treatment of iron overload). This was validated by determining the dissociation constant using the isothermal titration calorimetry (ITC) technique and calculating aggregation modes between ICG and ferric chloride molecules through the Gaussian 09 simulation. For the *in vitro* chelation effect and corresponding fluorescence, MR and PA imaging were verified in mouse primary hepatocytes. Based on *in vitro* findings, Lin *et al.* investigated the *in vivo* biodistribution of ICG and ICG/Leci in the liver through fluorescence and PA monitoring. After 4 hours injection of ICG/Leci, approximately 75 and 225% PA contrast enhancement was achieved in the liver of $Hfe^{-/-}$ and $Hjv^{-/-}$ mice, respectively. Similarly, the tissue specimens from diagnosed iron-overload patients were stained by ICG/Leci, and PA imaging clearly visualized the iron-deposited section and was in good agreement with the Prussian blue staining (21).

Beyond the bimodal diagnostic applications of ICG/Leci formulas in iron-overload conditions, ICG/Leci demonstrated a comparable iron depletion efficacy to DFO treatment. The pharmacokinetic study showed a 1.5-fold higher fecal iron content in mice after receiving ICG/Leci, which implies more favorable kinetics and biocompatibility

than DFO (21). The histochemical and blood biochemical analyses further verified that ICG or ICG/Leci formulas are promising as a new and safe iron chelator candidate for iron-overload diagnosis and treatment (21). The study of Lin *et al.* used this strategy via ICG-mediated chelation of ferric ions in the liver to amplify the MR diagnostic sensitivity and treatment efficacy in iron overload subjects (21).

Although the current concept is at an early stage for clinical translation, the initial findings are impressive regarding the high specificity and sensitivity of ICG/Leci in MR/PA-based LIC quantification and iron chelation therapy. Future clinical studies are needed to fully characterize the safety and efficacy of the ICG-guided theragnostic approach in iron overload patients. It is undoubtedly essential to monitor the potential effects of dose-response relationships and phototoxic effects during application. A lot of room for improvement may still exist for this new strategy of supramolecular approaches.

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