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Highlight

ICG/Lecithin: A promising theranostic agent for simultaneous therapy and diagnosis of MRI and PAI

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ABSTRACT

A chronic liver disease usually results in iron accumulation, and an excess of iron will further aggravate liver injury, forming a vicious circle. Likewise, it also plays a significant role in other organs when it comes to iron metabolism. A long time passes between the time it takes to break through to MRI-based iron diagnosis and its ability to distinguish the types of iron accumulation accurately and quickly. This work highlighted a new type of iron accumulation treatment solution integrated with diagnosis and treatment. A chelating method for ICG and Leci that can assist PAI and MRI to achieve better diagnostic and therapeutic effects. This work revealed biomaterial engineering techniques are being adapted to address clinical medical problems through cutting-edge research.

In the human body, iron is a basic inorganic nutrient, which is essential to physiological processes, such as cellular metabolism and cell growth [1]. However, studies have shown that iron excess can result in diseases as well as aggravated morbidity, including thalassemia [2], myelodysplastic syndromes (MDS) [3], non-alcoholic fatty liver disease (NAFLD) [4], metabolism disorders [5], and cancers [6]. Research examining the number of papers in PubMed indicates that reports of iron overload have been on the rise since 1990, and iron overload-related diseases are still attracting attention, as shown in Fig. 1. Early detection and timely removal of iron overload are the current methods for curing these diseases. However, there is no known method to diagnose and treat iron overload simultaneously. In terms of diagnosis, although magnetic resonance imaging (MRI) is the most commonly utilized to detect liver iron concentration (LIC), its clinical application is limited as it is imprecise, time-consuming, and lacks the specificity of tissue and iron type [7]. A new technology that is precise, rapid, probing-based, tissue-specific, and that simultaneously integrates diagnosis and therapeutic treatment is therefore urgently needed.

More recently, the supramolecular assembly system of ICG/Leci was demonstrated to be a probe for MRI and PAI diagnosis imaging with combined therapeutic effects on iron scavenging [8]. There were no methods available before that could diagnose iron overload while also treating it simultaneously. ICG, an FDA-approved clinical drug, was widely utilized as a MRI contrast agent for measuring liver function dynamically [9]. Lin *et al*. discovered a specific correlation between ICG probed MRI signal and ferric ions (Fe^{3+}) concentration, not with other ions such as zinc (Zn^{2+}), magnesium (Mg^{2+}), copper (Cu^{2+}), calcium (Ca^{2+}) and gadolinium (Gd^{3+}). The MRI results of the mouse liver area showed that a significant reduction of $1/T_1$ by approximate 50-fold in the presence of ICG indicated a linear relationship between the Fe^{3+} levels and $1/T_1$ signal. This relationship was not observed in T_2 values. Furthermore, two key genes of iron metabolism, HFE and HJV, were knocked out in mice, resulting in multi-organ iron overloaded $Hfe^{-/-}$ and $Hjv^{-/-}$ mice [10]. Compared to WT mice, $Hfe^{-/-}$ and $Hjv^{-/-}$ mice showed the significant changes of T_1SIR (T_1 signal intensity in muscle/T_1 signal intensity in liver) after ICG treatment in 1 and 4 h. Significant changes in T_1SIR were also observed in HBV transgenic mice (HBV-TG) 4 h after ICG injection. Atomic absorption spectroscopy was consistent with the MRI results, indicating that HBV transgenic mice have iron accumulation in the liver. Two patients with chronic viral hepatitis-related hepatocellular carcinoma were injected with ICG and imaged by MRI before tumor resection. These results support those obtained in mice, which suggest that ICG may act as a possible quantitative MRI contrast agent in iron-loaded conditions.

Photoacoustic imaging (PAI), a non-invasive, low-cost imaging technology, that acquires images by analyzing light absorption properties, has gained academic widespread popularity [11]. PAI compensates for the limitations of existing imaging modalities by

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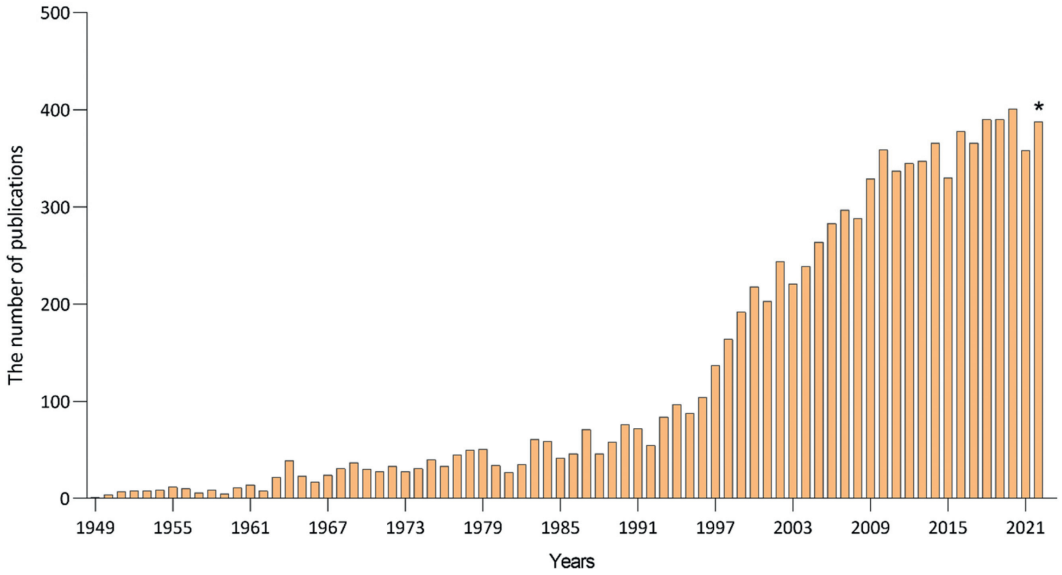

Fig. 1. The number of publications per year related to iron overloaded diseases since 1949. *: to the end of November 2022 (searched by "iron overloaded diseases").

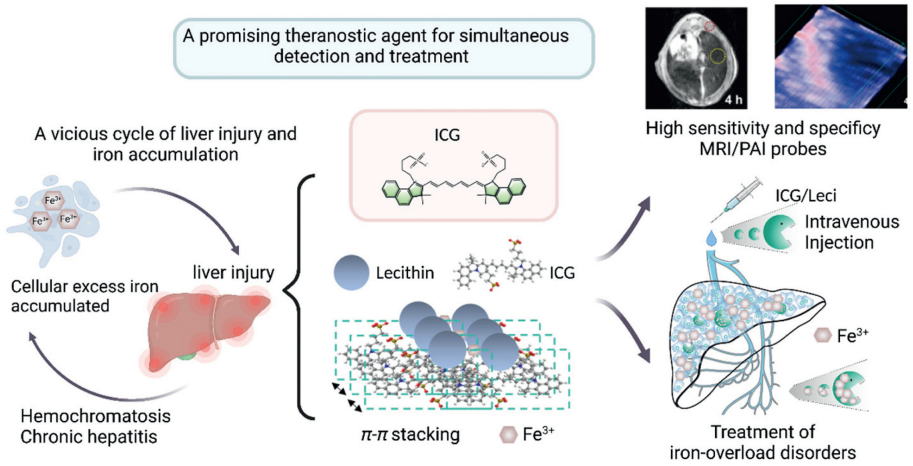

Fig. 2. The schematic illustration for ICG/Leci as a theranostic reagent of iron overload.

supplying diagnostic information that would otherwise be unavailable, such as tissue oxygenation levels and high-resolution vascular networks. Lin *et al.* innovatively proposed the use of PAI as an auxiliary MRI technique for diagnosing iron overload and developed a diagnostic probe compatible with both MRI and PAI. In spite of the fact that ICG can detect the dose of Fe^{3+} by its absorption peak at 875 nm, it is time consuming and has high detection limit (Fe^{3+} concentration >3 μmol/L). To solve this issue, supramolecular assembly technology was used to chelate ICG with lecithin, forming an ICG/Leci system. Chelation of metals can increase ordered aggregation and affect ICG's optical properties [12]. ICG/Leci chelation with Fe^{3+}, forming π-π stacking, is specifically characterized by a peak at 890 nm. It was shown that ICG/Leci could significantly improve the efficiency and specificity of Fe^{3+} detection of PAI at 890 nm. In comparison with ICG alone, ICG/Leci did not significantly alter the signal of MRI for hepatic iron overload diagnosis. Based on these results, ICG/Leci can serve as a multimodal probe for both MRI and PAI for iron overloaded detection.

For biosafety testing, primary hepatocytes were treated with different dose of Fe^{3+} for 4 h and then incubated with either ICG or ICG/Leci for another 20 h. It was found that ICG and ICG/Leci did not induce hepatocyte death and were capable of counteracting the damage caused by high Fe^{3+} concentrations. The Western blot of H-Ferritin showed that ICG/Leci could more efficiently protect the cells from the oxidative injury from Fe^{3+} than ICG only. Deferroxamine (DFO), one of the most prescribed iron scavenger drugs in the world, was introduced to this study as a positive control for *in vivo* toxicity and iron metabolism tests. $Hfe^{-/-}$ mice were grouped in placebo (PBS), DFO, ICG and ICG/Leci. The DFO and ICG groups depleted iron in liver and spleen in comparable measures, however, the ICG/Leci group showed the greatest improvement, with 100% lower iron levels than the other two groups. Regarding the iron excretion, ICG and ICG/Leci groups had 1.5-fold higher iron levels in feces than DFO groups. Having to do with DFO's main mechanism of excretion through urine, it is the main cause of kidney inflammation and damage. ICG/Leci treatment significantly reduced kidney inflammatory cells infiltration as assessed by HE stains. ICG/Leci, based on these results, is a safe and effective diagnostic and therapeutic reagent.

In summary, ICG/Leci can be used not only as a high-sensitivity and high-resolution imaging probe for MRI and PAI, but also for the treatment of hepatic iron overload (Fig. 2). It is the first method to integrate treatment and diagnosis, with high levels of safety. In order to implement the method, no high-tech equipment

is needed. This approach requires an MRI sequence that can be obtained in a routine clinic setting, so it has a high likelihood of being promoted clinically. Hemochromatosis and thalassemia as well as myelodysplastic syndromes that are associated with iron overload can be diagnosed and treated with this method. Over time, excess liver fat leads to iron accumulation, iron apoptosis, and ultimately necrosis of the liver cells [13]. Presently, many liver disease patients suffer from fatty liver, which is showing a younger age. Currently, there are no effective treatments for fatty liver, relying instead on diet and exercise metabolism. By making this technology available for patients with fatty livers, the number of people who can benefit greatly will increase in the future. Patients with liver cancer will be able to achieve chemotherapy through a combination of super-stable homogeneous iodinated formulation technology (SHIFT) [14–16] and anticancer drugs using this method.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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