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Research review paper

Indocyanine green delivery systems for tumour detection and treatments

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ABSTRACT

Indocyanine green (ICG) is a cyanine compound that displays fluorescent properties in the near infrared region. This dye is employed for numerous indications but nowadays its major application field regards tumour diagnosis and treatments. Optical imaging by near infrared fluorescence provides news opportunities for oncologic surgery. The imaging of ICG can be useful for intraoperative identification of several solid tumours and metastases, and sentinel lymph node detection. In addition, ICG can be used as an agent for the destruction of malignant tissue, by virtue of the production of reactive oxygen species and/or induction of a hyperthermia effect under irradiation. Nevertheless, ICG shows several drawbacks, which limit its clinical application. Several formulative strategies have been studied to overcome these problems. The rationale of the development of ICG containing drug delivery systems is to enhance the *in vivo* stability and biodistribution profile of this dye, allowing tumour accumulation and resulting in better efficacy. In this review, ICG containing nano-sized carriers are classified based on their chemical composition and structure. In addition to nanosystems, different formulations including hydrogel, microsystems and others loaded with ICG will be illustrated. In particular, this report describes the preparation, *in vitro* characterization and *in vivo* application of ICG platforms for cancer imaging and treatment. The promising results of all systems confirm their clinical utility but further studies are required prior to evaluating the formulations in human trials.

Contents

1. Introduction

Indocyanine green (ICG) is an amphiphilic, inert (nonionizing) and non-toxic compound having a molecular weight of 751.4 Da (Alander et al., 2012) and a hydrodynamic diameter of 1.2 nm (Polom et al.,

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2011). The tricarbocyanine dye is composed of two hydrophobic polycyclic parts connected to a carbon chain. Each polycyclic part is attached to a sulphate group, which results in hydrophilic properties (Desmettre et al., 2000). Kodak (Rochester, NY) developed ICG in 1955 using near infrared (NIR) technology (Dip et al., 2015) and the Food and Drug Administration (FDA) immediately commended this fluorescent agent for clinical use (Engel et al., 2008; Fox et al., 1956).

ICG, as well as other NIR fluorescent dyes, has achieved notable attention in many fields of biomedicine due to several advantages, such as decreased light scattering, as well as optimal level of tissue penetration and minimal levels of interference concerning auto-fluorescence from biological samples (Escobedo et al., 2010). In spite of the numerous benefits showed by different dyes within the NIR spectrum, the only fluorescent dye introduced in clinical application is ICG (Pauli et al., 2010). Its wide acceptance is due to its low toxicity (after intravenous administration LD_{50} of 50–80 mg/kg for animal subjects) (Costa et al., 2001), the fast binding with plasma proteins and quick excretion by the liver into bile juice. This dye fixes rapidly and intensely to serum proteins *in vivo* after intravenous injection, producing a fluorescence signal (Shimada et al., 2015) without alteration of protein structures (Kochubey et al., 2005). In addition, ICG exhibits absorption maximum at a hemoglobin isosbestic point, hence the spectrophotometric evaluation of the dye is not dependent on oxygen saturation and serum bilirubin level (Vos et al., 2014). In tissue and cells, the ICG emission peak is slightly moved to longer wavelengths compared with the ICG peak in aqueous solution (Desmettre et al., 2000). Fluorescent luminescence is emitted at 840 nm as an ICG–protein complex is exposed to an excitation source with a wavelength between 750 and 810 nm (Kokudo and Ishizawa, 2012). Because hemoglobin and water absorb intensely at this wavelength, structures in which ICG is present can be visualized through 5–10 mm of tissue by using an appropriate camera that is sensitive to infrared light, combined with an appropriate filter (Yamamichi et al., 2015). As reported in literature, halogen lamps (Meier et al., 2008) and LEDs (Tsujino et al., 2009) are used as light sources in imaging instruments for ICG detection. In this case, an appropriate filter is required to avoid the mixing of excitation and emission rays (Alander et al., 2012). On the contrary, no filter is usually needed when using a laser source (Raabe et al., 2003). The emitted fluorescence can be detected using specific cameras and identified on the monitor connected to the camera. With this mechanism, a fluorescence imaging device allows the detection of anatomical structures where ICG is present such as blood vessels, lymph nodes and biliary ducts (Boni et al., 2015).

Currently, ICG is widely employed for diagnostic and therapeutic applications by virtue of its fluorescent properties. During the twenty-first century, ICG applications have been remarkably increased, confirming the multifunctional role of ICG. For many years, this dye has been employed in medicine in several clinical settings such as ophthalmic angiography (Destro and Puliafito, 1989; Flower and Hochheimer, 1976; Stanga et al., 2003), cardiac output measurements (Desai et al., 2006; Lund-Johansen, 1990; Reuthebuch et al., 2004) and hepatic function studies (Caesar et al., 1961; Dorshow et al., 1998; Halle et al., 2014; Meijer et al., 1983), but recently many studies proving the potential of ICG in other fields have been published. The use of ICG has been extended to open and laparoscopic/robotic surgery, especially in angiography allowing the direct intraoperative visualization of the blood vessels in several surgical procedures. The use of ICG video angiography in vascular surgery was discussed for the first time by Raabe et al. (2003), proving that the new procedure is very promising.

Over the years, ICG became very popular in neurosurgery and it was found to be clinically useful for various diseases such as complex aneurysms, atero-venous fistulas and atero-venous malformations (Scerrati et al., 2014). Afterwards cardiovascular surgery represents another application field for ICG fluorescence imaging, as the dye can be used in simple angiography, in cardioplegia delivery, and other several procedures.

By exploiting the ICG excretion by the bile, it seems obvious that one of its applications is the visualization of the biliary tree (Boni et al., 2015). In addition to ICG application in open cholecystectomy (Ishizawa et al., 2009), Ishizawa et al. (2010) developed a new ICG fluorescent cholangiography technique to delineate the bile duct anatomy during laparoscopic cholecystectomy. The authors confirmed the potential of the ingenious procedure as a possible alternative for conventional radiographic cholangiography, preventing bile duct injury in laparoscopic surgery. Another study was conducted by Y. Kono et al. (2015) to evaluate the effect of clinical and technical conditions on the ability of the technique after preoperative intravenous injection of ICG. *Ex vivo* studies performed with five laparoscopic fluorescence systems and one conventional system for open surgery confirmed that it is necessary to optimize the fluorescence system to allow the use of ICG cholangiography as an essential tool for bile duct navigation.

Other experimental works support the applicability of ICG for additional purposes, including rheumatoid arthritis (Fischer et al., 2010; Werner et al., 2013), burns and other trauma (Fourman et al., 2014; Kamolz et al., 2006) and muscle perfusion (Habazettl et al., 2010; Vogiatzis et al., 2015), but nowadays ICG plays the main role in the diagnosis and treatments of tumours. On the one hand, ICG imaging permits confirming the targeting of the tumour region. On the other hand, ICG induces the production of a hyperthermia effect and/or reactive oxygen species (ROS) irradiating the specific region with a laser light having a suitable wavelength.

At present, ICG is extensively employed in NIR fluorescence (NIRF) cancer surgery because it has multiple roles within this context. In fact, ICG applications in this field concern sentinel lymph node (SLN) detection and identification of cancers during surgical procedure, as well as intraoperative angiography (Schaafsma et al., 2012). SLN biopsy using ICG fluorescence reduces time of surgery, and improves lymph node detection allowing a surgeon to make a minimal incision and prolonging high identification rate for hours (Schaafsma et al., 2012). To date, ICG-guided SLN mapping has been extensively considered for several cancers: breast cancer (Murawa et al., 2009; Samorani et al., 2015; Tagaya et al., 2008; Troyan et al., 2009; Verbeek et al., 2014), gastric cancer (Miyashiro et al., 2008; Kusano et al., 2008; Tajima et al., 2009; Takeuchi and Kitagawa, 2015), vulvar cancer (Crane et al., 2011; Hutteman et al., 2012), endometrial cancer (Holloway et al., 2012, Plante et al., 2015), cervical cancer (Crane et al., 2011; Schaafsma et al., 2012; Jewell et al., 2014), lung cancer (Moroga et al., 2012; Yamashita et al., 2011), head and neck cancer (Betz et al., 2009; Kogashiwa et al., 2015; Nakamura et al., 2015), skin cancer (Gilmore et al., 2013; van der Vorst et al., 2013b), colorectal cancer (Cahill et al., 2012; Hirche et al., 2012; Hutteman et al., 2011; Liberale et al., 2015), atero-venous fistulas and others (Handgraaf et al., 2015; Hirche et al., 2011). In addition, some combinations of NIRF and radioactivity for SLN mapping have been reported (Brouwer et al., 2012; Stoffels et al., 2015; Tsuchimochi et al., 2013; Van den Berg et al., 2012; Van der Poel et al., 2011).

Although ICG use in SLN mapping has introduced NIRF imaging in cancer surgery, its key application is in intraoperative tumour detection. Indeed, optical imaging represents a valuable non-invasive and high-resolution approach during surgical procedures. Among various cancers, the identification of hepatic tumours has received a lot of attention as a model of cancer-specific fluorescence imaging during open and laparoscopic surgery (Kudo et al., 2014). Particularly, selective uptake and retention of ICG by hepatocellular carcinoma (HCC) tumours has been revealed. As reported by Lim et al. (2014), the contrast between fluorescent cancer and non-fluorescent tissue depends on the time between ICG injection and the fluorescence measurement. In 2009, Ishizawa et al. first introduced the application of ICG imaging in hepatic surgery for the visualization of colorectal liver metastases and hepatocellular carcinomas (HCCs). Although further studies are needed to clarify the accumulation mechanism of ICG, the authors confirmed the ICG detection in non-cancerous hepatic parenchyma around metastases in the majority of poorly differentiated HCCs. On the contrary, biliary excretion disorders associated with cancer progression led to accumulation of the