European Journal of Pharmaceutics and Biopharmaceutics 117 (2017) 160–167

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Engineered polymeric microspheres obtained by multi-step method as potential systems for transarterial embolization and intraoperative imaging of HCC: Preliminary evaluation

Elena Piera Porcu [a,1], Andrea Salis [b,1], Giovanna Rassu [b], Marcello Maestri [c], Jacopo Galafassi [c], Giovanna Bruni [d], Paolo Giunchedi [b,*], Elisabetta Gavini [b]

[a] Experimental Medicine, Department of Clinical-Surgical, Diagnostic and Paediatric Sciences, University of Pavia, Pavia, Italy
[b] Department of Chemistry and Pharmacy, University of Sassari, Sassari, Italy
[c] IRCCS Policlinico San Matteo Foundation and Department of Clinical-Surgical, Diagnostic and Paediatric Sciences, University of Pavia, Pavia, Italy
[d] Department of Chemistry, Physical Chemistry Section, University of Pavia, Pavia, Italy

ARTICLE INFO

Article history:
Received 26 September 2016
Revised 6 April 2017
Accepted in revised form 11 April 2017
Available online 13 April 2017

Keywords:
Transarterial embolization
Microspheres
Indocyanine green
Fluorescence imaging
Chitosan
Cellulose acetate butyrate

ABSTRACT

The aim of this study was the development of novel fluorescent microspheres as embolic agent for transarterial embolization (TAE) of advanced stages of hepatocellular carcinoma (HCC). TAE is a minimally invasive procedure that induces tumour regression blocking the blood flow by injection of microparticles. The microspheres currently used in clinical application cannot be visualized *in vivo*. Surgeon could exploit the intraoperative detection of embolic agents during resection of the malignant mass. Biocompatible indocyanine green (ICG)-loaded microspheres (CAB-CS-ICG) were prepared using a multi-step method. Chitosan (CS)-ICG particles were prepared via spray-dryer and then loaded into cellulose acetate butyrate (CAB) microspheres, fabricated by emulsion solvent extraction method. Technological parameters such as yield, size, encapsulation efficiency and morphology were studied. CAB-CS-ICG microspheres showed spherical shape and smooth surface, as well as good injectability through a 21 G × 1½ needle. ICG release from CAB-CS-ICG was very low due to the strong interaction between CS and ICG. This result was also confirmed by *in vitro* fluorescence imaging studies, conducted using Photodynamic Eye (PDE) for the detection of particles incubated in human plasma. CAB-CS-ICG were capable to maintain the fluorescence selectivity for 4 weeks. Our data suggested the potential usefulness of CAB-CS-ICG in TAE application as embolic agents and following imaging of tumour during surgical procedure.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancer, with an increasing incidence in developed countries during the past 20 years [1]. Different treatment modalities are available for this malignancy, classified in palliative, potentially curative and symptomatic [2]. Although the hepatic surgery remains the most efficient method to treat HCC, the majority of patients are not suitable for surgical resection [3]. Indeed, the surgical approach is the primary method for early- and early-stage HCC, but most tumours are diagnosed at intermediate and advanced stages for which this curative treatment is not feasible [2,4,5]. In that case, the options are limited to palliative treatments, including transcatheter intra-arterial therapies and systemic chemotherapy [4,6]. Transarterial embolization (TAE) and chemoembolization (TACE) are loco-regional approaches widely used to treat HCC [7]. TACE implies the localized administration of chemotherapeutic drugs combined with the use of embolic materials, whereas TAE consists of embolization without chemotherapy [5,8]. Both procedures take advantage of the unusual structure of the liver that possess a dual blood supply. Although normal liver tissue receives its nutrient supply mainly from the portal vein, most HCCs are

Abbreviations: CAB, cellulose acetate butyrate; CS, chitosan; ICG, indocyanine green; CS-ICG, particles based on CS and ICG crosslinked with TPP; CS-ICG(L), CS-ICG microparticles obtained by freeze-drying; CS-ICG(SP), CS-ICG microspheres obtained by spray drying; CAB-ICG, CAB microspheres containing free ICG; CAB-CS-ICG, CAB microspheres containing CS-ICG(SP); HCC, hepatocellular carcinoma; PDE, photodynamic eye; TAE, transarterial embolization.

* Corresponding author at: Department of Chemistry and Pharmacy, University of Sassari, via Muroni 23/a, 07100 Sassari, Italy.

E-mail addresses: elenapiera.porcu01@ateneopv.it (E.P. Porcu), asalis@uniss.it (A. Salis), grassu@uniss.it (G. Rassu), marcello.maestri@unipv.it (M. Maestri), jacopo.galafassi01@universitadipavia.it (J. Galafassi), giovanna.bruni@unipv.it (G. Bruni), pgiunc@uniss.it (P. Giunchedi), eligav@uniss.it (E. Gavini).

[1] These two authors share the first position within the authorship.

http://dx.doi.org/10.1016/j.ejpb.2017.04.010
0939-6411/

almost exclusively vascularized by the hepatic artery [7,9,10]. The embolic agents are injected to the target site with a minimally invasive procedure, through a catheter facilitating artery occlusion. This strategy provides localized therapy directly to hepatic tumours, leading to cancer destruction and protecting the rest of the liver against necrosis [11,12]. Over the years, a variety of embolic agents has been employed as liquids, coils, and particulate systems, classified as spherical or non-spherical [13].

Polymeric microparticles, as well as the other embolic agents, can generate a permanent or temporary occlusion, depending on their biocompatible composition. For their fabrication, natural polymers, such as gelatin, starch and chitosan and synthetic polymers such as polylactic-co-glycolic acid have been used [8,14,15].

Besides their chemical constituents, other critical parameters of embolic microparticles are their shape and size. Unlike non-spherical particles, microspheres have precisely controlled sizes as well as being smooth and symmetrical systems. This structure avoids particle aggregation and ensures compression of particles during the injection into microcatheter [13]. As regard the size, microparticles ranging from 40 to 1000–1200 μm in diameter are required. Generally, larger particles occlude mainly proximal vascular areas, increasing the risk of reflux and non-specific embolization. For this reason, small particles (less than 100 μm) are advantageous over larger particles as they are capable to block terminal vessels, producing higher hypoxia rate [16,17]. Microparticles less than 40 μm in diameter can spread to non-targeted organs, such as the lungs [18]. Different commercial products have been successful in human embolization [19,20], but they still show some limitations. One of the main drawbacks of these systems is the lack of imaging capability regarding the location and distribution of embolic material. Therefore, it is important to explore a new kind of multi-functional embolic agent. During the last years, several research groups have focused their attention on the development of detectable embolic systems by using different traceability modalities [21–24].

Indocyanine green (ICG) is a cyanine dye that displays fluorescent properties in the near infrared region [25]. This compound has achieved remarkable attention in many clinical applications due to its favourable properties [26]. During the last years, ICG has been employed for the identification of HCC during surgical procedures [27–29] proving that ICG is a promising tool for intraoperative imaging during hepatic resection. The fluorescence of ICG can be detected by using different instruments, including Photodynamic Eye (PDE), which is widely employed in clinical applications [30–33]. After bonding with plasma proteins in the blood, ICG is excited with near infrared light at 760 nm and emits fluorescence at a slightly longer near infrared wavelength below 820 nm [26].

In 2015, Salis et al. [34] loaded ICG into *in situ* gelling thermosensitive chitosan/glycerophosphate solutions for TAE and following intraoperative fluorescence imaging of HCC, demonstrating a strong interaction between chitosan and ICG. On the basis of these preliminary results, we decided to develop a novel microparticulate system containing ICG-chitosan (CS-ICG) microspheres, with the aim of obtaining suitable embolic agents, in terms of size and fluorescent properties, for the treatment and the intraoperative visualization of HCC. Chitosan, a polysaccharide derivatized from chitin by deacetylation, has been shown to be biocompatible and biodegradable [35]. Several studies reported the crosslinking of chitosan with sodium tripolyphosphate (TPP), a very popular and non-toxic [36], in order to form safe polymeric systems through electrostatic interactions [37–41]. Cellulose acetate butyrate (CAB), used to fabricate several delivery systems [42–44] was chosen to form microsphere matrix due to its biocompatibility. It is a hydrophobic and thermoplastics polysaccharide obtained by esterification of hydroxyl groups of cellulose [45]. The use of CAB as polymer suitable for the embolization of blood vessels has been already proposed [46]. Nevertheless, CAB-based microspheres are not reported in literature yet as embolic agents.

In this work, ICG-chitosan microspheres, obtained by spray drying (CS-ICG(SP)), have been subsequently incorporated into CAB microspheres (CAB-CS-ICG) by emulsion solvent extraction method.

After preparation, microparticles were characterized for size distribution, morphology, loading and release properties. In addition, the potential selective fluorescence imaging of the embolic microspheres was evaluated *in vitro* by using plasma.

2. Materials and methods

2.1. Materials

Chitosan (CS) (Chitoclear® 1360, MW 35 kDa, 96% deacetylated) was supplied from Primex Ltd (Iceland). The material was used to prepare the corresponding hydrochloride salt. Briefly, it was obtained by the dissolution of the purified CS (1% p/V) in 0.1 N hydrochloric acid solution with magnetic stirring, followed by freeze-drying.

Sodium triphosphate pentabasic (TPP), corn oil, Tween 80, N-methyl-2-pyrrolidone (NMP) and Cardiogreen® (ICG) were obtained from Sigma Aldrich (St. Louis, USA). Cellulose acetate butyrate (CAB) was an Eastman Chemical Company (Kingsport, USA) product (CAB 553–0.4). Plurol diisostearique CG was gently provided by Gatefossé (Saint Priest, France). All other chemicals and reagents were of analytical grade. Ultrapure bi-distilled water was obtained by a MilliQ R4 system, Millipore (Milan, Italy).

Drug free plasma from a bag of fresh-frozen plasma was kindly provided by IRCCS Policlinico San Matteo Foundation, Pavia.

2.2. Preparation of formulations

2.2.1. Preparation of CS-ICG particles

CS-ICG particles were prepared by ionic gelation technique [47] using TPP as cross-linking agent. Briefly, a precisely weighed quantity of CS hydrochloride (0.06% w/v) was dissolved in bidistilled water by magnetic stirring and after its complete dissolution, aqueous ICG (0.03 w/v) was dispersed uniformly into the CS solution. After that, a volume of TPP (0.1% w/v), corresponding to a CS-TPP weight ratio 4:1, was added dropwise to mixture under magnetic stirring at room temperature. Green particles were instantaneously formed after the complete addition of TPP solution. In order to collect particles, two methods of drying (freeze drying and spray drying) were compared.

In the first case, the suspension obtained was then centrifuged at 4400 rpm for 20 min at room temperature (Centrifuge 5702 R, Eppendorf, Hamburg, Germany) and washed several times with distilled water and then freeze-dried with Lio 5P Cinquepascale (Trezzano sul Naviglio, Italy). The freeze-dried particles (CS-ICG (L)) were stored in dark within a desiccator for further use.

In the second case, CS-ICG suspension was concentrated until a fifth of initial volume and mixed with dichloromethane (ratio 1:2 v/v) under homogenization at 12,000 rpm (Ultra-Turrax T25 basic, IKA, Germany) in an ice bath. The emulsion, kept under magnetic stirrer, was then sprayed through the nozzle (0.7 mm) of a spray dryer (co-current flow type) model Mini Spray Dryer Büchi B-191 (Büchi Labortechnik-Technik AG, Flawil, Switzerland), within the nebulization chamber. The conditions of the spray-drying process were: inlet air temperature 120 °C; outlet air temperature 56 °C–78 °C; pump ratio 23%; aspirator ratio 73% and flow control 600 L/h. After preparation, microspheres (CS-ICG(SP)) were collected and stored in desiccator at 20 °C in dark conditions.