Journal of Controlled Release 243 (2016) 342–356

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Synthesis, characterization and *in vivo* evaluation of a magnetic cisplatin delivery nanosystem based on PMAA-graft-PEG copolymers

Efstathia Voulgari [a,1], Aristides Bakandritsos [b,c,1], Sotiris Galtsidis [d], Vassilis Zoumpourlis [d], Benjamin P. Burke [e], Gonçalo S. Clemente [e], Christopher Cawthorne [e], Stephen J. Archibald [e], Jiři Tuček [c], Radek Zbořil [c], Vasiliki Kantarelou [f], Andreas Germanos Karydas [f], Konstantinos Avgoustakis [a,*]

[a] Department of Pharmacy, University of Patras, Patras 26500, Greece
[b] Department of Materials Science, University of Patras, Patras 26500, Greece
[c] Regional Centre for Advanced Technologies and Materials, Department of Physical Chemistry, Faculty of Science, Palacky University in Olomouc, 17.listopadu 1192/12, 771 46 Olomouc, Czech Republic
[d] Institute of Biology, Medicinal Chemistry & Biotechnology, NHRF, Athens, Greece
[e] Department of Chemistry and Positron Emission Tomography Research Centre, University of Hull, Cottingham Road, Hull HU6 7RX, UK
[f] Institute of Nuclear and Particle Physics, NCSR "Demokritos", Athens, Greece

ARTICLE INFO

Article history:
Received 12 August 2016
Received in revised form 15 October 2016
Accepted 23 October 2016
Available online 26 October 2016

Keywords:
Nanocarriers
Drug delivery
Magnetic targeting
Cisplatin
Theranostics
Cancer therapy

ABSTRACT

The development of anticancer drug delivery systems which retain or enhance the cytotoxic properties of the drug to tumorous tissues, while reducing toxicity to other organs is of key importance. We investigated different poly(methacrylic acid)-*g*-poly(ethyleneglycol methacrylate) polymers as *in situ* coating agents for magnetite nanocrystallites. The obtained magnetic nano-assemblies were in turn thoroughly characterized for their structural, colloidal and physicochemical properties (drug loading capacity/release, magnetic field triggered drug release, cell uptake and localization) in order to select the best performing system. With the focus on *in vivo* validation of such magnetic drug delivery systems for first time, we selected cisplatin as the drug, since it is a potent anticancer agent which exhibits serious side effects due to lack of selectivity. In addition, cisplatin would offer facile determination of the metal content in the animal tissues for biodistribution studies. Alongside *post-mortem* Pt determination in the tissues, the biodistribution of the drug nanocarriers was also monitored in real time with PET-CT (positron emission tomography/computed tomography) with and without the presence of magnetic field gradients; using a novel chelator-free method, the nanoparticles were radiolabeled with ^{68}Ga without having to alter their structure with chemical modifications for conjugation of radiochelators. The ability to be radiolabeled in such a straightforward but very robust way, along with their measured high MRI response, renders them attractive for dual imaging, which is an important functionality for translational investigations. Their anticancer properties were evaluated *in vitro* and *in vivo*, in a cisplatin resistant HT-29 human colon adenocarcinoma model, with and without the presence of magnetic field gradients. Enhanced anticancer efficacy and reduced toxicity was recorded for the cisplatin-loaded nanocarriers in comparison to the free cisplatin, particularly when a magnetic field gradient was applied at the tumor site. *Post mortem* and real-time tissue distribution studies did not reveal increased cisplatin concentration in the tumor site, suggesting that the enhanced anticancer efficacy of the cisplatin-loaded nanocarriers is driven by mechanisms other than increased cisplatin accumulation in the tumors.

1. Introduction

cis-Diamminedichloridoplatinum (II) (cisplatin or *cis*-Pt) is widely used in cancer treatment [1,2], however it exhibits serious side effects such as nephrotoxicity, auditory toxicity, nausea and vomiting due to lack of selectivity [3,4]. Therefore, drug delivery systems (DDS) for *cis*-Pt are pursued in order to increase selectivity and reduce side-effects. Drug carriers have significantly ameliorated the side-effects, as evidenced by advanced clinical trials of the modified *cis*-Pt derivative lipoplatin [5] and other studies [2]. However, evidence of low-toxicity and high-therapeutic systems still remain elusive.

In general, preclinical observations on successful tumor targeting through the enhanced permeability and retention (EPR) effect and enhanced therapeutic efficacies have not yet translated to similarly high therapeutic indices in clinical trials [6–11]. Therefore, alternative selective drug delivery approaches are under investigation, such as those facilitated by hybrid nanostructures [12,13], which should be accompanied by studies to give in depth understanding of cancer

* Corresponding author.
E-mail address: avgoust@upatras.gr (K. Avgoustakis).
[1] E.V. and A.B. have equally contributed.

http://dx.doi.org/10.1016/j.jconrel.2016.10.021
0168-3659/

biology, pharmacokinetics, biodistribution, and spatiotemporal drug release properties. Among the hybrid therapeutic nanostructures, magnetic drug delivery systems (MDDS) are particularly attractive due to their additional properties [14]. Tissue imaging can be achieved *via* magnetic resonance imaging (MRI) with additional contrast at areas of high nanostructure concentration [15–17], allowing for diagnosis and potential therapeutic response imaging. Magnetically triggered drug release (MTDR) and induced magnetic fluid hyperthermia (MFH) in the tumor by application of external alternating magnetic fields also allows for additional therapeutic effects [18,19].

cis-Pt MDDS are of high interest, but have largely only been studied using *in vitro* systems [20–34]. Currently, the magnetic properties of MDDS are usually low to moderate, as indicated by magnetic measurements [23,24,27,28,30–32,35], or as dictated by the low MION content [20,33]. This poses limitations in exploiting the unique attribute of MDDS to facilitate magnetic drug targeting (MDT), MTDR, and will also compromise T_2 weighted MR imaging and MFH. In other cases, low *cis*-Pt loading has been the limiting aspect [25,29]. Such drawbacks have significantly hampered *in vivo* evaluation of MDDS. There are few examples [13,36] where *cis*-Pt MDDS have been studied *in vivo*, as also evidenced from preclinical evaluation of MDDS recently discussed in a review article [37]. One of the example systems tested displayed saturation magnetization (M_s) of only 3 emu/g_{hybrid} [13]. The nanocarrier was developed through elaborate procedures, including the synthesis of a Pt(IV) prodrug, use of an adamantine derivatized RGD targeting peptide and multiple reaction steps in order to immobilize these previous molecules, in addition to the cyclodextrin (which was acting as a pore gate keeper and controlling drug release). Despite the low M_s, encouraging therapeutic results were obtained with MDT, probably due to the sophisticated engineering of these nanocarriers. Other studies, reported enhanced therapeutic results on A549 human lung cancer xenograft model [36], by developing nanocapsules from poly(vinyl alcohol) and poly(acrylic acid) with relatively high magnetization (40 emu/g_{hybrid}) and at the same time simple synthetic approach. However, the encapsulation of magnetic material introduces batch variability in magnetic material loading and the double emulsification preparation process increases the difficulties of scaling-up the preparation of nanocapsules. The number-weighted hydrodynamic diameter (z-average values not reported) was as high as 190 nm, increasing the clearance from the mononuclear phagocyte system (MPS) [38–41] or reducing stability during storage. In addition, as the size shifts to larger values, sterilization through filtration becomes problematic. This poses serious limitations for scaling up to large preclinical or clinical studies, since filtration remains the first-choice technique for sterilization [26].

Taking into account the obstacles for clinical (and commercial) translation of a nanomedicine, such as high manufacturing and materials costs and the complexity of the production process [11,42,43], it is considered of paramount importance to identify cost-effective procedures to improve production of MDDS in order to offer (i) synthetic simplicity, (ii) appropriate crystallite size of the MIONs and mass content thereof that would not compromise the magnetic properties of the products, (iii) high drug-loading capacity and controlled release, and (iv) dense PEGylation and small size. With this rationale, we envisioned that taking advantage of our previous expertise in the development of simple synthetic pathways of PEGylated and highly magnetic MION-based nano-assemblies [44], a simple yet effective *cis*-Pt MDDS could be developed. In particular, the MDDS selected here are based on the *in situ* precipitation of a single ferrous precursor in the presence of the *graft*-copolymer of poly(methacrylic acid)-*g*-poly(ethyleneglycol methacrylate) or P(MAA-*g*-EGMA) (Fig. 1a). The single ferrous precursor route has been established as a highly appropriate method in the production of high magnetization colloidal nano-assemblies [21,44–47], whereby both size and MION content can be controlled within the desired parameters. The P(MAA-*g*-EGMA) graft-copolymer is very effective in providing a dense PEG canopy to the particles, and at the same time a carboxylate rich inner polymeric corona for binding both to the MION surfaces, drug molecules (Fig. 1b) and fluorescent dyes (rhodamine in the present case). Similar conclusions have been drawn by other groups, thus strengthening our choice of this class of polymers. For example, copolymers with PEG grafts were reported to impart high colloidal stability and blood circulation to MIONs [48,49] and also to carbon nanotubes [50]. Use of block copolymer analogues, such as of PMAA-*b*-PEG, have also led to high colloidal stability of MIONs, with high M_s [51,52]. Nevertheless, they have been never studied in the frame of *in vivo* therapeutic applications. On the other hand, polycarboxy-PEG copolymers have been evaluated as a single component DDS for *cis*-Pt in *in vivo* studies [53,54]. It would be therefore highly interesting to study whether the incorporation of the magnetic core towards magnetic hybrid analogues retains or even enhances the performance of the polymeric *cis*-Pt DDS. This would additionally allow for exploitation of the MDT, MTDR, MFH and MRI properties provided by the magnetic cores.

2. Materials and methods

Random copolymers of poly(methacrylic acid)-*graft*-poly(ethyleneglycol methacrylate) were synthesized by radical copolymerization of methoxy-PEG-methacrylate with methacrylic acid, as

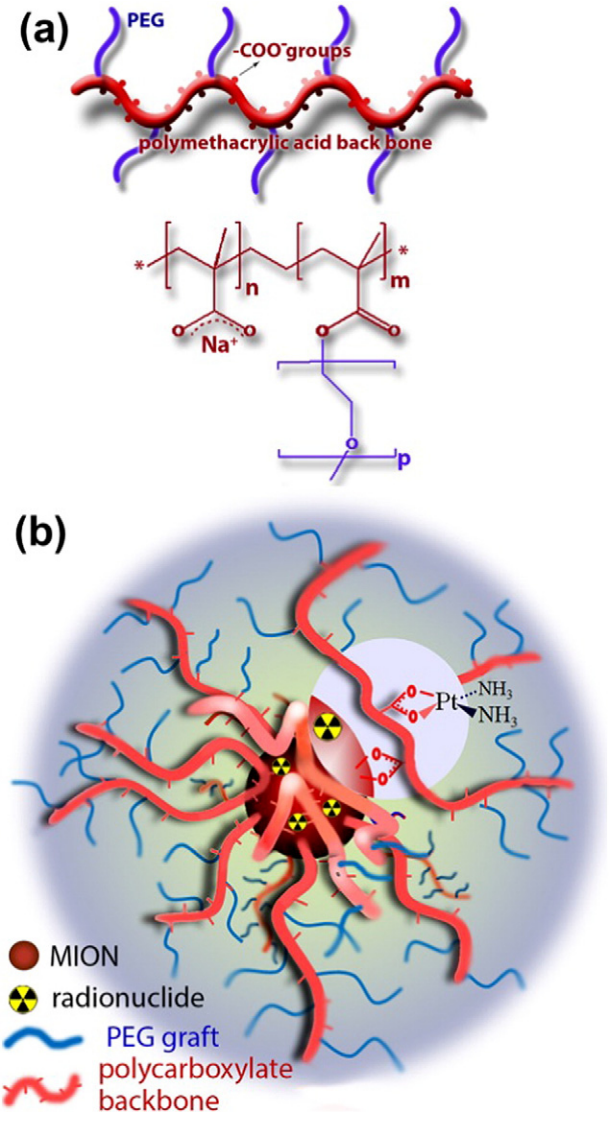

Fig. 1. (a) Chemical structure of p(MAA-*g*-EGMA) copolymers. (b) schematic structure of the studied magnetic drug delivery systems.