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Porphyrin-containing polyaspartamide gadolinium complexes as potential magnetic resonance imaging contrast agents

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ABSTRACT

Porphyrin-containing polyaspartamide ligands (APTSPP–PHEA–DTPA) were synthesized by the incorporation of diethylenetriaminepentaacetic acid (DTPA) and 5-(4′-aminophenyl)-10,15,20-tris(4′-sulfonatophenyl) porphyrin, trisodium salt (APTSPP) into poly-α,β-[N-(2-hydroxyethyl)-L-aspartamide] (PHEA). These ligands were further reacted with gadolinium chloride to produce macromolecule-gadolinium complexes (APTSPP–PHEA–DTPA–Gd). Experimental data of ^{1}H NMR, IR, UV and elemental analysis evidenced the formation of the polyaspartamide ligands and gadolinium complexes. *In vitro* and *in vivo* property tests indicated that APTSPP–PHEA–DTPA–Gd possessed noticeably higher relaxation effectiveness, less toxicity to HeLa cells, and significantly higher enhanced signal intensities (SI) of the VX2 carcinoma in rabbits with lower injection dose requirement than that of Gd–DTPA. Moreover, APTSPP–PHEA–DTPA–Gd was found to greatly enhance the contrast of MR images of the VX2 carcinoma, providing prolonged intravascular duration, and distinguished the VX2 carcinoma and normal tissues in rabbits according to MR image signal enhancements. These porphyrin-containing polyaspartamide gadolinium complexes can be used as the candidates of contrast agents for targeted MRI to tumors.

1. Introduction

Magnetic resonance imaging (MRI) is a non-invasive clinical imaging modality, which has been widely used in the diagnosis of human diseases such as tumors (Caravan et al., 1999; Lauffer, 1987; Lauterbur, 1973). One important way to improve the contrast in MRI is to introduce contrast agents. MRI contrast agents are a unique class of pharmaceuticals that enhance the image contrast between normal and diseased tissue and indicate the status of organ function or blood flow after administration by increasing the relaxation rates of water protons in tissue in which the agent accumulates (Yan and Zhuo, 2001; Yan et al., 2007b, 2008c, 2010a).

However, the clinically used MRI contrast agents, for example, gadolinium diethylenetriaminepentaacetic acid (Gd–DTPA), are small ionic complexes that can diffuse freely through the extracellular space and be excreted rapidly through kidney. Selectively tagging was difficult and do not work well in some organs, tissue or pathologic conditions such as cancer. The injection of large quantities of the ionic complex raises ion concentration *in vivo* and causes localized disturbances in osmolality, which, in turn lead to cellular and circulatory damage (Van Beer et al., 1997; Wen et al., 1998; Yan et al., 2008b). An ideal contrast agent will have the high relaxivity and specificity, low toxicity and side effects, suitable long intravascular duration and excretion time, high contrast enhancement with low dose, *in vivo*, and with minimal cost (Yan et al., 2001, 2004, 2005).

According to the Solomon–Bloembergen–Morgan theory (Caravan et al., 1999; Lauffer, 1987), macromolecular contrast agents are anticipated to increase rotational correlation times and, hence, to improve the relaxivity per ion atom. Compared with the low molecular weight metal complex Gd–DTPA, macromolecular contrast agents may show more effective relaxation rates and prolonged intravascular retention due to their bulky molecular volume (Yan et al., 2007b, 2010a). They offer the opportunity for measuring changes in regional blood volume and can be used clinically as the blood pool contrast agents in MRI and magnetic resonance angiography (MRA). In addition, when a tissue or organ-targeting group is tagged with this macromolecular contrast agent, it can be endowed with tissue or organ-targeting property (Yan et al., 2001, 2007b, 2008c, 2010b).

Generally, two main groups of macromolecular agents were developed, based on the covalent or non-covalent binding between Gd–DTPA and macromolecules. Some biological molecules including monoclonal antibodies, albumin, protein, polysaccharide and

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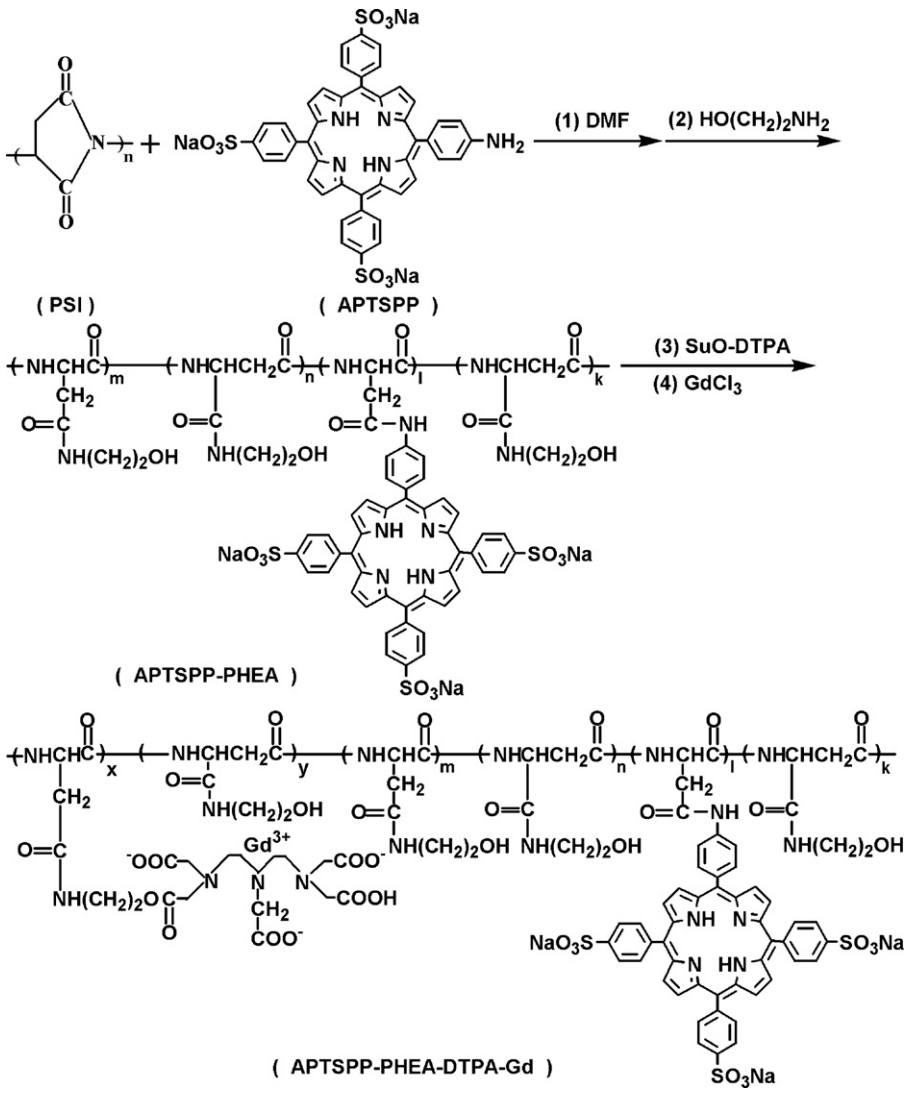

Scheme 1. Synthetic route to polyaspartamide MRI contrast agents.

dextran, and some synthetic polymers such as poly (amino acid) and dendrimer, have also been investigated as polymer carriers for MRI contrast agents (Aime et al., 1997; Accardo et al., 2004; Bai and Zhuo, 1996; Brasch, 1991; Duarte et al., 2001; Gruell et al., 2010; Judd et al., 1999; Lu et al., 2003, 2004; Mohs et al., 2004; Ouyang et al., 1996; Schuhmann-Giampieri et al., 1991; Tóth et al., 1998; Uzgiris et al., 2004; Wallace et al., 1998; Wen et al., 2004; Vaccaro et al., 2007; Yan et al., 2002, 2010b).

Polyaspartamide is a water-soluble, biocompatible synthetic polymer with a protein-like structure that is understood as a plasma extender and polymeric carrier in drug delivery because it is nontoxic or no antigenic, degradable in living systems and modified easily by reactions with the side chain (Giammona et al., 1987, 1990, 1991). As reported, antiviral drugs and anti-inflammatory agents were covalently linked to poly-α,β-[N-(2-hydroxyethyl)-D,L-aspartamide] (PHEA) forming drug–polymer conjugates capable of increasing drug stability and bioavailility. Therefore polyaspartamide can also be used as the polymer carrier for MRI contrast agents (Giammona et al., 1992, 1998; Yan et al., 2001, 2007c).

One promising approach for increasing the local concentration of paramagnetic conjugates in tumor tissue is to conjugate Gd–DTPA with targeting molecules that have a high affinity to tumor cells. Target specific molecules including monoclonal antibodies, protein, avidin, vitamin, peptide, and porphyrin have been investigated as means to increase the site specific accumulation of MRI contrast agents in tumor cells (Aime et al., 1997; Brasch, 1991; Caravan et al., 2007; De Leon-Rodriguez et al., 2010; Schuhmann-Giampieri et al., 1991; Vaccaro et al., 2009; Yan et al., 2002). Contrast agent enhanced MRI has already emerged as a promising technique for the detection, diagnosis, and characterization of cancer (Van Beer et al., 1997; Wen et al., 1998; Yan and Zhuo, 2001; Yan et al., 2007b, 2008a,b,c, 2010a).

Some porphyrins and their metal complexes play important roles in magnetic resonance imaging (MRI), photodynamic therapy (PDT), anticancer drug and fluorescence imaging because of their preferential selective uptake and retention by tumor tissues. A possible mechanism of uptake is that the porphyrins are incorporated into the tumor cell via receptor mediated endocytosis of low-density lipoproteins (LDL), as cancer cells express elevated levels of LDL receptors. The water-soluble meso-tetrasulfonatophenyl porphyrin, tetrasodium salts (TPPS) was found to be highly concentrated in Walker carcinosarcoma (Nelson and Schmiedl, 1991; Chen et al., 1984). Meso-tetra [4-(carboxymethyleneoxy) phenyl] porphyrin (H_2T_4CPP) can accumulate in the Sarcoma 180 in mice and in mammary tumors of Sprague–Dawley rats (Chatterjee et al., 1999). Several research groups have made efforts towards synthesis of porphyrin-antitumor drug conjugates and study on their tumor