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Radiopaque iodinated copolymeric nanoparticles for X-ray imaging applications

Hagit Aviv [a], Sonke Bartling [b], Fabian Kieslling [c], Shlomo Margel [a,*]

[a] Dept. of Chemistry, Bar-Ilan University, Ramar-Gan 52900, Israel
[b] Dept. of Medical Physics in Radiology, German Cancer Research Center, Heidelberg, Germany
[c] Dept. of Experimental Molecular Imaging, Aachen University, Aachen, Germany

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ABSTRACT

Recently we described iodinated homopolymeric radiopaque nanoparticles of 28.9 ± 6.3 nm dry diameter synthesized by emulsion polymerization of 2-methacryloyloxyethyl(2,3,5-triiodobenzoate) (MAOETIB). The nanoparticle aqueous dispersion, however, was not stable and tended to agglomerate, particularly at weight concentration of dispersed nanoparticles above ~0.3%. The agglomeration rate increases as the concentration of nanoparticles in aqueous phase rises and prevents the potential *in vivo* use as contrast agent for medical X-ray imaging. Here we describe efforts to overcome this limitation by synthesis of iodinated copolymeric nanoparticles of 25.5 ± 4.2 nm dry diameter, by emulsion copolymerization of the monomer, MAOETIB, with a low concentration of glycidyl methacrylate (GMA). The surface of resulting copolymeric nanoparticles is far more hydrophilic than that of polyMAOETIB (PMAOETIB) nanoparticles. Therefore, P(MAOETIB-GMA) nanoparticles are significantly more stable against agglomeration in aqueous continuous phase. After intravenous injection of P(MAOETIB-GMA) nanoparticles in rats and mice (including those with a liver cancer model) CT-imaging revealed a significant enhanced visibility of the blood pool for 30 min after injection. Later, lymph nodes, liver and spleen strongly enhanced due to nanoparticle uptake by the reticuloendothelial system. This favorably enabled the differentiation of cancerous from healthy liver tissue and suggests our particles for tumor imaging in liver and lymph nodes.

1. Introduction

In recent years, a growing need has risen for radiopaque polymers for a variety of medical uses as contrast agents for X-ray imaging [1–21]. Among these are the imaging of blood pool [1,2] or certain body organs [3], monitoring embolization processes [4–10], radiopaque vessel grafts [11–16] and dental composition [16–18].

Different techniques have been reported for preparing radiopaque polymers of various types. For example, radiopaque polymer blends have been prepared by incorporating various radiopacifying agents within an appropriate polymer [6–8]. Radiopaque polymer–salt complexes have been produced by the incorporation of radiopaque heavy metal salts into an appropriate polymer ligand via chelation. Radiopaque polymers have also been formed by the polymerization of methyl methacrylate with metal salts of vinyl monomers such as barium or zinc acrylates [16], or by grafting iodine-containing molecules onto preformed high molecular weight polymers [5–7,15].

Another approach for preparing radiopaque polymers is based on the homo or copolymerization of aromatic iodine-containing vinylic monomers with other vinylic monomers such as 2-hydroxyethyl methacrylate (HEMA) or methyl methacrylate (MMA) [4,11–21]. One of the first iodinated monomers of that type was triiodophenyl methacrylate [13,16]. This iodinated monomer is highly resistant to both homo and copolymerization, and only low molecular weight polymers (oligomers) could be formed [13,16]. This was attributed to the bulky nature of the iodinated aromatic nucleus sterically hindering the propagation step during attempted polymerization. In the next generation of radiopaque monomers, the steric hindrance was reduced by introducing a spacer arm between the bulky iodinated aromatic nucleus and the vinyl group [11,12,14,16–21]. This change allowed the facile homo and copolymerization of monomers such as 2-methacryloyloxyethyl (2,3,5-triiodobenzoate) [MAOETIB], 2-hydroxy-3-methacryloyloxypropyl(2,3,5-triiodobenzoate), and 3-(methacryloyl-amidoacetamido)-2,4,6-triiodobenzoic acid with HEMA or MMA, by dispersion, suspension, and bulk polymerization processes [11,12,16–19,21].

Radiopaque iodinated copolymeric microparticles have been prepared by the precipitation of iodinated copolymeric chains

* Corresponding author. Tel.: +972 3 531 8861; fax: +972 3 635 5208.
E-mail address: shlomo.margel@mail.biu.ac.il (S. Margel).

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[19,21], or by the suspension copolymerization of aromatic iodinated vinylic monomers with HEMA or ethylene glycol dimethacrylate [11]. Recently, radiopaque homopolymeric micrometer-sized particles were prepared by our research group [22–24] by dispersion polymerization of the vinylic monomer, MAOETIB. Radiopaque micrometer-sized particles can be used for X-ray imaging needs such as embolization and implants but are not suitable for blood pool and body organs imaging because of the danger of plugging blood vessels. For these purposes nanometer-sized particles are essential.

Iodinated nanoparticles of 28.9 ± 6.3 nm dry diameter were recently prepared by us via the emulsion homopolymerization of MAOETIB [25]. However, the produced nanoparticle aqueous dispersion was not stable and the particles tended to agglomerate, particularly when the concentration of the dispersed nanoparticles was above approximately 0.3%. *In vivo* CT-imaging was performed in a dog after the intravenous administration of the PMAOETIB nanoparticles dispersed in saline in a concentration of 3 mg/ml (0.3%) [25]. Because of the low particle concentration, in order to observe any radiopacity, 260 ml of the nanoparticle dispersion were injected into a 20 kg dog. The injection was performed by a slow drip IV infusion. Unfortunately, this method is inconvenient and not practical for clinical use. Furthermore, despite the large volume that was injected, only a slight change in the X-ray opacity of tissues was observed [25]. In addition, this low concentration of PMAOETIB homo nanoparticles in saline did not allow any blood pool imaging.

The present article describes the synthesis of iodinated copolymeric nanoparticles of narrow size distribution by the emulsion polymerization of the monomers, MAOETIB, and a relatively low concentration of GMA. Stability assessment and the influence of the weight ratio between the monomers on the diameter of the P(MAOETIB-GMA) nanoparticles have been elucidated.

In vivo CT-imaging trials with rats and mice models were performed by IV injection into the animals' tails of the optimal P(MAOETIB-GMA) nanoparticles dispersed in an aqueous continuous phase containing 5% of dextrose.

2. Experimental

2.1. Materials

The following analytical-grade chemicals were purchased from Aldrich and used without further purification: 2,3,5-triiodobenzoic acid (98%), HEMA (99%), 1,3-dicyclohexylcarbodiimide (DCC, 99%), 4-pyrrolidinopyridine (98%), diethyl ether anhydrous (99.7%), $MgSO_4$ (99%), ethyl acetate (99.5%), GMA (97%), potassium persulfate (PPS, 99%), sodium dodecyl sulfate (SDS, 90%), toluene (HPLC grade and dextrose) (99.5%). Water was purified by passing deionized water through an Elgastat Spectrum reverse osmosis system (Elga Ltd., High Wycombe, UK).

2.2. Methods

2.2.1. Synthesis of the monomer, MAOETIB

The iodinated monomer MAOETIB was synthesized according to Fig. 1, as described in the literature [17]. Briefly, 2,3,5-triiodobenzoic acid (50 g, 0.10 mol), HEMA (15 g, 0.11 mol), DCC (23 g, 0.11 mol) and 4-pyrrilidinopyridine (1.5 g, 0.010 mol) were dispersed in ether (500 ml), and then stirred at room temperature for 18 h. The formed solid was filtered off and the residue washed with fresh ether. The ether solution was then washed with HCl (2 N) and saturated $NaHCO_3$. The organic phase was dried over $MgSO_4$, filtered, and evaporated to produce an orange solid. Pure white crystals of MAOETIB (m.p. 95 °C) were obtained by the two-fold recrystallization of the orange solid from ethyl acetate (yield 84%).

^{1}H NMR ($CDCl_3$) δ 1.97 (s, 3H, CH_3), 4.57 and 4.48 (m, 4H, OCH_2CH_2O), 5.61 (s, 1H, olefinic), 6.16 (s, 1H, olefinic), 7.33 (d, 1H, $J = 1.68$ Hz, Ar–H), 8.30 (d, 1H, $J = 1.68$ Hz, Ar–H). ^{13}C NMR ($CDCl_3$) δ 18.33 (C-3), 61.92 (C-5), 63.93 (C-6), 93.64 (C-12), 106.56 (C-9), 113.39 (C-10), 126.41 (C-1), 135.72 (C-2), 137.13 (C-13), 148.86 (C-11), 165.60 (C-4), 166.97 (C-7). MS (ES+): m/z 635 (MNa^+, 100). The molecular weight of the monomer, MAOETIB, was confirmed by mass spectrometry. Elemental analysis-Calculated: C, 25.52; H, 1.81; O, 10.46; I, 62.21; Experimental: C, 25.65; H, 1.82; O, 10.49; I, 62.04.

2.2.2. Synthesis of the PMAOETIB nanoparticles

PMAOETIB nanoparticles were prepared by the emulsion polymerization of MAOETIB, as described in the literature [25]. Briefly, PMAOETIB nanoparticles of 28.9 ± 6.3 nm dry diameter were prepared by introducing 5 ml of a toluene solution containing 400 mg MAOETIB into a vial containing 20 ml of 1% SDS aqueous solution and 0.05% PPS (10 mg). The mixture was then shaken at 73 °C for 12 h. The organic phase containing the toluene and the excess monomer was then extracted from the aqueous phase. Excess SDS was then removed from the aqueous dispersion by extensive dialysis.

2.2.3. Synthesis of the P(MAOETIB-GMA) nanoparticles

P(MAOETIB-GMA) nanoparticles were prepared by the emulsion copolymerization of MAOETIB with GMA, according to a procedure similar to that described for the PMAOETIB nanoparticles. Briefly, radiopaque copolymeric nanoparticles of 25.5 ± 4.2 nm dry diameter were formed by introducing them into a vial containing 20 ml of a 1% SDS aqueous solution and 0.05% PPS (10 mg) 5 ml of a toluene solution containing 396 mg MAOETIB and 4 mg GMA. The mixture was then shaken at 73 °C for 12 h. The organic phase containing the toluene and excess monomers was then extracted from the aqueous phase. Excess SDS was then removed from the aqueous dispersion by extensive dialysis. Dried radiopaque P(MAOETIB-GMA) nanoparticles were then obtained by lyophilization. The effect of the weight% ratio [MAOETIB]/[GMA], while maintaining the total monomer (GMA + MAOETIB) weight constant (400 mg) on the size and size distribution of the P(MAOETIB-GMA) nanoparticles, was also elucidated.

2.2.4. In vivo CT-imaging

Two male Copenhagen rats (ca. 500 g weight of each rat) were used to evaluate the distribution and X-ray visibility of P(MAOETIB-GMA) nanoparticles in the blood, lymph nodes, liver and spleen. For this purpose, a 5% dextrose aqueous dispersion of the P(MAOETIB-GMA) nanoparticles (80 mg/ml) was injected (400 mg/kg) into the rat's tail vein. CT scans were performed 2 and 30 min after administration of the radiopaque nanoparticles.

Similarly, two male Black6 mice (ca. 30 g weight of each mouse) with a liver cancer model were used to evaluate the potential of the nanoparticles for liver imaging. For this purpose, the nanoparticles were injected into the mouse's tail vein in a dosage of 600 mg/kg. P(MAOETIB-GMA) nanoparticles (80 mg/ml) were dispersed in 5% dextrose aqueous solution. CT scans were performed before the injection and 3 min and 4 h post injection.

2.3. Characterization

^{1}H and ^{13}C NMR spectra were obtained with a Bruker DPX-300 spectrometer. Chloroform-d and tetrahydrofuran-d_8 (THF-d_8) chemical shifts are expressed in ppm downfield from tetramethylsilane used as an internal standard.

Mass spectra were obtained with a Finnigan 4021 spectrometer (electrospray and desorption chemical ionization).

TEM pictures were obtained with a JEOL-JEM 100SX electron microscope with 80–100 kV accelerating voltage. Samples for TEM were prepared by placing a drop of a diluted sample on a 400-mesh carbon-coated copper grid. Negative staining was performed by placing a drop of 2% aqueous phosphotungstic acid (PTA) of pH 7.0 [26]

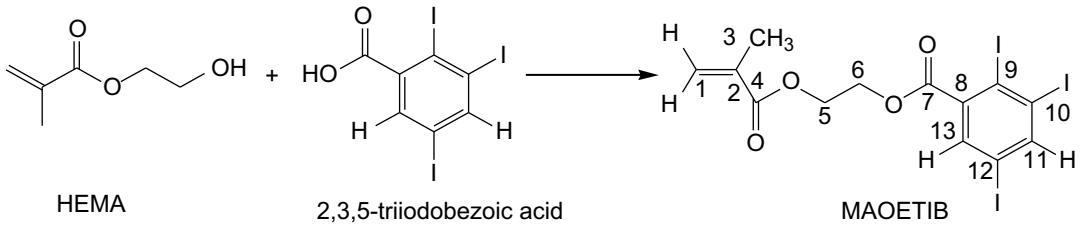

Fig. 1. A scheme illustrating the synthesis of the monomer, MAOETIB.