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Impact of ionizing radiation on physicochemical and biological properties of an amphiphilic macromolecule

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ABSTRACT

An amphiphilic macromolecule (AM) was exposed to ionizing radiation (both electron beam and gamma) at doses of 25 kGy and 50 kGy to study the impact of these sterilization methods on the physicochemical properties and bioactivity of the AM. Proton nuclear magnetic resonance and gel permeation chromatography were used to determine the chemical structure and molecular weight, respectively. Size and zeta potential of the micelles formed from AMs in aqueous media were evaluated by dynamic light scattering. Bioactivity of irradiated AMs was evaluated by measuring inhibition of oxidized low-density lipoprotein uptake in macrophages. From these studies, no significant changes in the physicochemical properties or bioactivity were observed after the irradiation, demonstrating that the AMs can withstand typical radiation doses used to sterilize materials.

1. Introduction

Amphiphilic macromolecules (AMs) can self-assemble into micelles in aqueous media due to their amphiphilicity. Given the micellization behavior of AMs, they are capable of encapsulating hydrophobic molecules in the micelle inner core and transporting the molecular cargo within the aqueous environment [1–9]. An amphiphilic macromolecule (AM) (Fig. 1) previously developed by Uhrich et al. is composed of a hydrophobic, alkylated mucic acid backbone and hydrophilic poly(ethylene glycol) (PEG) coupled to the hydrophobic core via an ester bond [10]. This AM has been demonstrated as a nano-carrier for water-insoluble drugs and *in vitro* evaluation for systemic delivery was also performed [11–13]. The AM has also been incorporated into liposomes by electrostatic and hydrophobic interactions with lipids. The formulation of these AM-lipid complexes results in increased biocompatibility, ability to load and deliver anti-cancer therapeutics, and preferential uptake in cancer cells [14]. By chemically incorporating oligoethylenimines into the AM backbone, cationic AMs can be obtained with capabilities to complex with and deliver siRNA *in vitro* [15]. In addition to these drug delivery applications, AMs have also demonstrated unique properties in the management of atherosclerosis, a main trigger for cardiovascular disease [16]. The AM has been tested as a therapeutic agent to inhibit the atherosclerotic cascade by inhibiting the uptake of oxidized low-density lipoprotein (oxLDL) in macrophages [17–20]. To optimize the inhibition effectiveness, other AMs with modified structures have also been synthesized and tested [16]. Due to the broad biomedical applications of these AMs, they could potentially be developed into a medical device or drug and therefore, must be rendered sterile.

Common sterilization methods include heat sterilization [21,22], chemical sterilization [23,24], sterile filtration [25,26], and ionizing sterilization [27–30]. Heat sterilization usually requires high temperatures (120 °C) which could possibly lead to decomposition or degradation of AMs [21,22,31,32]. Chemical sterilization requires high concentrations of reactive sterilants, such as hydrogen peroxide, or ozone, which could lead to potential oxidative reaction with the AM [23,33,34]. Sterile filtration is a convenient and efficient way to sterilize samples; however, it requires a filtration membrane which will absorb highly viscous drug components or nanoparticles [25,26,35]. Ionizing sterilization includes electron beam (e-beam) and gamma radiation. Gamma

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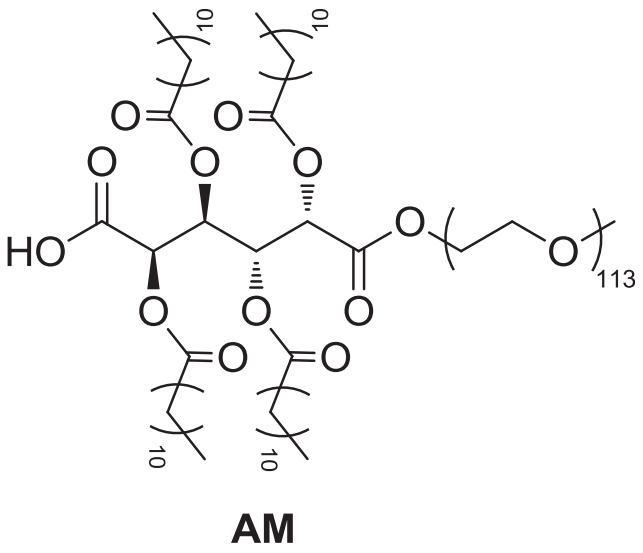

Fig. 1. Structure of AM.

radiation uses Cobalt-60 to continuously emit gamma rays with a high penetrating effect. Electron beam radiation is generated as electrons from a high-energy accelerator and has a higher dose rate but less penetration than gamma radiation. As both e-beam and gamma sterilization methods use ionizing energy to irradiate samples without inducing high temperature or using chemical sterilants, these methods were chosen to test the stability of AMs to typical sterilizing conditions. Despite their positive attributes, both e-beam and gamma radiation exposure can cause slight degradation due to the high-energy of the ionizing source [24,28,36].

In this work, both e-beam and gamma radiations are used to irradiate the AM in powder form to study the effect of both sterilization methods on the AM properties. The AM was exposed to each irradiation process at target doses of 25 kGy and 50 kGy, as 25 kGy is a typical sterilization dose and 50 kGy is a typical maximum processing dose [30]. After the exposures, proton nuclear magnetic resonance (^{1}H NMR) spectroscopy was performed to determine the impact on chemical structure of the AM. Gel permeation chromatography (GPC) was used to investigate changes in molecular weight. Dynamic light scattering (DLS) was utilized to determine the impact on micelle behavior of AM. *In vitro* oxLDL uptake inhibition studies were then performed to determine the impact on the inhibiting activity of AMs in macrophages.

2. Materials and methods

2.1. Materials

All chemicals and reagents were purchased from Sigma–Aldrich (Milwaukee, WI) and used as received.

2.2. Sample preparation

The AM (Fig. 1) was synthesized using previous methods [10]. In short, mucic acid was acylated with lauroyl chloride followed by coupling with mono-hydroxy PEG. The AM powder (2.5 g) was divided into five BD Falcon 5 mL polystyrene round-bottom tubes (12 × 75 mm style; Becton Dickinson Bioscience Discovery Labware, Bedford, MA), one was for the untreated control and another four were for the radiation exposures. All the samples were stored at 4 °C for 24 h prior to being sent to Johnson & Johnson Sterile Process Technology (SPT) for radiation processing. After exposure, samples were stored at 4 °C for 24 h until physicochemical and biological characterization studies were performed in triplicate.

2.3. Radiation exposure

Gamma irradiation was conducted in a Cobalt-60 source MDS Nordion Gamma Cell 220 Research irradiator at SPT. The temperature during exposure ranged from 30 °C to 37 °C with a dose rate of approximately 0.002 kGy/s for a maximum of 9 h. Samples for e-beam irradiation were processed under ambient conditions in the Mevex 5 MeV, 2 kW electron beam linear accelerator. Samples were placed upright in an Ethafoam® jig and presented single-sided to the beam. Doses for both of the radiation processes were 25 kGy and 50 kGy. The dose rate for e-beam was approximately 12.5 kGy/s. The temperature ranged from 38 °C (25 kGy) to 55 °C (50 kGy) during the e-beam exposures. Samples designated as controls were not exposed to ionizing radiation.

2.4. Physicochemical characterization

Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained using a Varian 500 MHz spectrometer. AM samples (10–15 mg) were dissolved in deuterated chloroform. Each spectrum was an average of 16 scans. Molecular weights (M_w) were determined using gel permeation chromatography (GPC) with respect to PEG standards (Sigma–Aldrich) on a Waters Stryagel® HR 3 THF column (7.8 × 300 mm). The Waters LC system (Milford, MA) was equipped with a 2414 refractive index detector, a 1515 isocratic HPLC pump, and 717 plus autosampler. Samples (10 mg/mL) were prepared in THF and filtered using 0.23 μm pore PTFE syringe filters (Fisher Scientific). Dynamic light scattering (DLS) analysis was carried out on a Zetasizer nanoseries nano ZS90 (Malvern instruments). Samples (1–2 mg/mL) were prepared in HPLC water and filtered using 0.23 μm pore size PTFE syringe filters (Fisher Scientific).

2.5. Cell culture

Peripheral blood mononuclear cells (PBMC) were isolated from human buffy coats (Blood Center of New Jersey, East Orange, NJ) by density gradient centrifugation over Ficoll–Paque. Monocytes were selected by plastic adherence as follows. PBMCs suspended in Roswell Park Memorial Institute (RPMI) 1640 medium (ATCC) with 10% fetal bovine serum were incubated in 96-well plates for 4 h. Non-adherent cells were removed by washing three times with phosphate-buffered saline, and adherent cells were cultured for 7 days in RPMI supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin and 50 ng/mL macrophage colony-stimulating factor (M-CSF) (PeproTech) for differentiation into macrophages. Media was changed every 2–3 days.

2.6. oxLDL uptake by PBMC macrophages

PBMC macrophages were co-incubated with 10 μg/mL of 3,3′-dioctadecyloxacarbocyanine (DiO) labeled oxLDL (Kalen Biomedical; Montgomery Village, MD) and AM (10^{-6} M) for 24 h in RPMI 1640. Cells were fixed with and counterstained with Hoechst 33342 prior to epifluorescent imaging on a Nikon Eclipse TE2000-S. oxLDL uptake was quantified using ImageJ and normalized to cell count. Results are the average of three experiments performed in biological triplicate.