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Cisplatin delivery from poly(acrylic acid-*co*-methyl methacrylate) microparticles

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Abstract

To develop a platform for tumor chemotherapy, poly(acrylic acid-*co*-methyl methacrylate) microparticles have been synthesized. Carboxylate containing monomers were included to complex therapeutic agents, specifically cisplatin. Microparticles were prepared by free radical emulsion polymerization in aqueous media. Particle diameter, ζ-potential, in vitro cytotoxicity, and in vivo acute toxicity were characterized for both cisplatin-loaded microparticles and unloaded microparticles. In vitro cytotoxicity and FT-IR were used to characterize cisplatin released from cisplatin-loaded microparticles. Acrylic acid feed mole fraction determined several key microparticle properties, including particle size, ζ-potential, and yield. A burst release of cisplatin (40%) in the first day was followed by a zero-order release phase. The interaction between cisplatin and microparticles allowed approximately 20% additional cisplatin release in the next five days. Cisplatin-loaded and unloaded microparticles are non-toxic (LC_{50}>15 mM) to the cell line used in in vitro tests. Cisplatin released from cisplatin-loaded microparticles retained activity, but that activity was slightly lower than freshly prepared cisplatin. Other than a slight reduction in cisplatin activity, microparticles exhibited low in vivo acute toxicity (LD_{50}>170 mg/kg), which suggests that this hydrogel particulate system and the hydrogel complexation mechanism should further be studied for drug delivery.

Keywords: Polymer; Microparticle; Cisplatin; Cytotoxicity; Acute toxicity

1. Introduction

Cisplatin (*cis*-dichlorodiammineplatinum(II), CDDP) is a commonly used chemotherapeutic agent for treatment of various cancers, including testicular cancer, ovarian cancer, lymphoma, and glioma [1,2]. After both passive and active cellular uptake, cisplatin coordinates with the N7 atom of guanine in DNA to form

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adducts and causes cellular apoptosis [3]. Chronic cisplatin usage results in cellular cisplatin accumulation which induces resistance by several possible mechanisms including increased interactions with metallothionein and glutathione and increased DNA repair [4]. Resistance lowers the efficiency of cisplatin significantly. To counteract resistance, clinical dosing of cisplatin generally will be elevated to higher levels. The higher dose inevitably causes systemic toxicity including gastrointestinal problems, ototoxicity, nephrotoxicity and visual abnormality [5,6]. As such, mechanisms of delivering cisplatin to the local environment of a tumor are desirable.

To localize cisplatin to tumors, many approaches have evolved. Cisplatin attachment to monoclonal antibody results in tumor targeting and reduced toxicity [7]. Due to reduced toxicity and increased activity, polymeric delivery systems are attracting increasing attention due to their low toxicity, ease of tailoring, and variety of properties; polymeric systems have been studied for delivering various types of bioactive substances, i.e. small molecules, vaccines, proteins, DNA, and RNA [9–11]. Cisplatin entrapped in polymeric implants has reduced systemic toxicity and increased activity [8]. Poly(acrylic acid) and poly(methyl methacrylate) were chosen as building blocks for the platform of the described system because these two polymers are known to be biocompatible in many cases [12]. Acrylic polymers have exhibited high in vivo tolerance in rats after subcutaneous implantation for up to 24 days [13]. In addition, the carboxylic acid groups in poly(acrylic acid) can form hydrogen bonds with mucin, a glycoprotein secreted locally that coats the mucosal surfaces [12]. This bioadhesive property makes poly(acrylic acid) a good candidate for many different delivery routes including oral, ocular, and nasal. More importantly, carboxylic groups in poly(acrylic acid) can interact with various groups thus creating attachment sites for a wide range of therapeutics. Addition of poly(methyl methacrylate) modifies the carboxylic group density and distribution of acrylic acid in the copolymer and assists in making microparticles of an acceptable size [14,15].

As a preliminary study investigating polymeric microparticles as a drug delivery platform, the objectives of this study were to test the physicochemical properties, the complexation of cisplatin, and the in vitro and in vivo toxicity of poly(acrylic acid-*co*-methyl methacrylate) (p(AA-MMA)) microparticles. Cisplatin was complexed with poly(acrylic acid) residues in poly(acrylic acid-*co*-methyl methacrylate) copolymer microparticles. Carboxylic acid is exchanged with water or chloride forming aqua-platinum and chloroplatinum, respectively. The strength and rate of these interactions is determined by the geometry and multivalency of the complex [16]. The absolute exchange rate of carboxylic groups with water molecules is slow due to the more favorable carboxylate interaction and multivalency of the carboxylate polymer–platinum interaction. The complexation provides sufficient interaction between cisplatin and poly (acrylic acid) residues that reduce the burst-effect, prolong the release of cisplatin, and further reduce the systemic toxicity of cisplatin. Characterization of the microparticles supports the proposed mechanism. In vitro release, cytotoxicity, and acute animal toxicity further confirmed that poly(acrylic acid-*co*-methyl methacrylate) copolymer microparticles exhibit many characteristics of a successful carrier system for therapeutic molecules.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA) and used without further purification unless otherwise described. Solvents were HPLC grade and chemicals were ACS grade unless noted.

2.2. Microparticle preparation

Poly(acrylic acid-*co*-methyl methacrylate) microparticles were synthesized in 200 ml distilled deionized water by a free radical emulsion polymerization [15]. Briefly, AA and MMA were polymerized using 100 mg ammonium persulfate (APS) and 100 μl poly(ethylene glycol) diacrylate (FW = 575 g/mol; PEGDA), as initiator and crosslinker, respectively. The monomers and crosslinker were added directly into a 250-ml three-necked flask. While stirring with an overhead stirrer at 1000 rpm, the reaction mixture

was purged with nitrogen for 30 min and reacted at 70 °C under nitrogen for 5 h. The AA feed mole fraction (X_{AA}) ranged from 0.59 to 0.91 with the total monomer concentration kept constant at 0.3 mM. The p(AA-MMA) microparticles were collected by centrifugation at 10,000 rpm for 30 min followed by washing and lyophilization. Theoretical microparticle recovery was determined by dividing the final mass of washed and dried microparticles by the mass of monomers added to the flask.

2.3. Microparticle characterization

Microparticles were sonicated in distilled water for 15 min before analysis by quasi-elastic light scattering (NICOMP™ 380 particle sizer). Surface morphology of the microparticles was observed by scanning electron microscopy (SEM, Hitachi S3000N). Prior to SEM observation, microparticles were sputter-coated with gold vapor under vacuum. Zeta potential (ζ-potential) was measured in distilled water at pH 7.4 prepared by adjustment with 0.1 N sodium hydroxide (NaOH) (Lazer Zee Meter Model 501, Pen Kem, Inc.). Carboxylate content was determined by potentiometric titration with 0.1 N NaOH with pH monitored by a pH meter (Corning, model 440). Microparticles (100 mg) were accurately weighed and suspended in distilled water. Sodium hydroxide (0.1 N) was added into the suspension with continuous stirring. The equivalence point was determined by regression of the potentiometric titration curve.

2.4. Cisplatin loading

Cisplatin was loaded into p(AA-MMA) microparticles by complexation with pendant carboxylate ions. In this study, only p(AA-MMA) microparticles with a feed mole fraction of 0.63 were used for cisplatin loading, release, in vitro, and in vivo experiments; therefore, all results presented represent data obtained with this composition. Microparticles were suspended and stirred in deionized water. Two methods of cisplatin loading were utilized. In the first method (Method I), cisplatin (265.2 mg) was dispersed as a suspension in 106 ml deionized water and mixed with 235.8 mg microparticles as an aqueous suspension. This concentration is above the solubility of cisplatin (1–2 mg/ml) at this temperature. Since only dissolved cisplatin will complex to microparticles, a second method of loading cisplatin in the microparticles was necessary to improve the efficiency of complexation. In the second method (Method II), cisplatin (265.2 mg) was dissolved in 108 ml water–dimethyl sulfoxide (98.4 : 1.6, v/v) which solubilized all cisplatin. The remainder of the procedures for cisplatin loading was the same for both methods. Complexation was continued overnight at room temperature in the dark. The suspension was centrifuged at 10,000 rpm for 30 min, washed with deionized water, and the pellet was lyophilized. Residual cisplatin in the supernatant was analyzed by a modified colorimetric *o*-phenylenediamine method at 704 nm [17]. *O*-phenylenediamine (oPDA) was purified by recrystalization in dimethylformamide. The loading efficiency of cisplatin was calculated by Eq. (1).

$$\eta_{cis} = \frac{m_{total} - m_{supernatant}}{m_{total}} \times 100\% \quad (1)$$

where, η_{cis} is the loading efficiency of cisplatin, m_{total} is the total cisplatin in the loading solution, and $m_{supernatant}$ is the amount of cisplatin in the supernatant. Theoretical loaded-microparticle recovery was determined by dividing the final mass of washed and dried loaded-microparticles by the mass of microparticles and platinum added to the beaker.

2.5. In vitro cisplatin release

Cisplatin-loaded p(AA-MMA) microparticles (50 mg) were suspended in 5 ml of phosphate buffered saline (PBS). At room temperature, the suspension was placed into a chamber mounted with a 500 g/mol molecular weight cut-off dialysis membrane (DispoDialyzer™, Harvard Apparatus) and subsequently placed in a beaker containing dissolution media, i.e. phosphate buffered saline (PBS). The volume of the dissolution media was maintained at 60 ml while constant stirring (~100 rpm) was maintained at room temperature (~25 °C). Aliquots (2 ml) of dissolution media were sampled at specified time points and replaced with fresh media immediately after sampling. Two concentrations of free cisplatin were used to characterize the apparatus, thus removing mixing and solubility effects. Cisplatin release was measured by the colorimetric oPDA method with appropriate dilution as necessary. Released cisplatin that was not

used for concentration determination was collected at the time of sampling and saved for chemical and biologic characterization. All remaining collected cisplatin from one experiment was pooled, lyophilized, and used for chemical characterization, and in vitro cytotoxicity determination.

2.6. Characterization of released cisplatin

Attenuated total reflectance Fourier transformed infrared (ATR-FTIR) spectra of CDDP were recorded on a Thermo Nicolet NEXUS 870 spectrometer (4000–1000 cm^{-1}). Cisplatin species, including lyophilized cisplatin (lyo-CDDP), intact cisplatin (CDDP), and released cisplatin (CDDP-R) were recorded as references compounds. Lyo-CDDP was made by lyophilizing an aqueous cisplatin suspension.

2.7. Cytotoxicity

Cytotoxicity was tested on NIH/3T3 fibroblast cells (ATTC CRL 1658) using a modified MTT assay (Cell Titer 96® Aqueous One Solution Cell Proliferation Assay kit; Promega, Madison, WI) [18]. All cells were maintained in Dulbecco's Modification of Eagle's Medium (DMEM) supplemented with 10% calf bovine serum and 1% penicillin streptomycin media under 5% CO_2 at 37 °C. Twenty-four hours following plating on 96-well plates at a density of 5×10^4 cells/ml, cells were treated with serial concentrations of p(AA-MMA) microparticles, cisplatin-loaded p(AA-MMA) microparticles, or cisplatin released from cisplatin-loaded p(AA-MMA) microparticles. DMEM (supplemented with 10% calf bovine serum and 1% penicillin streptomycin) and equimolar cisplatin solutions were used as negative and positive controls, respectively. Cytotoxicity was expressed as a percentage of negative control.

2.8. In vivo acute toxicity

The Up-and-Down Procedure (UDP) with slight modification was used to determine in vivo acute toxicity following the Organization for Economic Cooperation and Development (OECD) guidelines for the testing of chemicals, OECD 425, as approved by University of Illinois at Chicago Animal Care Committee; NIH guidelines for the care and use of laboratory animals (NIH Publication #85-23 Rev. 1985) have been observed. Briefly, Sprague–Dawley female rats (Harlan, Indianapolis, IN) were injected intravenously with cisplatin or microparticles in PBS at specified doses. As an initial dose, cisplatin was administered at 2.5 mg/kg while microparticles and CDDP-containing microparticles were administered initially at 175 mg/kg. The pH and osmolarity of test substances were adjusted to approximately 7.4 and 290 mOsm. As all microparticles are suspensions, microparticles were suspended in PBS and autoclaved while cisplatin solutions were filtered through a 0.22 μm membrane (Micron Separations Inc.; Westboro, MA). Sterilized test materials were injected into the lateral tail vein of rats while the rats were restrained but not anesthetized. The animals were observed closely for at least 4 h post-injection and three times a day thereafter. Animals were euthanized by intraperitoneal injection of pentobarbital (50 mg/ml; Abbott Laboratories; North Chicago, IL) after one week or if moribund status (inability to ambulate, inflammation, anorexia, dehydration, or more than 20% weight loss) was observed. The weight of each animal was recorded immediately before intravenous injection, daily after injection, and at the time of euthanasia. All outcomes were recorded and processed using aot425statpgm (United States Environmental Protection Agency, http://www.epa.gov/oppfead1/harmonization). The calculation of LD_{50} is based on the maximum likelihood method with sigma set at 0.5.

2.9. Statistical analysis

Particle diameters, zeta potentials, in vitro release, and in vitro cytotoxicity studies were performed in triplicate unless specified. The data are represented as mean ± standard deviation (SD). Data was analyzed using the Student t test and one-way analysis of variance (ANOVA) and considered significantly different at the level of $p < 0.05$.

3. Results

Lyophilized p(AA-MMA) microparticles appeared as a fine white powder. Lyophilized cisplatin-loaded p(AA-MMA) microparticles appeared as gray-yellow-

ish to grayish powder. In either case, the powder was free flowing and could be easily manipulated. The microparticle theoretical recovery ranged from 10 to 40% following polymerization. The loaded-microparticle theoretical recovery following cisplatin-loading was 40 to 80% with little variation between batches of a specific microparticle formulation. The loading efficiency of cisplatin (η_{cis}) in p(AA-MMA) microparticles was determined to be approximately 90% of solubilized cisplatin (Method II) which was significantly better than suspended cisplatin (~40–50%; Method I). This was due to the cisplatin being available to interact with the carboxylic groups of the polymer when Method II was employed.

The volume-weighted particle diameter distribution was measured by quasi-elastic light scattering and plotted against acrylic acid feed mole fraction ($0.63 < X_{AA} < 0.83$) (Fig. 1A). The major peak and deviation from that peak are presented. The diameter distributions of all compositions of microparticles were bimodal with peaks at around 500 nm and 2 μm, but the fraction of particles falling into each of the two populations differed for each of the compositions. The p(AA-MMA) microparticles were spherical with rough surfaces (Fig. 1B) as observed under scanning electron microscopy. The particle diameters ranged from approximately 500 nm to 2 μm which confirmed the results obtained by quasi-elastic light scattering.

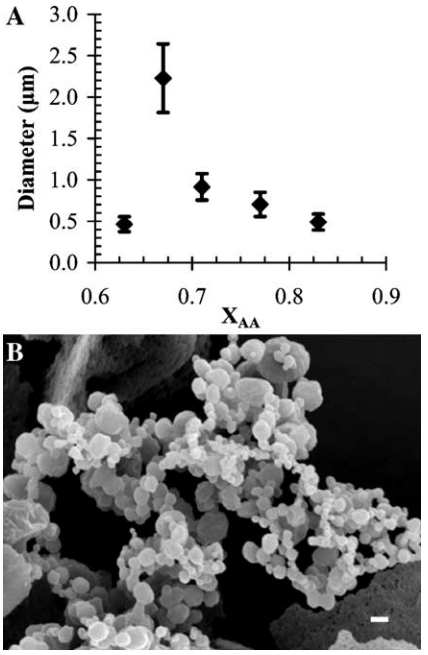

Fig. 1. Size and morphology of poly(acrylic acid-*co*-methyl methacrylate) microparticles. (A) Volume-weighted particle diameter distribution of poly(acrylic acid-*co*-methyl methacrylate) microparticles with various acrylic acid feed mole fraction (X_{AA}). (B) Scanning electron micrographs of poly(acrylic acid-*co*-methyl methacrylate) (X_{AA}=0.63) microparticles. The scale bar in the micrograph is 1 μm.

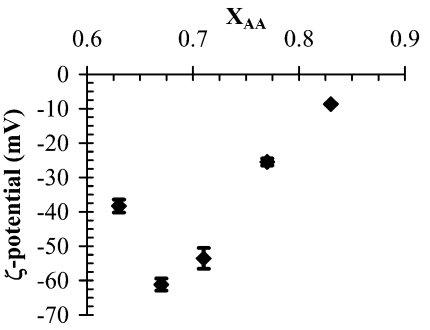

Fig. 2. Zeta potential (in pH 7.4 deionized water) as a function of acrylic acid feed mole fraction (X_{AA}) in poly(acrylic acid-*co*-methyl methacrylate) microparticles.

Zeta potentials of p(AA-MMA) microparticles ranged from −8.67 to −61.17 mV depending upon composition. The highest ζ-potential range was observed for p(AA-MMA) microparticles whose mole fractions were 0.65 to 0.75 while mole fractions outside this range were less electronegative (Fig. 2). The content of carboxyl groups at the equivalence point was calculated based on the mole number of NaOH consumed (Table 1). With increasing acrylic acid mole fraction, the content of carboxyl groups increased from 2.4 to 8.2 mmol/g of p(AA-MMA) microparticles. For cisplatin-loaded p(AA-MMA) microparticles with different loading efficiency, the ζ-potentials are significantly different ($p < 0.05$) (Fig. 3). The ζ-potential of cisplatin-loaded p(AA-MMA) microparticles with higher loading (Method II) was more electropositive compared to that of either p(AA-MMA) micro-

Table 1
Carboxylic acid content of various p(AA-MMA) microparticles formulations

X_{AA}	0.59	0.63	0.67	0.71	0.77	0.83	0.91
Carboxylic Acid (mmol/g)	2.4	3.4	4.2	4.1	4.4	6.2	8.2

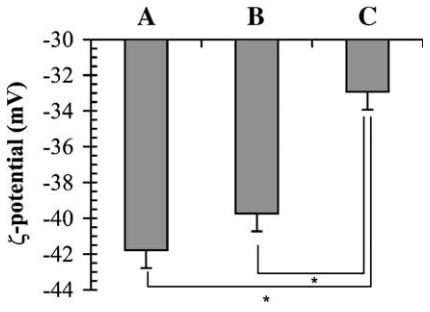

Fig. 3. Zeta potential of (A) unloaded microparticles (X_{AA}=0.63) and (B) cisplatin-loaded poly(acrylic acid-*co*-methyl methacrylate) microparticles using a saturated solution of cisplatin in water (Method I) and (C) cisplatin-loaded microparticles using an equivalent concentration of cisplatin dissolved in water–dimethyl sulfoxide (Method II). The asterisks (*) indicate statistical significance ($p<0.05$).

particles or cisplatin-loaded p(AA-MMA) microparticles with lower loading (Method I).

Release of cisplatin from p(AA-MMA) microparticles (X_{AA}=0.63) was prolonged with a slight burst release in the first few hours (Fig. 4). The release from microparticles continued for at least six days and fit a zero-order release pattern. Free cisplatin equilibrated within the compartments within 12 h indicating that there was not a significant artifact from the apparatus. Within six days, about 60% of loaded cisplatin in p(AA-MMA) microparticles was released with cisplatin continuing to be released. The ATR-FTIR spectrum of released CDDP was similar to lyo-CDDP examined (Fig. 5). Other than the characteristic amine stretching peak of CDDP (3400–3200 cm^{-1}), the asymmetric amine bending (1600–1500 cm^{-1}) and the symmetric amine bending (1300–1200 cm^{-1}) similar to those of intact CDDP, both lyo-CDDP and CDDP-R samples exhibited hydroxyl stretching and bending peaks (wave numbers 3600–3000 cm^{-1} and 1420–1400 cm^{-1}, respectively).

In vitro cytotoxicity of cisplatin has shown that cisplatin and CDDP-R have similar activity while p(AA-MMA) microparticles and cisplatin-loaded p(AA-MMA) microparticles showed only minimal toxicity at the highest concentration (Fig. 6). Compared to values for cisplatin in various glioma cell lines and in the NIH/3T3 cell line (0.18 μg/ml or 0.6 μM) [19], the LC_{50} of cisplatin and CDDP-R were comparable at 26.6 μg/ml (88.6 μM) and 112 μg/ml (373 μM), respectively, in which CDDP-R concentration has been corrected for platinum content as determined by the oPDA method. The LC_{50} of p(AA-MMA) microparticles could not be calculated from the cytotoxicity results due to the inability to reproducibly suspend the microparticles at the elevated concentrations. The LC_{50} of cisplatin-loaded p(AA-MMA) microparticles was 4688 μg/ml (15.6 mM). Considering the cisplatin loading, the maximum non-toxic concentration of cisplatin-loaded in the microparticles was equivalent to 2344 μg/ml (7.8 mM) of free cisplatin.

After intravenous injection in rats, cisplatin was lethal at levels similar to that reported in the literature, 14.51 mg/kg (Fig. 7) [20]. Cisplatin-loaded and unloaded p(AA-MMA) microparticles were equally lethal as having an LD_{50} of 175 mg/kg. Considering the cisplatin loading efficiency and cisplatin weight percentage in cisplatin-loaded p(AA-MMA) microparticles, the LD_{50} of cisplatin-loaded p(AA-MMA) microparticles was equivalent to 83.82 mg/kg cisplatin, substantially higher than that possible for free cisplatin.

4. Discussion

The synthesis of p(AA-MMA) microparticles in this study may lead to a new approach for delivery of cisplatin and many other therapeutic agents which can strongly complex with carboxylic groups. Current

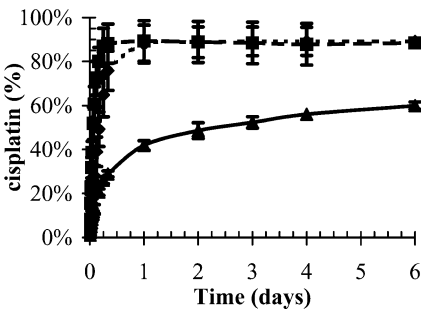

Fig. 4. Cumulative in vitro release in terms of percent theoretical loading of (▲) cisplatin from poly(acrylic acid-*co*-methyl methacrylate) microparticles loaded from water-dimethyl sulfoxide (Method II) solution of cisplatin. Free cisplatin, (♦) 25 mg and (■) 5 mg, was also placed in the donor chamber to validate the apparatus and used as a dissolution standard. A single data point (6th day for cisplatin-loaded poly(acrylic acid-*co*-methyl methacrylate) microparticles) is the average of two data points, remaining samples are presented as the mean and standard deviation of three independent samples.

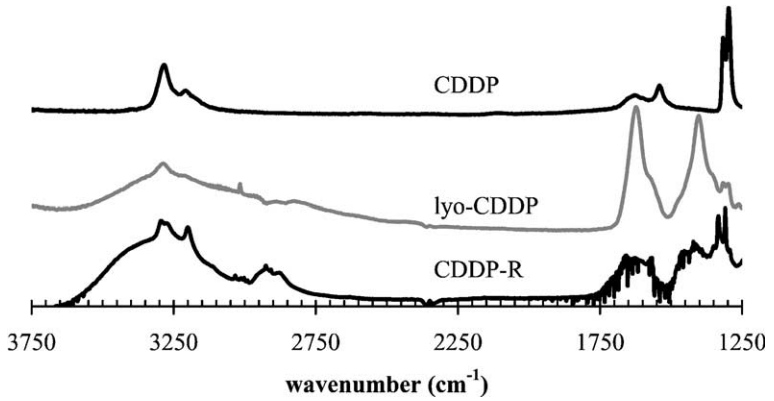

Fig. 5. Attenuated total reflectance Fourier transformed infrared spectra of cisplatin (CDDP), lyophilized cisplatin (lyo-CDDP), and released cisplatin (CDDP-R).

clinical administration of cisplatin is mainly *i.v.* infusion or *i.p.* injection. The systemic toxicity in either case is generally high. By utilizing this complexation-based system, high does of cisplatin can be initially injected and cisplatin will be slowly released from the microparticles. Since cisplatin will be released at a rate lower than typical for *i.v.* or *i.p.* solutions, the microparticles minimize the systemic toxicity and reduce the number of injections necessary to maintain systemic concentrations of cisplatin or other chemotherapeutic agent. This type of system could also be utilized for local therapy if injected at the site of a tumor due to the particle diameter which ranged from 500 nm to 2 ìm. This sized microparticle can also be applied to many forms of drug delivery including oral, nasal, and parenteral routes.

To form microparticles that can be applied to so many routes of delivery with these monomers, a micellar model of block copolymer of oligo(methyl methacrylate) (oMMA) and poly(acrylic acid) with hydrophobic core of oMMA and hydrophilic shell of pAA has been proposed [14]. This model explained some physicochemical properties of the synthesized p(AA-MMA) colloidal microparticles including ζ-potential. The ζ-potential is very important to predict the stability of colloidal system [21]. The ζ-potentials of p(AA-MMA) microparticles differed with the AA feed mole fraction. The highest ζ-potentials occurred when the acrylic acid feed mole fraction ranged from 0.65 to 0.75. Based on the model of Inoue et al.

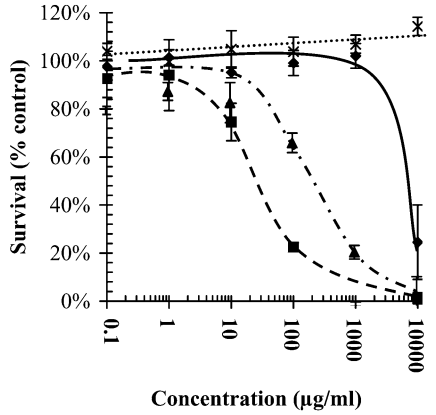

Fig. 6. Cytotoxicity of (×) poly(acrylic acid-*co*-methyl methacrylate) microparticles (X_{AA}=0.63), (♦) cisplatin-loaded poly(acrylic acid-*co*-methyl methacrylate) microparticles, (▲) released cisplatin and (■) cisplatin in NIH/3T3 cells.

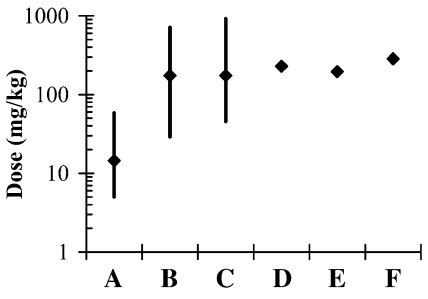

Fig. 7. Acute toxicity (LD_{50}) of (A) cisplatin, (B) cisplatin-loaded poly(acrylic acid-*co*-methyl methacrylate) microparticles, (C) poly (acrylic acid-*co*-methyl methacrylate) microparticles, (D) poly(butyl cyanoacrylate) nanoparticles, (E) poly(isobutyl cyanoacrylate) nanoparticles, and (F) poly(hexyl cyanoacrylate) nanoparticles from Kreuter [26].

propagating AA does not form on the surface of growing pMMA at high AA feed mole fraction due to the high water solubility of AA and low pMMA seed solubility [14]. The yield of copolymer p(AA-MMA) microparticles supported this idea; the yield of microparticles was quite low particularly in cases where the acrylic acid content was high due to the large content of uncrosslinked or loosely crosslinked polymer formed in the aqueous phase. When the mole fraction of acrylic acid was less than 0.65, there was less AA available for polymerization and thus lower ζ-potential, but the yield of microparticles were higher in the lower AA feeds. In both cases, ζ-potentials were decreased compared to intermediate AA mole fractions. The mole fraction of two components played an important role in ζ-potential values and furthermore the stability of aqueous suspension of p(AA-MMA) microparticles. The main reason that p(AA-MMA) microparticles with an acrylic acid feed ratio of 0.63 was chosen for this preliminary study was the high electronegativity; this mole fraction also exhibited the largest fraction of 500 nm particles which further suggested this formulation for use in the preliminary studies reported here. However, further studies are necessary to understand how all compositions of microparticles interact with cisplatin and the body.

The ζ-potentials of cisplatin-loaded p(AA-MMA) microparticles were decreased compared to unloaded p(AA-MMA) microparticles. The reason was that the complexation of cisplatin with carboxylic groups in acrylic acid residues partially disturbed the hydration layer of the particle surface combined with masking of negatively charged acrylic acid with neutral platinum complex, specifically mono- or bi-carboxyl cisplatin. By solubilizing CDDP before complexation (Method II), the high loading efficiency of cisplatin increased and caused more carboxylic acid occupation and a subsequent decrease of ζ-potential. The ζ-potential differences partially support the proposed complexation mechanism (Fig. 8) and suggested a strong, multivalent interaction between cisplatin and p(AA-MMA) microparticles. Other groups have investigated polymer therapeutics utilizing a multivalent anionic complexation between platinum and polymer [22,23] as do carboplatin [24] and other platinum-based chemotherapeutics [25].

Potentiometric titration confirmed that acrylic acid content in p(AA-MMA) microparticles increased from acrylic acid feed mole fraction (X_{AA}) of 0.59 to 0.91. Considering the proposed complexation mechanism, the titration could thus guide the cisplatin loading capacity estimation. Theoretically, higher acrylic acid content would allow higher loading capacity of complexed drug. This idea needs to be confirmed in future studies.

The release of cisplatin from p(AA-MMA) microparticles was prolonged compared to cisplatin suspension or solution in the device that was used. Cisplatin is first released as a burst over the first day. This can be explained by cisplatin entrapped in the microparticles but not complexed to the polymer. Free cisplatin is expected because the microparticles are not thoroughly washed following loading. The free cisplatin molecules quickly diffuse from the small microparticles. Cisplatin release from p(AA-MMA) microparticles after the first day followed approximately zero-order kinetics. At the end of sixth day, only about 60%

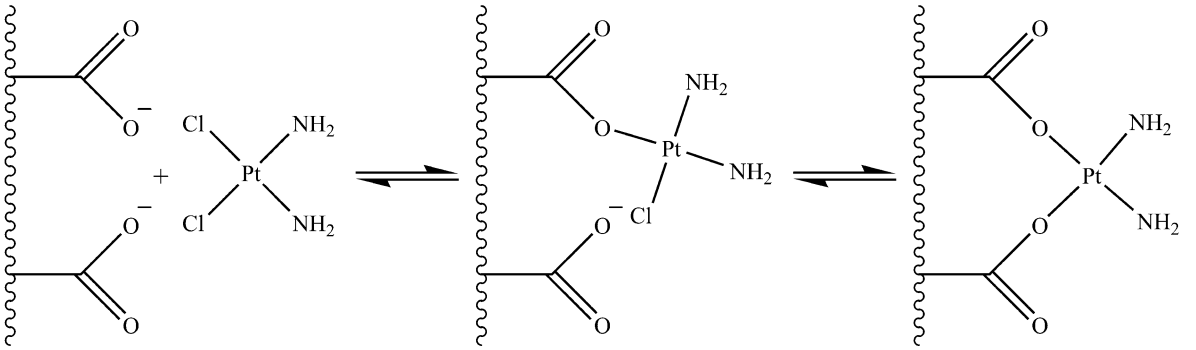

Fig. 8. Schematic of the proposed complexation mechanism of cisplatin and carboxyl group from acrylic acid residues.

of loaded cisplatin was released from the microparticles. Cisplatin-loaded p(AA-MMA) microparticles will release cisplatin for a longer period considering the release profile has not yet reached plateau. The prolonged release profiles is due to the interaction of cisplatin with carboxylic groups [7] combined with diffusion of cisplatin from the polymeric matrix [14]. The prolonged release suggests that complexation with functional groups can increase the loading efficiency and change the release profile of cisplatin from a particulate delivery system, but release alone does not indicate that active drug is being released.

ATR-FTIR spectra confirmed that released CDDP maintained its characteristic amino groups and was partially hydrated. It is expected that chloride groups are rapidly replaced by carboxylic groups from acrylic acid residues or hydroxyl groups from water. The ATR-FTIR spectra do not perfectly match cisplatin. Low molecular weight poly(acrylic acid), poly(acrylic acid)-CDDP complex, and other impurities in the released cisplatin sample are expected. These impurities are responsible for much of the difference between the free cisplatin spectra and released cisplatin spectra. The fact that the characteristic peaks in released cisplatin remain suggests that the structure of released cisplatin has not been greatly altered. HPLC retention times confirmed that released cisplatin was not chemically altered in a significant way (unpublished data). Combined, these results suggest that released CDDP was not associated with polymers or associated only with low molecular weight species.

As expected, prolonged release of cisplatin reduced toxicity of the drug. The toxicity of cisplatin-loaded p(AA-MMA) microparticles (LC_{50}=4688 μg/ml; 15.6 mM) was comparable to that of p(AA-MMA) microparticles. The results showed that the complexation of cisplatin reduced the cytotoxic concentration for NIH/3T3 cells by at least one order of magnitude. Cisplatin released from cisplatin-loaded p(AA-MMA) microparticles remained active following release, but had slightly lower activity (LC_{50}=112 μg/ml; 373 μM). The slightly lower activity compared to intact cisplatin (LC_{50}=26.6 μg/ml; 88.6 μM) may have been caused by the interaction of cisplatin with soluble small molecular weight oligomers with acrylic acid residues, but HPLC and ATR-FTIR suggest that this is not the case. There are still other possibilities to explain the lower cytotoxicity. More likely, aquated/hydrated platinum may be excluded from cells. Cisplatin is typically in the intact chlorinated state outside the cell (in the blood) due to a high serum chloride concentration, but upon entry aquation/hydration takes place rapidly [4,16]. Aquated platinum, being positively charged, is not rapidly transported into cells. Also, aquated cisplatin is in equilibrium with a neutral, but inactive hydroxylated species (pKa ≈ 6.56) [16]. Thus, several reasons can explain the reduced activity of cisplatin released from microparticles. However, the minimum toxicity of p(AA-MMA) microparticles and cisplatin-loaded p(AA-MMA) microparticles make high dose administration possible and overcome the slight decrease of cisplatin activity.

Side effects of cisplatin could also be minimized by use of this, or similar, delivery system. This idea was supported by in vivo acute toxicity studies in rats. In acute toxicity studies, no animal injected with either p(AA-MMA) microparticles or cisplatin-loaded p(AA-MMA) microparticles showed acute toxicity at a dose level similar to cisplatin. Both p(AA-MMA) microparticles and cisplatin-loaded p(AA-MMA) microparticles had comparable toxicity to poly(cyanoacrylate) nanoparticles in this type of model and are considered to be non-toxic [26]. Cisplatin-loaded p(AA-MMA) microparticles reduced the toxicity of cisplatin six fold compared to a solution of free cisplatin. This result is very promising and suggests that further development of a polymeric particulate delivery system to control the release of toxic therapeutics is possible. Unfortunately, the described system only retains a portion of the activity of cisplatin. It has been suggested, however, that low-dose chemotherapy is effective in vivo even though in vitro studies show marginal effect [27]. Further studies examining the in vivo efficacy of this polymer-based microparticulate drug delivery system are ongoing based upon these preliminary studies.

5. Conclusion

A non-toxic microparticulate drug delivery system has been synthesized and examined in vitro and in vivo. The carboxylic acid content of microparticles can be controlled based upon the feed composition of

the monomers even though the yield of microparticles decreases dramatically at high acrylic acid feed. The physical properties of these microparticles are determined predominantly by the acrylic acid feed mole fraction. The diameter of the microparticles is acceptable for administration via various routes, including intravenous, intraperitoneal, intravitreal, or intrathecal administration.

Acrylic acid was included to allow complexation of certain transition metal drugs, including cisplatin. In vitro release of cisplatin was prolonged which will allow localization of drug delivery. The binding of cisplatin to microparticles was sufficient to reduce the activity (in vitro) and toxicity (in vivo) of cisplatin. Cisplatin released from cisplatin-loaded p(AA-MMA) microparticles retained its activity to a large extent. This system shows great promise as a drug delivery platform and as a model for targeted (activated) drug delivery due to the favorable characteristics identified in these studies.

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