Encapsulation and Release of Rhodium(II) Citrate and Its Association Complex with Hydroxypropyl-β-cyclodextrin from Biodegradable Polymer Microspheres

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Received November 4, 1998. Final revised manuscript received February 5, 1999.
Accepted for publication February 8, 1999.

Abstract □ Rhodium(II) carboxylates and their derivatives constitute a promising class of second-generation transition metal compounds with anticancer properties. While most transition metal anticancer compounds chelate DNA and cause extensive chromosomal damage, rhodium(II) carboxylates act on the enzyme DNA polymerase α and hence cause minimal chromosomal damage. Rhodium(II) citrate, a recent member of the rhodium(II) carboxylate family is highly promising as an antitumor agent. However, due to its high water solubility, a high systemic dose is necessary to achieve efficacy. In this paper, we have explored the complexation of rhodium(II) citrate with hydroxypropyl-β-cyclodextrin as a means to improve encapsulation and release kinetics from poly(*dl*-lactic-co-glycolic) acid (PLGA) and poly(anhydride) microspheres. We observed that complexation of rhodium(II) citrate with hydroxypropyl-β-cyclodextrin significantly increased both the encapsulation efficiency and duration of release in both polymer systems.

Introduction

A class of promising second-generation transition metal anti-cancer compounds are the rhodium(II) [Rh(II)] carboxylates.[1−4] Most anti-cancer drugs induce chromosomal damage in treated cells (e.g., cisplatin), resulting in the formation of chromosomal lesions.[5] In contrast, rhodium(II) butyrate only slightly increases the incidence of chromatid gaps and breaks.[3] It is believed that its mode of inhibition of DNA synthesis is via the inhibition of enzyme(s) essential for DNA synthesis, such as DNA polymerase α, rather than DNA chelation. In general, inhibition of DNA synthesis by R-substituted Rh(II) carboxylates derivatives in vitro increases with increasing side chain lipophilicity in the order: Rh(II) methoxyacetate < acetate < propionate < butyrate.[2,5] However, the simple extension of the carboxylate chain (R group) is not effective in increasing the therapeutic effects of the drug. Thus it appears that for Rh(II) compounds to be efficacious, they have to exhibit some degree of water solubility.[6] However, Rh(II) butyrate and analogues are very toxic; Rh(II) carboxylates irreversibly inhibit enzymes that possess sulfydryl groups.[4] Recently, a new class of Rh(II) carboxylates [i.e., Rh(II) citrates] have been synthesized and shown to be promising compounds for chemotherapy.[7−9] However, due to the high water solubility of Rh(II) citrate, high systemic doses of the drug would be required to achieve efficacious concentrations in tumor sites. Therefore, it is useful to develop controlled release systems wherein the Rh(II)-complex is shielded from the extracellular milieu to minimize local toxicity and prolong drug action.

To address this problem we have examined the host: guest complexation approach using cyclodextrins to alter solubility and improve the encapsulation and release from polymer microspheres.

Experimental Section

Materials—Rhodium(II) citrate was prepared as described elsewhere.[7] Hydroxypropyl-β-cyclodextrin (HPBCD) was generously donated by Cerestar Company to VPS and had a degree of substitution of hydroxypropyl groups between 5 and 7. Poly(vinyl alcohol) (PVA) was purchased form Aldrich (MW, 70 000; 90% hydrolyzed) and used as received. The poly((*dl*)lactic-*co*-glycolic)-acid (PLGA; RG503, MW, 30 000) was purchased from Boehringer Ingelheim (Indianpolis, IN) and poly(1,3-bis(*p*-carboxyphenoxy)-propane-*co*-sebacic acid) (CPP:SA, 20:80; (MW, 70 000) was prepared as described elsewhere.[10] All other chemical were purchased from Aldrich Chemical and used as received.

Methods—*Preparation of the Association Compound between Rhodium(II) Citrate and HPBCD*—The association compound was prepared by mixing 292 mg of HPBCD and 200 mg of Rh(II) citrate in 20 mL of distilled water and stirring overnight. The resulting solution was then frozen in liquid N_2 and lyophilized into a free flowing powder.

Characterization of Rh(II) Citrate−HPBCD Association Complex—the formation of the association complex of Rh(II) citrate with HPBCD was studied by infrared (IR) spectroscopy and thermogravimetric (TG) analyses. The IR spectra were obtained on a Nicolet Magna IR-550 FTIR equipped with a liquid nitrogen cooled helium−neon laser. All samples were pressed as KBr pellets. The TG and differential thermogravimetric (DTG) curves were obtained on 5−10 mg of sample under dynamic nitrogen atmosphere using a Perkin-Elmer Series 7 thermogravimetric analyzer interfaced to a PC. The curves were analyzed using Perkin-Elmer TGA software.

Preparation of the Microspheres—Polymer microspheres were prepared by a solvent evaporation technique.[11] In brief, 200 mg of the polymer (PLGA or CPP:SA) was dissolved in 1 mL of

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10.1021/js9804314 CCC: $18.00
Published on Web 04/02/1999

methylene chloride. To this, 50 mg of the Rh(II) citrate or the association compound (corresponds to ≃20 mg of Rh(II) citrate) dissolved in 200 mL of water was added, and the solution was sonicated using a Vibra Cell ultrasonicator (Sonics and Materials, Danbury, CT, six pulses, 50% duty cycle, output 3) to prepare the first w/o microemulsion. The microemulsion was then dispersed in 100 mL of a 1% PVA solution with an homogenizer (Silverson L4R, Nottingham, England, 3/4 in. homogenization tip) at 5200 rpm for 1 min to stabilize the second emulsion (w/o/w). The PVA solution containing polymer microspheres was then stirred for 1.5 h to facilitate evaporation of methylene chloride (solvent) and induce hardening of the polymer microspheres. The microspheres were isolated by centrifugation at 1500 rpm for 5 min, subsequently washed three times with water to remove surface-adsorbed PVA, and finally resuspended in 2 mL of water and freeze-dried to a free powder. The size distribution of the microspheres was determined with a Coulter Counter multisizer.

Rhodium(II) Citrate Release Studies—the release studies were carried out on 10 mg of microspheres in 1.5 mL Eppendorf tubes containing 1 mL of phosphate buffer release medium (pH 7.4) at 37 °C. To ensure thorough mixing, the tubes were placed on a Labline orbital shaker. Sink conditions were maintained by periodically replacing the release medium with fresh release buffer throughout the duration of the study.

Quantification of Rhodium—The Rh(II) citrate released was quantified by visible spectroscopy (Beckman DU-6) and inductively coupled plasma mass spectrometry (ICP-MS). The visible absorbance was measured at 591 nm in 1X PBS using a quartz sample cell (1 cm path length). The calibration curve was established using known concentrations of Rh(II) citrate in 1X PBS. The loading in microspheres was determined by solvent extraction. In brief, 5.0 mg of microspheres (in triplicate) was first placed in 1 mL of methylene chloride to dissolve the polymer phase and then extracted with 1 mL of 1X PBS. The Rh(II) citrate, which was extracted in the aqueous phase, was quantified as described above.

Results and Discussion

Characterization of the Association Compound between the Rhodium (II) Citrate and HBCD—The formation of the association compound between Rh(II) citrate and HPBCD was verified by IR, TG, and DTG. The IR spectra of the association compound showed two important characteristics consistent with the formation of an associative complex between the Rh(II) citrate and HPBCD: (a) displacement of the IR band associated with the stretching the −OH bond to 3400 cm^{-1} in comparison with free HPBCD (3440 cm^{-1}) and (b) narrowing of this band due to the breakdown of hydrogen bonding in the CD cavity upon the release of the resident water molecules.[12,13]

The TG and DTG curves of Rh(II) citrate, HPBCD, and the association compound between Rh(II) citrate and HPBCD are shown in Figures 1a and 1b, respectively. The TG and DTG curves of Rh(II) citrate revealed an onset of mass loss beginning at 25 °C. However, significant changes in mass presumably due to the decomposition of the metal complex, occurred at 220 and 350 °C. In contrast, the TG and DTG curves of the Rh(II) citrate−HPBCD association compound showed only one thermal transition at higher temperature, around 310 °C, which was accompanied by an 80% mass loss. Thus, it appears that the thermal stability of Rh(II) citrate is improved significantly upon association with HPBCD. The increased thermal stability could have significant benefits when using high-temperature polymer processing techniques, such as compression or injection molding to manufacture polymer delivery devices.

Release of Rh(II) Citrate and its Association Complex with HPBCD from PLGA and CPP:SA Microspheres—The size distributions of PLGA and CPP:SA microspheres containing Rh(II) citrate and its association complex with HPBCD are shown in Table 1. An interesting observation was the significant enhancement of Rh(II) citrate encapsulation efficiency upon complexation with HPBCD. The encapsulation efficiency of the free Rh(II) citrate in PLGA microspheres was 30%, whereas that of the HPBCD complex system was 83%. Similarly, the encapsulation efficiencies of free Rh(II) citrate and Rh(II) citrate−HPBCD association complex in CPP:SA were 22 and 79%, respectively. This increase in loading efficiency upon complexation with HPBCD can be explained on the basis of the following observations.

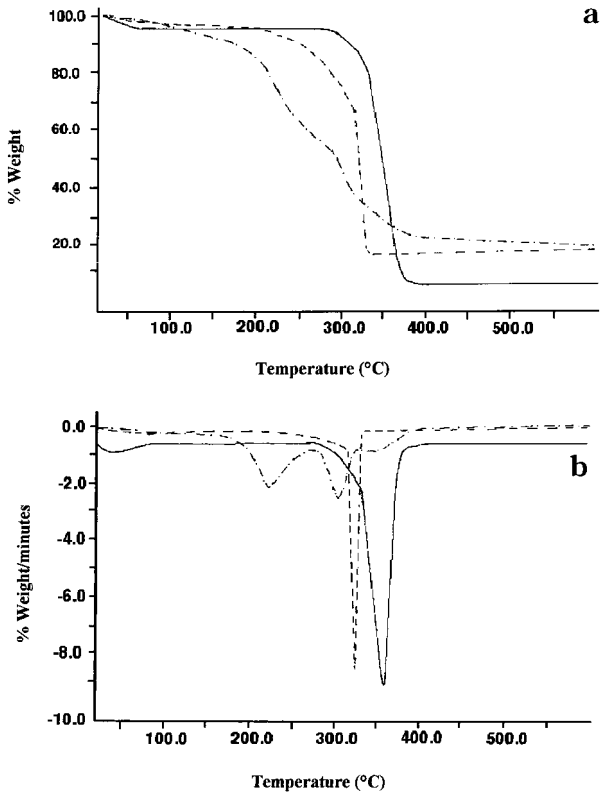

Figure 1—(a) TG curves of HPBCD (–), Rh(II) citrate (–•–•), and association complex between Rh(II) citrate and HPBCD (- - -). (b) DTG curves of HPBCD (–), Rh(II) citrate (–•–•), and association complex between Rh(II) citrate and HPBCD (- - -).

Table 1—Size Distribution and Percent Loading of PLGA and CPPSA Microspheres Containing Rh(II) Citrate and Rh(II) Citrate–HPBCD Association Complex

compound	polymer	size, μm	loading of Rh(II) citrate, %
Rh(II) citrate	PLGA	10.0	30.0
Rh(II) citrate	CPP:SA	20.5	22.4
Rh(II) citrate–HPBCD	PLGA	15.4	83.4
Rh(II) citrate-HPBCD	CPP:SA	25.0	79.2

The enhanced solubility of alkyl-substituted β-CD in comparison with the unsubstituted β-CD has been attributed to several factors, including breakdown of hydrogen bonding between CD molecules, changes in ring conformation, and lowering of the lattice free energy due to its enhanced interaction with surrounding water molecules.[14,15] However, the introduction of alkyl groups such as hydroxypropyl, can also increase the lipophilicity of substituted β-CDs in comparison with the unsubstituted β-CD. One possible mechanism could involve the delocalization of the hydrophobic core in the alkyl substituted β-CD toward the primary 6-CH_2OH hydroxy face.[16] Thus, it is plausible that the complexation of Rh(II) citrate with HPBCD can alter the partition coefficient of the Rh(II)

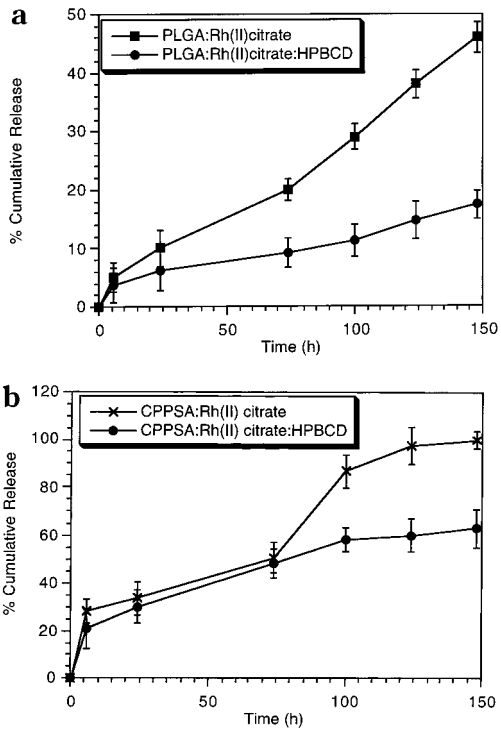

Figure 2—(a) Release of Rh(II) citrate and Rh(II) citrate–HPBCD from PLGA microspheres. (b) Release profiles of Rh(II) citrate and Rh(II) citrate–PBCD association complex from CPP:SA (20:80) microspheres. The error bars are represented as the standard deviation from the mean.

citrate molecule so as to bias its distribution in the hydrophobic polymer phase during microsphere preparation

The release profiles of Rh(II) citrate and its association complex with HPBCD from PLGA microspheres are shown in Figure 2a. The 'burst effect' (8 h) was almost identical in both the cases although the loading was nearly three times higher in the case of the Rh(II) citrate−HPBCD association complex. Beyond 24 h, near zero-order release kinetics was observed, with 45% of the encapsulated Rh-(II) citrate released at 148 h in comparison with 18% of the HPBCD complexed molecule. Thus, it appears that one can prolong the duration of release of small molecules from polymer microspheres by association with CDs.

Release profiles of Rh(II) citrate and Rh(II) citrate−HPBCD association complex from CPP:SA (20:80) microspheres are shown in Figure 2b. As in the PLGA system, the 'burst effect' was almost identical in both the cases, although the loading of the Rh(II) citrate−HPBCD association complex in the microspheres was nearly four times higher. As observed in the PLGA system, the association of Rh(II) citrate with HPBCD prolonged the duration of release from the CPP:SA microspheres as well. Whereas 100% of the encapsulated Rh(II) citrate was released at the end of 148 h, only 60% of HPBCD complexed molecule was released during the same period.

Finally, in this study we observed that although the size of the PLGA and CPP:SA microspheres containing Rh(II) citrate−HPBCD was larger (20.5 and 25.0 μm, respectively) when compared with microspheres containing uncomplexed Rh(II) citrate (10.0 and 15.4 μm, respectively), the cumulative release was lower in the former system. If one were to assume that the surface roughness of all the microspheres were similar, the lower cumulative release from the PLGA and CPP:SA microspheres containing the complexed Rh-(II) citrate is surprising, considering the fact that the surface area of these microspheres would be larger in comparison with the microspheres containing uncomplexed Rh(II) citrate. This result could be due to the decrease in the solubility of the Rh(II) citrate upon complexation with HPBCD, as discussed earlier.

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Acknowledgments

The authors acknowledge the Ministry of Education of the Brazilian Government (CAPES) for partial support through a sabbatical Fellowship to RDS and the National Institutes of Health (GM 26698−18).

JS9804314