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Controlled release of anti-cocaine catalytic antibody from biodegradable polymer microspheres

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Abstract

Recent reports have shown that anti-cocaine catalytic monoclonal antibody 15A10 reduces the toxic effect of cocaine by increasing its breakdown to systemically inert products ecgonine methylester and benzoic acid. This study reports the microencapsulation of antibody 15A10 using biodegradable poly (lactic-glycolic) acid (PLGA) by double emulsion technique. Formulation parameters such as protein loading, polymer molecular weight and the presence of zinc carbonate were studied for their effects on in-vitro release of antibody from microspheres. The initial burst release was decreased by the reduction of the protein (as % of total ingredients) in the formulation. Although changing the polymer molecular weight did not cause a reduction in initial burst release, it was effective in improving the release rate. The inclusion of zinc carbonate in microsphere preparation resulted in increase in initial burst release. An in-vivo study in mice revealed the presence of antibody in blood up to ten days following subcutaneous injections. These data demonstrate a potential for a sustained-release formulation of monoclonal antibody 15A10 for treatment of cocaine addiction.

Introduction

Cocaine abuse, a leading cause of death among young adults, continues to be a significant social and medical problem (Marzuk et al 1995). The most severe adverse reactions to cocaine include cardiac arrhythmia, myocardial infarction, stroke, seizures and sudden death. Current therapies are generally not effective against all the toxic effects of cocaine. Moreover, some effects of cocaine can be exacerbated by concurrent treatment of other illness, such as myocardial infarction, seizures and cardiovascular abnormalities (Nelson & Hoffman 1995).

A more satisfactory approach might be to reduce the toxicity of cocaine by accelerating its metabolic inactivation. In recent years, various groups have examined the possibility of using either vaccination or catalytic antibodies for the treatment of cocaine addiction. Landry et al (1993) developed an antibody capable of catalysing the cocaine molecule, which proved to extinguish cocaine self-administration behaviour in a selective manner in a rat model (Mets et al 1998). Since use of a catalytic antibody would require multiple injections in a drug abuser and patient compliance is always a problem, a local delivery of a sustained-release formulation could be of great advantage.

Controlled-release parenteral formulations, using biodegradable polymer, have been developed for numerous therapeutic agents (Lewis 1990). One of the advantages of these formulations is that no follow-up surgical removal is required once the drug supply is depleted. Among different polymers, poly (DL-lactic-co-glycolic acid) (PLGA) copolymers have been extensively studied as microparticle carriers for many bioactive molecules. PLGA copolymers are biocompatible, biodegradable and approved by the FDA for certain human clinical uses (Suggs & Mikos 1996). The degradation times of PLGA can be altered from days to years by varying the polymer molecular weight, the ratio of lactic to glycolic acid in the copolymer, or the structure of the microparticle (Hollinger & Leong 1996; Anderson & Shive 1997). Release characteristics of several proteins, such as luteinizing hormone-releasing hormone analogue (Sanders et al 1985), human serum albumin (Hora et al 1990), somatostatin

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(Herrman & Bodmeier 1995, 1998), bovine serum albumin (Cohen et al 1991; Sah et al 1994; Marchais et al 1996), hIGF-1 (Lam et al 2000), αVEGF (Kim & Burgess 2002), TGFβ (Lu et al 2000) and ovalbumin (O'Hagan et al 1994; Uchida et al 1995) have been investigated. These microspheres were prepared by a water-in-oil-in-water (W/O/W) type emulsion solvent evaporation method. The drug release rates from PLGA microspheres are affected by various factors including the structure, size and solubility of the encapsulated molecules as well as the structure and degradation of the polymer.

In this study, we investigated the feasibility of encapsulating the anti-cocaine catalytic antibody using PLGA for parenteral delivery. We also determined the effect of copolymer molecular weight, protein concentration and the presence of additive zinc carbonate on the release characteristics of the antibody.

Materials and Methods

Materials

The copolymer poly (DL-lactide-co-glycolide) (502H or 506) was obtained from Boehringer Ingelheim, (Germany). The surfactant, L-α-phosphatidylcholine was purchased from Avanti Polar-Lipid, Inc. (Alabaster, AL). Antibody 15A10 and TSA-1 were by courtesy of Dr Landry. Solvents, reagent grade, were obtained from Sigma Chemical Co. (St Louis, MO); ABTS from Pierce (Rockford, IL); HRP-goat/mouse IgG from Zymed (San Francisco, CA). Balb/c mice were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN).

Microsphere preparation

Microspheres were prepared according to the W/O/W-solvent evaporation method. Four separate preparations with different characteristics were made (Table 1). For preparation A, 500 mg of poly (DL-lactide-co-glycolide), PLGA, was dissolved in 5 mL of dichloromethane. One hundred microlitres of phosphatidylcholine (PC) solution, (8 mg mL^{-1}) was added and vortexed for 1 min. A concentrated antibody solution (90 mg in phosphate-buffered saline, PBS) was then injected in the dichloromethane solution and vortexed for 1 min. The mixture was then added to 1.25 mL of poly vinyl alcohol (PVA) (1%) and mixed for 1 min. Dichloromethane was then removed by addition of 100 mL of PVA (0.3%) and stirring for 3 h. PVA was removed by multiple washes with water and centrifugation. Microspheres were freeze-dried and kept at −20 °C.

For preparing B, the method was similar to that used for A, but the amount of protein was reduced to 10 mg.

For C, microspheres were prepared by a modification suggested by Westedt et al (2000). In addition to changing the proportion of ingredients, 2.3% (of total weight) of zinc carbonate was added (Table 1).

For preparation D, the modified method of Westedt et al (2000) was also used. Ingredient proportions were similar to preparation C but no zinc carbonate was added. In addition, the emulsion was sprayed in cold PVA (0.3%), through a sonicator (40 set for 1 min). Also, all the steps were carried out under nitrogen stream. All preparations were made at least three times.

Protein loading

The total antibody content of the microspheres was determined by dissolving 10 mg of the samples in 3 mL of 1 M NaOH containing 0.5% of sodium dodecyl sulfate (SDS), incubating at 37 °C overnight and measuring by a modification of Lowry method (1951). The mean particle size (by volume) of the microspheres was determined from the particle size distribution using a Coulter Counter (Beckman Coulter Inc., Miami, FL). Morphology and surface appearance of microspheres was examined by electron microscopy.

In-vitro study

In an Eppendorf tube, 10 mg of microspheres were incubated at 37 °C in 200 μL of PBS buffer, pH 7.4, containing 0.4% BSA and 0.05% Tween 20. After various times, the tubes were removed and centrifuged at 201 g for 2 min and the supernatant was recovered and kept at 4 °C until analysis. Microspheres were resuspended with 200 μL fresh buffer and incubation was continued. All the assays were run in triplicate.

ELISA assay

ELISA assay was performed according to the method developed by Landry et al (1993) with modifications. Briefly, in a 96-well plate, each well was covered by 100 μL of antigen (TSA-1) and incubated for 2 h or overnight at 4 °C. The antigen was then discarded and

Table 1 Preparation characteristics of different batches of microspheres.

Batch	Dichloromethane (mL)	PLGA (type)	PLGA (mg)	Antibody (mg)	Additive $ZnCO_3$ (mg)	Protein recovery (μg/10 mg)
A	5.0	RG506	500	90	—	191.9 ± 24.5
B	5.0	RG506	500	10	—	269.3 ± 13.5
C	1.5	RG502H	607	10	—	235.8 ± 2.8
D	1.5	RG502H	607	10	15.3	241.7 ± 25.4

200–300 μL of blocking solution (1% BSA in PBS, pH 7.4) was added and incubated for 2 h at room temperature (23 °C). Blocking solution was then discarded and wells were washed once with PBS. One hundred microlitres of diluted primary antibody (15A10) or unknown samples was added and incubated for 1 h at room temperature. Wells were emptied and washed three times with washing solution (PBS containing 0.4% BSA and 0.05% Tween-20, pH 7.4) and 100 μL of secondary antibody (HRP-goat/mouse IgG) was added and incubated for 1 h. After discarding the secondary antibody and washing the wells with washing solution (3 ×) and PBS (2 ×), 100 μL of ABTS (one-step solution) was added and incubated for 30 min. Reactions were stopped with 1% SDS and absorbance was read with a plate reader (Molecular Device) at 405 nm.

In-vivo study

Mice were housed in a 12-h light–dark condition for two weeks before the test. Mice were injected subcutaneously with anti-cocaine antibody 15A10 (0.615 mg 250 μL PBS) or 6.15 mg of microsphere (preparation D) dispersed in 250 μL of PBS. Ocular blood samples were collected at different times following antibody injection. Serum was then prepared and analysed for the presence of antibody using ELISA technique.

Statistical analysis

In-vitro data are presented as mean ± s.e.m. Comparisons were made between different preparations for the quantity of antibody released in-vitro at each time point using one-way analysis of variance followed by post-hoc Tukey's test. $P < 0.05$ was considered as the significant level. In-vivo results are the mean ± s.d. from two mice in each group and the assays were run in triplicate.

Results and Discussion

This study reports the feasibility of microencapsulation of anti-cocaine catalytic antibody (15A10) using an established double-emulsion technique. Several parameters, such as polymer molecular weight, protein loading and the presence of additive (zinc carbonate), were evaluated for their effect on the release characteristics of antibody 15A10. Electron microscopic evaluations showed that all preparations had smooth surface morphologies (Figure 1). Microsphere diameter was in the range 40–70 μm. Figure 2 shows cumulative release of antibody from different preparations. Microspheres prepared with high-molecular-weight PLGA (RG506) and 15.3% protein (% of total ingredients) showed an initial burst release of 13 μg (mg microspheres)$^{-1}$ (about 81% of total release) within the first 24 h of in-vitro incubation (Figure 2, preparation A).

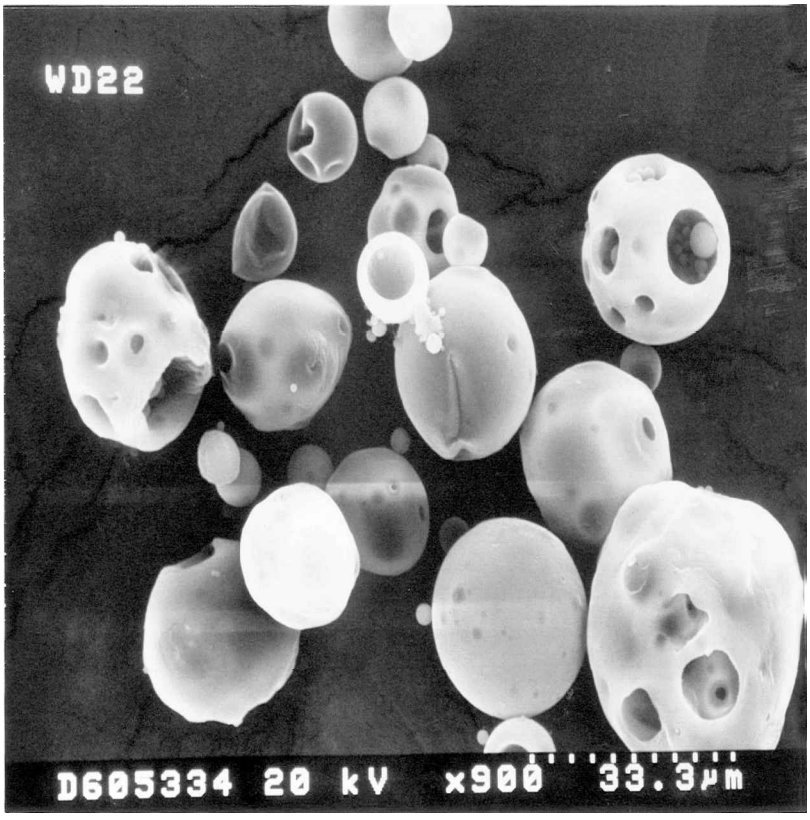

Figure 1 Electron micrographic image of anti-cocaine antibody 15A10-loaded PLGA microspheres.

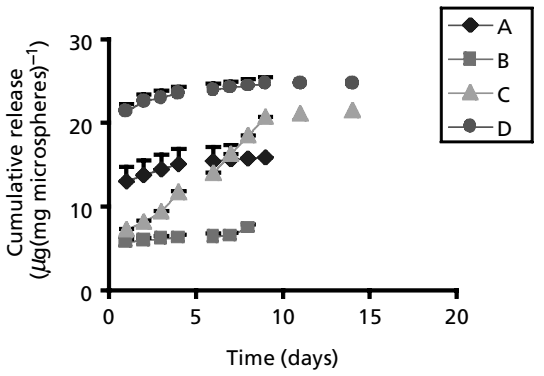

Figure 2 The cumulative release of anti-cocaine antibody 15A10 from microspheres prepared with 15.3% antibody and high-molecular-weight PLGA (A), 1.96% antibody and high-molecular-weight PLGA (B), 1.96% antibody and low-molecular-weight PLGA (C) or 1.96% antibody, low-molecular-weight PLGA and 2.3% zinc carbonate (D). Data are the mean ± s.e.m. of three preparations measured in triplicate.

Slight and gradual release was observed until day 10 of incubation. By lowering the amount of protein in the formulation (to 1.96% of total ingredients in the formulation; Figure 2, preparation B), the initial release decreased to 5.08 μg (mg microspheres)$^{-1}$ (about 76% of total release). This initial release was significantly lower than the burst release from preparation A ($P < 0.05$). Slightly higher amount of antibody was released at 48 h. The release reached a plateau after 72 h.

Polymer molecular weight has been reported to affect the release profile (Lam et al 2000). In preparation C, the protein content was kept constant (10 mg), but the PLGA polymer was changed from high-molecular-weight resomer RG 506 (MW 100 000) to low-molecular-weight resomer RG 502H (MW 10 000). In addition, we have reduced the volume of dichloromethane and increased the quantity of polymer. The initial burst release from this preparation was slightly higher than from preparation B, but significantly lower than from preparation A ($P < 0.05$). The release of antibody from preparation C increased from 24 h up to the 8th day of incubation. The rate of release remained significantly higher than from preparation B ($P < 0.05$) but lower than from preparation A until the 5th day of incubation. After 5 days of incubation, the release of antibody from preparation C was also higher than from preparation A.

The contact of protein in solution with water/organic interface in the primary emulsion for the preparation of PLGA-microspheres by W/O/W-solvent evaporation method can lead to unfolding and denaturation of the protein (Sah et al 1994). Additives such as zinc carbonate have been used to stabilize proteins during microencapsulation (Pean et al 1998). The inclusion of 2.3% (w/w) zinc carbonate in preparation D resulted in slight increase in incorporation of antibody in microspheres (Table 1). The release of antibody from this preparation is shown in Figure 2. The initial burst release was significantly increased ($P < 0.05$). However, the amount of antibody released after the first 24 h was significantly lower than for preparation C (at 2, 4, 7 and 8th day of incubation).

The double-emulsion technique proved to be useful for encapsulation of a number of drugs. With regard to anti-cocaine antibody 15A10, very low levels of loading were obtained. Although lower quantities of protein were used for these preparations as compared with the amounts used by other investigators (Lam et al 2000), nevertheless the incorporation rate seemed to be very small. Perhaps the spray freeze-drying technique, as suggested by Lam et al (2000), could improve the efficiency of encapsulation. The initial release of anti-cocaine antibody could be reduced by decreasing the proportion of protein in the formulation, although subsequent release rate was not affected by this change. The rate of release, however, could be improved by changing the type of PLGA polymer. Zinc carbonate has been used as a release modifier and is suggested to slow the hydration of PLGA microspheres, thereby reducing the initial release (Cleland et al 1997; Johnson et al 1997). However, in this study, the inclusion of 2.3% zinc carbonate in preparation D caused a significant increase in burst release.

A preliminary study was performed to determine whether antibody could be released in-vivo from microsphere preparations. Figure 3 shows the serum content of antibody following subcutaneous injections of pure antibody or microsphere solutions in PBS to mice. Antibody was detected in serum at one hour following injection, although slightly above background (0.05 μg mL^{-1}). In mice injected

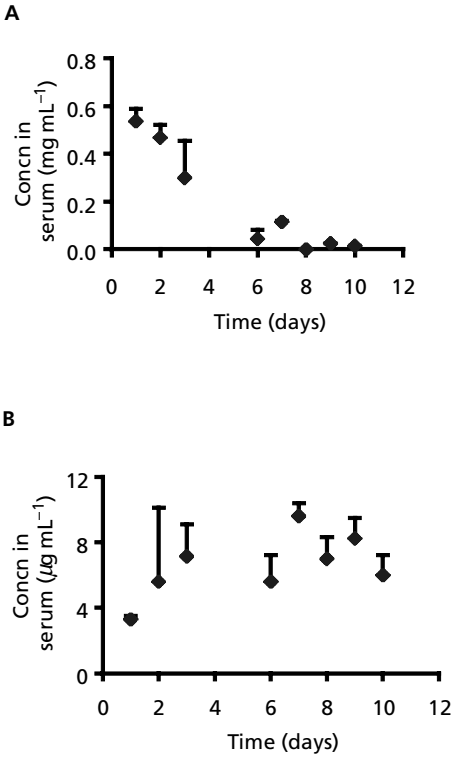

Figure 3 Antibody detected in mice serum after the subcutaneous injection of pure antibody (A) or microspheres (B). Data are the average of two mice measured in triplicate ± s.d.

with pure antibody, the serum level reached 0.54 mg mL^{-1} at 24 h following injection and decreased thereafter to reach microgram levels by day 5. Release of anti-cocaine antibody from microspheres was slow (Figure 3). The serum level was at 3 μg mL^{-1} after 24 h and increased gradually, reaching about 10 μg mL^{-1} on the 7th day post injection. As compared with the in-vitro release of this particular batch of microspheres (preparation D), the in-vivo release within the first 24 h was only a fraction of the release under in-vitro conditions (1.2% vs 85%, respectively). One possible explanation could be the delivery route of microspheres.

A pharmacological intervention for cocaine abuse would be preferred over the traditional receptor-mediated therapy if it provided longer duration of effectiveness and consequently reduced the need for frequent dosing, therefore increasing the patient's compliance. Anti-cocaine catalytic antibody 15A10 is proved to be effective in reducing cocaine-induced increase in mean arterial pressure for at least 3 days in mice (Brisco et al 2001). Preliminary results from this study suggest that antibody could be released from long-lasting microsphere preparation, although to a very limited amount. A reason for the low release of antibody could be the choice of injection route, and other delivery routes should be tested. However, for this preparation to be more effective, not only an antibody variant with greater affinities would be of great interest, but also technological improvement in microsphere production is necessary for increasing the level of antibody encapsulation and augmenting the rate of in-vivo release.

Conclusions

Using an established double-emulsion technique, we have demonstrated that anti-cocaine antibody (15A10) could be used in the preparation of a sustained-release formulation and it could be released in a controlled manner from PLGA polymers. The protein content and the molecular weight of the polymer were both found to have significant effects on the antibody release kinetics. Although the strategy was to deliver higher levels of antibody to break cocaine molecules in-vivo, the use of high percentages of antibody in the formulation did not lead to a greater release (at least in-vitro). Because using lower-molecular-weight polymer caused an improvement in the amount of antibody release, manipulation of polymer molecular weight would be a better strategy for controlling release characteristics. This study also shows that following subcutaneous injection, while the level of pure antibody decreased after 3 days, its release from microspheres was continuous and remained relatively constant for up to at least 10 days. Slow-release formulation of this antibody may therefore be a preferable way for providing the antibody over a long period of time.

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