Synthesis and Characterization of Albumin Microparticles as Carriers for Antibiotic Levofloxacin

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ABSTRACT

Levofloxacin is an antibiotic used to treat a number of bacterial infections including acute bacterial sinusitis, pneumonia, urinary tract infections, chronic prostatitis, gastroenteritis, tuberculosis, meningitis, or pelvic inflammatory disease. However, the use of levofloxacin has some side effects. The accumulation of Levofloxacin in tendon may be the reason for tendon incidents. Modifications in drug delivery to redirect the antibiotic from the circulation and target it to cells, tissues, or organs where infection occurs may lessen the chance for the Fluoroquinolone to travel to bone and cartilage. To address this issue, a microparticulate delivery platform for the levofloxacin should be developed and characterized. Albumin can also serve as a transporting agent for the delivery of copper, zinc, and calcium ions. This plasma protein is able to carry medicines such as warfarin, ibuprofen, chlorpromazine, and naproxen. Binding of the compounds to albumin changes their targeting effects as well as the circulation time. The mean particle size and zeta potential of levofloxacin-loaded microparticles was determined. Higher encapsulation efficiency was observed till the addition of up to 10% levofloxacin. Beyond that percentage, the encapsulation efficiency reduced significantly. In vitro release profile of levofloxacin-loaded microparticles exhibited an initial burst followed by a period of slow release. The levofloxacin-loaded albumin microparticles possess potential as antibiotic carriers for sustained release.

Key Words: *Albumin, Levofloxacin, Microparticles, Particle Size.*

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INTRODUCTION

Devices and vehicles for drug delivery have made excellent contributions to the improvement of therapeutic outcomes by enhancing the efficacy of established and emerging drugs [1–4]. One major milestone in the field of medicine is the development of advanced carriers capable of delivering therapeutic payloads in significant quantities to specific sites [5, 6]. Much of the research in this area has focused on particle-based technologies, such as liposomes, micelles, microparticles and nanoparticles [7–9].

Microparticles are micro-sized. Micoparticles have been used as a cargo space for the encapsulation of a variety of therapeutic and diagnostic agents. Such encapsulation substantially increases their bioavailability and improves their pharmacokinetics and biodistribution. The size of microparticles permit their accumulation in a variety of pathological sites. This fact provides an opportunity for physiology-based targeting of drugs and/or drug-loaded pharmaceutical carriers, such as microparticles to these pathological areas. Microparticles are also easy to prepare on a large scale, providing an additional practical advantage.

Levofloxacin is an antibiotic.[10] It is used to treat a number of bacterial infections including acute bacterial sinusitis, pneumonia, urinary tract infections, chronic prostatitis, and some types of gastroenteritis. Along with other antibiotics it may be used to treat tuberculosis,

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meningitis, or pelvic inflammatory disease. It is available by mouth, intravenously, [10] and in eye drop form. [11] Its common side effects include nausea, diarrhea, and trouble sleeping. [10] Serious side effects may include tendon rupture, tendon inflammation, seizures, psychosis, and potentially permanent peripheral nerve damage. Tendon damage may appear months after treatment is completed. People may also sunburn more easily. In people with myasthenia gravis, muscle weakness and breathing problems may worsen. [10] Levofloxacin is a broad-spectrum antibiotic of the fluoroquinolone drug class. [12] It usually results in death of the bacteria.

In blood plasma, albumin is one of the multifunctional proteins which is found abundantly. Drugs bind to two primary sites in subdomains, namely, IIA and IIIA. These sites are similar and contain six helixes. However, recent work showed that the steroid antibiotic, fusidic acid binds specifically to subdomain IB. Albumin can also serve as a transporting agent for the delivery of copper, zinc, and calcium ions. This plasma protein is able to carry medicines such as warfarin, ibuprofen, chlorpromazine, and naproxen. Binding of the compounds to albumin changes their targeting effects as well as the circulation time. Compared to synthetic polymers, proteins possess several advantages: They might be degraded into the peptides by naturally occurring enzymes; in comparison with the chemically synthesized nanomolecules, they may accumulate in the body and result in toxic degradation products. Electrostatic interactions, hydrophobic attractions, and covalent bindings are the mechanisms allowing attaching the drug with the carrier.

We think that albumin nanoparticles carrying levofloxacin will not possess negative side effects. Also, the frequency of the treatment after utility of the levofloxacin-coated particles in comparison with the free levofloxacin will be less, which will improve the efficiency of this medicine. Binding with the albumin particles will prolong circulation time of this medicine and, consequently, will reduce the metabolic rate and prolong time of its gradual influence without necessity of second dosage uptake.

Chemicals and Reagents

Albumin, levofloxacin, methylene chloride, corn oil, gelatin, formaldehyde, 2,3-butanedione, glutaraldehyde, diethyl ether, phosphate buffered saline, cuvettes for particle size and zeta potential analysis.

Preparation of the microparticles

Albumin microparticles were prepared by multiple emulsion method. Varying concentrations of levofloxacin 0 (blank), 1%, 5%, 10% or 20% of total polymer composition were dispersed in 0.5 ml corn oil respectively. The dispersion was then mixed with 1 ml of various aqueous solution containing (1, 5 and 15%) albumin and 1% gelatin. The mixture was stirred for 10 min to produce an o/w emulsion. The emulsion was added to 3 ml of corn oil and stirred again for 2 min to obtain the corresponding o/w/o multiple emulsion. Thermal crosslinking was used to harden the albumin dissolved in the aqueous phase. The emulsion was frozen to 0º C and subsequently added to 100 ml of corn oil heated to 120°C and stirred for 20 min. The microparticles obtained were separated by centrifugation and washed with 100 ml diethyl ether. In the presence of corn oil as the inner phase of the multiple emulsion, the permanence of a liquid phase within the particles suggested the formation of microcapsules.

Size determination by particle analyzer

The average particle size was measured using the PSS particle size analyzer. Particles were suspended in particle free water (2 mg/mL) in a cuvette. The cuvette was placed in the instrument for measurement reading. An average of three measurements was recorded.

Zeta potential Measurement

In this study, the zeta potential of the particles was measured using the particle size analyzer (PSS). Levofloxacin-loaded particles were suspended in PBS. The sample was transferred into the measurement cuvette and loaded into the instrument for zeta potential measurement. The zeta potential of the levofloxacin-loaded particles was measured at pH 7.4, so that the colloidal stability of the particles could be determined at physiological pH.

Encapsulation efficiency Determination

Each sample of particles was weighed and added to distilled water, to generate a 1 mg/mL sample. To release levofloxacin from the particles, the sample were crushed in a mortar with a pestle and then sonicated for 1 min. The absorbance of the drug was measured by the spectrophotometric instrument, using the UV-vis application. A standard curve of levofloxacin was prepared and used to compare the absorbance values of sample. The encapsulation efficiency was calculated using the following equation:

$$\text{encapsulation efficiency} = \frac{\text{actual drug loading x } 100\%}{\text{theoretical drug loading}}$$

In vitro release studies

Microparticle samples were placed into dialysis bags and suspended in 20 mL of pH 7.4 phosphate buffer solution. Then, microparticles were shaken horizontally in a shaking incubator at 50 rpm and 37±0.5°C. At various time points, samples (1 mL) were withdrawn with a syringe filter (0.45 µm pore size) from the release media, and replaced with an equal volume of the corresponding fresh media. The samples were analyzed at 287 nm spectrophotometrically. The in vitro release experiments were conducted in triplicate.

RESULTS AND DISCUSSION

Particles containing levofloxacin were prepared from various percentages of albumin (Table 1). Diluting the total polymer concentration further decreased the particle size. With all three polymer formulations (1% albumin, 5% albumin and 15% albumin), the zeta potential became less negative as the amount of levofloxacin increased. Some formulations had colloidal stability, since the zeta potential for these formulations were more negative than -30 and colloidal suspensions are stable when zeta potential is more negative than -30 or more positive than +30. We have also shown that a decrease in total polymer concentration results in decreased particle size range, when preparing albumin microparticles. The amount of levofloxacin influences particle size; there is a trend towards increase in particle size, as the concentration of levofloxacin is increased. This trend has been reported when preparing PACA nanoparticles, containing Ofloxacin, by emulsion polymerization (incorporation method), as well (Fresta et al., 1995). Particle size was significantly increased when levofloxacin concentration reached 20%. The zeta potential of the particles became less negative with increased addition of levofloxacin. For the zeta potential to be influenced by the addition of levofloxacin, it is suggestive that the drug compound must be present at the surface as well as contained within the particle. Additionally, the decrease in negative zeta potential with the addition of levofloxacin signified the dissociation of acidic groups for levofloxacin, at the surface of the particle. Since colloidal suspensions are stable when the zeta potential is more negative than -30 or more positive than +30, some formulations would have good colloidal stability at physiological pH in the body.

Table 1: Particle size and zeta potential of albumin microparticles.

Polymer	Levofloxacin Concentration	Particle size range (µm)	Zeta Potential (mV)
1% Albumin	Blank	0.3-2.5	-33
	1	0.5-2.8	-31
	5	0.5-2.7	-27
	10	0.6-3.0	-27
	20	0.6-2.9	-25
5% Albumin	Blank	3.5-5.5	-35
	1	3.5-5.5	-35
	5	3.5-6.0	-30
	10	3.5-6.5	-26
	20	4.0-7.0	-21
15% Albumin	Blank	4.5-8.0	-37
	1	4.5-8.5	-35
	5	5.0-8.5	-33
	10	5.0-8.5	-31
	20	5.0-9.0	-26

The encapsulation efficiency was found in the range of 96% for all three polymer formulations, containing any amount of levofloxacin, except 20% (Table 2). The 20% levofloxacin formulation probably becomes saturated and therefore reduces encapsulation efficiency of the drug. When levofloxacin concentration reaches 20%, encapsulation efficiency dropped significantly, to between 65% and 75%. At this concentration, the suspension became saturated and therefore reduced encapsulation efficiency of the drug. The large particles observed in the 20% levofloxacin formulation were probably drug precipitates. All other formulations, reached an encapsulation efficiency of 96–97%, dictating that the preparation process of these formulations was efficient.

Table 2: Encapsulation Efficiency of Albumin Microparticles

Polymer (%)	Levofloxacin Concentration (%)	Encapsulation Efficiency (%)
1% Albumin	Blank	-
	1	96
	5	96
	10	95
	20	75
5% Albumin	Blank	-
	1	97
	5	96
	10	96
	20	70
15% Albumin	Blank	-
	1	97
	5	97
	10	97
	20	65

To investigate the drug release pattern of microparticles, in vitro release studies were carried out in pH 7.4 phosphate buffer solution with 0.1% (w/v) Tween 80 at 37±0.5°C. Because of favorable properties such as particle size and encapsulation efficiency, 5% albumin microparticles containing 10% drug formulation was used for in vitro release studies. As shown in Figure 1, microparticles displayed biphasic drug release pattern with a burst release within 1 h, followed by a sustained release afterward. In the first hour, free levofloxacin released was 43%. The reason for the initial burst in release profile may be due to the levofloxacin associated on and just beneath the surface of microparticles. The slow release in the later stage was attributed to the fact that the solubilized or dispersed drug can only be released slowly from the polymer matrices compared with free drug.

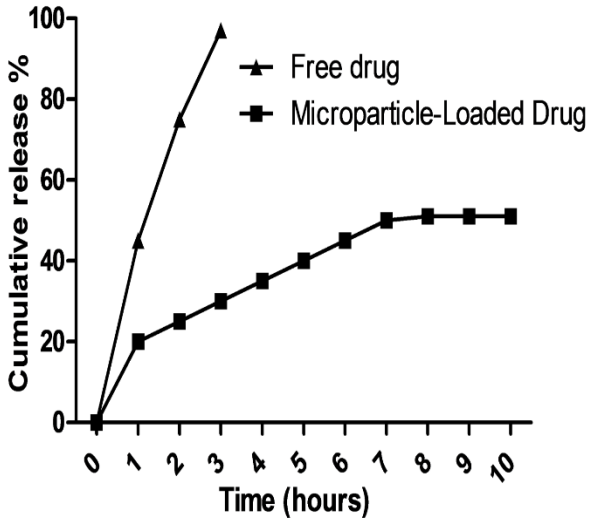

Fig. 1: Cumulative levofloxacin release from microparticles

CONCLUSION

Levofloxacin loaded Albumin microparticles were prepared by using emulsion methods. Higher encapsulation efficiency was obtained till the addition of up to 10% levofloxacin. Beyond that percentage, the encapsulation efficiency reduced significantly. In vitro release profile of levofloxacin-loaded microparticles exhibited an initial burst followed by a period of slow release. The results of our current study showed promising capabilities of levofloxacin-loaded albumin microparticles as carriers of antibiotics.

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REFERENCES

[1] Langer R, Tirrell DA (2004) Designing materials for biology and medicine. Nature 428: 487-492.

[2] Ulery BD, Nair LS, Laurencin CT (2011) Biomedical Applications of Biodegradable Polymers. J Polym Sci B Polym Phys 49: 832–864.

[3] Luckachan GE, Pillai CKS (2011) Biodegradable Polymers- A Review on Recent Trends and Emerging Perspectives. J Polym Environ 19: 637–676.

[4] Li Y, Rodrigues J, Toma´s H (2012) Injectable and biodegradable hydrogels: gelation, biodegradation and biomedical applications. Chem Soc Rev 41: 2193–2221.

[5] Chaudhuri P, Soni S, Sengupta S (2010) Single-walled carbon nanotubeconjugated chemotherapy exhibits increased therapeutic index in melanoma. Nanotechnology 21 (2), 025102. doi:10.1088/0957-4484/21/2/025102.

[6] Ali-Boucetta H, Al-Jamal KT, McCarthy D, Prato M, Bianco A, et al. (2008) Multiwalled carbon nanotube-doxorubicin supramolecular complexes for cancer therapeutics. Chem Commun 4: 459–461.

[7] Davis ME, Chen Z (Georgia), Shin DM (2008) Nanoparticle therapeutics: an emerging treatment modality for cancer. Nat Rev Drug Discov 7: 771–782.

[8] De M, Ghosh PS, Rotello VM (2008). Applications of Nanoparticles in Biology. Adv Mater 20: 4225–4241.

[9] Mora-Huertas CE, Fessi H, Elaissari A (2010) Polymer-based nanocapsules for drug delivery. Int J Pharm 385: 113–142.

[10] "Levofloxacin". The American Society of Health-System Pharmacists. Archived from the original on 1 May 2016. Retrieved 25 August 2016.

[11] "Levofloxacin ophthalmic medical facts from Drugs.com". www.drugs.com. Archived from the original on 2 February 2017. Retrieved 23 January 2017.

[12] Yaffe, Gerald G. Briggs, Roger K. Freeman, Sumner J. (2011). Drugs in pregnancy and lactation : a reference guide to fetal and neonatal risk (9th ed.). Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins. p. 828. ISBN 9781608317080. Archived from the original on 1 February 2016.