Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 26 (2005) 109–115
Biomaterials
www.elsevier.com/locate/biomaterials

Microspheres of corn protein, zein, for an ivermectin drug delivery system

Xinming Liu[a], Qingshen Sun[b], Huajie Wang[b], Lei Zhang[a], Jin-Ye Wang[a,b,*]

[a] *Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Road, Shanghai 200032, China*
[b] *Biomedical Engineering, Shanghai Jiao Tong University, 1954 Huashan Road, 200030, China*

Received 9 December 2003; accepted 3 February 2004

Abstract

A novel microsphere drug delivery system of ivermectin (IVM) using hydrophobic protein zein was prepared by the phase separation method and characterized by a scanning electron microscope and laser light scattering particle size analyzer. Releases of model drug IVM from zein microspheres, tabletted microspheres and pepsin degradation of tabletted microspheres were also performed in vitro to investigate the mechanism of model drug release. The results show that the zein microspheres and tabletted microspheres are suitable for use as a sustained-release form of IVM. The microspheres may also be useful in drug targeting system since the diameter of the microspheres is appropriate for phagocytosis by macrophages. Moreover, the release of IVM from enzymatic degraded tabletted microspheres shows a zero-order release, implying a potential application in tissue engineering for preparing scaffold, which is composed of microspheres encapsulating bioactive components for stimulating cell differentiation and proliferation.

Keywords: Biodegradation; Controlled drug release; Drug delivery; Electron microscopy; Microspheres

1. Introduction

In recent years, microspheres have been proposed to treating many diseases needing a constant drug concentration in blood or drug targeting to specific cells or tissues [1,2]. Various synthetic or natural biodegradable polymers have been developed as materials of microspheres for drug delivery system [3]. Synthetic polymers such as poly(lactic acid) (PLA) and poly(lactic-*co*-glycolic acid) (PLGA) are widely studied because of their superior biodegradability and regulatory physiochemical properties [4]. However, PLGA undergoes bulk erosion that produces a very low pH environment within PLGA matrices, which may adversely affect sensitive therapeutic agents such as proteins [5,6]. As to natural polymers, natural proteins such as gelatin, albumin, casein and soluble monomolecular collagen represent good raw materials since they have the advantages of synthetic polymers own and the advantages of absorbability and low toxicity of the degradation end products. In spite of these possible advantages, they present a main drawback of a rapid solubilization in aqueous environments, thus resulting in fast drug release profiles. In order to overcome this problem, chemical cross-linking procedures (e.g. glutaraldehyde and formaldehyde treatment) have been considered [7–10]. Unfortunately, the presence of residual cross-linking agents could lead to toxic side effects, in addition, unwanted reactions between the drug and cross-linker could result in the formation of toxic or inactivated derivatives [11,12]. Furthermore, as a hydrophilic polymeric system, these protein microspheres have difficulties to achieve sustained drug release. When the system absorbs water and swells, drugs will rapidly diffuse out [7–9]. In contrast, the hydrophobic polymeric system has the capability of yielding sustained drug release [4,5].

As to drug targeting, many works focus on the macrophage drug delivery, which means using the

Abbreviations: IVM, ivermectin; SEM, scanning electron microscope; SR, sustained-release; PLA, poly(lactic acid); PLGA, poly(lactic-*co*-glycolic acid)

*Corresponding author. Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Road, Shanghai 200032, China. Tel.: +86-21-64163300x1225; fax: +86-21-64376697.
E-mail address: jywang@mail.sioc.ac.cn (J.-Y. Wang).

0142-9612/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2004.02.013

macrophage as a vehicle for the drug carrying device [13]. Uptake of microspheres by the macrophage cells heavily depends on the nature of the microspheres such as size, hydrophobicity, and charge. The size plays a great role in controlling drug delivery to the target tissues and the subsequent uptake of drugs into tissues; microspheres with an average diameter of 1 μm are most effectively taken up by macrophages. In addition, a more hydrophobic microsphere surface is desirable for better uptake by macrophages [14–17].

Microspheres with an average diameter of 1 μm have been developed for the i.v. or oral administration of drugs [18]. For per oral delivery, the microspheres could be administered in the form of an aqueous dispersion. However, poor stability of the drug or polymer in an aqueous environment or poor taste of the drug may require the incorporation of the microspheres into solid dosage forms, e.g. tablet. Tablet still accounts for more than 80% of all dosage forms administered to man. The principal reasons for its continued popularity include its ease of manufacture, its convenience of dosing, its stability and the release mechanism compared with liquid and semi-solid presentations [19].

In order to overcome the disadvantages of drug delivery system using hydrophilic protein and offer a drug targeting system, we chose a hydrophobic protein zein to prepare microsphere drug delivery system with an average diameter of 1 μm which can be most effectively taken up by macrophages, and then we used the microspheres to prepare tablet which can obtain zero-order release, an often desired property of a controlled release device. Ivermectin (IVM) was chosen as a model drug.

Zein is a major storage protein of corn. As indicated by SDS-PAGE, zein with biochemical purity used in the present study is mainly composed of two distinct bands, with molecular weight of 23 and 21 kDa. The minor bands are 13 and 9.6 kDa. The high proportion of non-polar amino acid residues is responsible for its solubility behavior. The molecular structure is helical wheel conformation that nine homologous repeating units are arranged in an anti-parallel form stabilized by hydrogen bonds [20]. Zein can form tough, glossy, hydrophobic coatings and has anti-bacterial activity, which has been used in food industry [21]. Furthermore, zein can also prevent avermectin from photo-degradation [22]. Zein has also been used to form microspheres by cross linking a zein solution containing the drug, but it is quite heterogeneous in size [23,24]. In our group, zein has been used to prepare films composed of microspheres with good biocompatibility [25].

IVM, a semi-synthetic derivative of avermectin B1 produced by the soil-dwelling actinomycete *Streptomyces avermitilis*, is a highly effective parasiticide that belongs to the macrocyclic lactone class of compounds. It has a broad spectrum of activity against endoparasites and ectoparasites of sheep, cattle, pigs and dogs [26–28]. Its low toxicity, high efficiency and safety also make it for treating onchocerciasis of human [29]. Many liquid oral, topical and injectable formulations of IVM are currently available for use in cattle. But these formulations have the disadvantages of injury to user or multiple injection or significant systemic side effects. Pour-on formulations of IVM are particularly convenient for single-dose applications in cattle. Unfortunately, assuming total bioavailability of subcutaneous formulations, the highest relative bioavailability for a pour-on formulation of IVM does not exceed 15% [30]. IVM sustained-release (IVM SR) bolus has been shown to have excellent anti-parasitic activity with a steady-state delivery rate over 6 months, and offer an excellent alternative to multiple injections [31,32]. But the IVM SR bolus was regarded as potentially more ecotoxic than the more rapidly excreted oral formulations for horses and sheep [33]. Furthermore, IVM SR bolus was prepared from polyvinyl chloride that cannot be degraded. So modification of the dosage form is expected to increase the therapeutic effect of IVM.

2. Materials and methods

2.1. Microsphere preparation

IVM-loaded microspheres were prepared using a phase separation procedure. Typically, 600 mg zein (Wako Pure Chemical Industries LTD., Japan) and 60 mg IVM (Tongren Drug Company, Shanghai, China) were dissolved in 12 ml of 66.7% ethanol. Then, 8 ml of ultrapure Milli-Q water was immediately added with vigorous mixing using an agitator (IKAMAG RCT basic, IKA, Germany) set at 9 at room temperature. The resulted microspheres were lyophilized overnight before use.

2.2. Tabletted microsphere preparation

Tabletted microspheres, in diameters of 10 mm, were prepared by compressing 220 mg of microspheres containing IVM with a mold, and then the tabletted microspheres were placed into wet box at 37°C for 3 days to acquire certain toughness.

2.3. Morphology analysis

The morphologies of the microspheres and tabletted microspheres were observed using a scanning electron microscope (SEM, S-450, Hitachi, Japan). The zein microspheres and tabletted microspheres were vacuum-dried at room temperature, mounted onto brass stubs and sputter-coated with gold in an argon atmosphere prior to examination under SEM.