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ORIGINAL ARTICLE

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Taxol encapsulation in poly(ε-caprolactone) microspheres

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Abstract Poly(ε-caprolactone) (PCL) microspheres containing taxol were prepared by the solvent evaporation method and tested for angiogenesis inhibition using the chick chorioallantoic membrane (CAM) model. Very high encapsulation efficiencies (95%) for taxol in PCL microspheres were obtained. In vitro release studies showed about 25% of the loaded drug was released in 6 weeks from microspheres containing 5% taxol. Studies with the CAM showed that taxol released from the microspheres induced vascular regression and inhibited angiogenesis.

Key words Taxol · Microspheres · Antiangiogenesis

Introduction

Taxol is a diterpenoid isolated from the western yew, *Taxus brevifolia*, and has been shown to exhibit a broad range of antitumor activity [13] . Taxol binds directly and with high affinity to polymerized tubulin, preventing depolymerization and thus stabilizing microtubules [7]. Taxol is a potent inhibitor of cell replication in the late G_2 or M phases of the cell cycle, it alters cellular attachment/detachment and interferes with cell motility, migration, intracellular transit and type IV collagenase secretion [6, 17, 18]. Recent work using the chick chorioallantoic membrane (CAM) model has shown that taxol also possesses potent antiangiogenic activity.

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Arterial chemoembolization using drug-loaded microspheres has been used to target antineoplastic agents to tumors of the urinary tract, oral cavity and liver [3, 8, 9]. This technique offers the advantages of elevated local concentration of drug and blockade of the blood supply (and nutrients) to the tumor. However, secretion of angiogenic factors by the tumor induces the development of a collateral circulation and the blood supply to the tumor is re-established. To attempt to counteract this effect, Kamei et al. [5] used embolic microspheres containing an angiogenesis inhibitor, TNP-470 (a synthetic derivative of fumagillin), and reported a dramatic but short-lived regression of VX-2 carcinomas in rabbits. The TNP-470 was released rapidly from the poly(DL-lactic-co-glycolic acid) biodegradable polymeric microspheres with less that 10% of the drug remaining after 60 h.

In the present study the in vitro release rate profile of taxol from biodegradable microspheres of poly(ε-caprolactone) (PCL) was evaluated and the antiangiogenic activity of taxol released from microspheres placed on the CAM was demonstrated.

Material and methods

Chemicals

PCL (molecular mass 35,000–45,000 Da) was purchased from Polysciences (Warrington, Pa.); dichloromethane (DCM) from Fisher Scientific Co., Canada; polyvinyl alcohol (PVA; molecular mass 124,000–186,000 Da, 99% hydrolysed) from Aldrich Chemical Co. (Milwaukee, Wis.). Taxol was obtained from Sigma Chemical Co. (St. Louis, Mo.). Unless otherwise stated all chemicals and reagents were used as supplied. Distilled water was used throughout.

Preparation of microspheres

Microspheres were prepared using the solvent evaporation method [1]. Briefly, 5%w/w taxol-loaded microspheres were prepared by dissolving 10 mg taxol and 190 mg PCL in 2 ml DCM, adding the

resulting solution to 100 ml 1% PVA aqueous solution and stirring at 1000 rpm at 25 °C for 2 h. The suspension of microspheres was centrifuged at 1000 *g* for 10 min (Beckman GPR), the supernatant removed and the microspheres washed three times with water. The washed microspheres were air-dried overnight and stored at room temperature. Control microspheres (taxol-free) were prepared as described above. Microspheres containing 1% and 2% taxol were also prepared. Microspheres were sized using an optical microscope with a stage micrometer.

Encapsulation efficiency

A known weight of drug-loaded microspheres (about 5 mg) was dissolved in 8 ml acetonitrile and 2 ml distilled water was added to precipitate the polymer. The mixture was centrifuged at 1000 *g* for 10 min and the amount of taxol encapsulated was calculated from the absorbance of the supernatant measured in a UV spectrophotometer (Hewlett-Packard 8452A Diode Array Spectrophotometer) at 232 nm.

Drug release studies

About 10 mg taxol-loaded microspheres were suspended in 20 ml of 10 m*M* phosphate buffered saline (PBS), pH 7.4, in screw-capped tubes. The tubes were tumbled end-over-end at 37 °C, and at given time intervals 19.5 ml of supernatant was removed (after allowing the microspheres to settle at the bottom), filtered through a 0.45-μm membrane filter and retained for taxol analysis. An equal volume of PBS was replaced in each tube to maintain sink conditions throughout the study. The filtrates were extracted with 3 × 1 ml DCM, the DCM extracts evaporated to dryness under a stream of nitrogen, redissolved in 1 ml acetonitrile and analyzed by HPLC using a mobile phase of water/methanol/acetonitrile (37:5:58) at a flow rate of 1 ml min^{-1} (Beckman Isocratic Pump), a C8 reverse phase column (Beckman), and UV detection (Shimadzu SPD A) at 232 nm.

CAM studies

Fertilized, domestic chick embryos were incubated for 4 days prior to shell-less culturing. The egg contents were incubated at 90% relative humidity in an atmosphere containing 3% CO_2 for 2 days. On day 6 of incubation, 1-mg aliquots of 5% taxol-loaded or control (taxol-free) microspheres were placed directly on the CAM surface. After a 2-day exposure the vasculature was examined using a stereomicroscope interfaced with a video camera. The video signals were displayed on a computer and video printed.

Scanning electron microscopy

Microspheres were placed on sample holders, sputter-coated with gold and then placed in a Philips 501B Scanning Electron Microscope operating at 15 kV.

Results

The size range for the microsphere samples was between 30 and 100 μm, although there was evidence in all taxol-loaded or control microsphere batches of some microspheres falling outside this range. The efficiency of loading the microspheres with taxol was always greater than 95% for all drug loadings studied. A representative scanning electron micrograph of 5% taxol-loaded PCL microspheres is shown in Fig. 1. The microspheres were all spherical and many showed a rough or pitted surface morphology. There appeared to be no evidence of solid drug on the surface of the microspheres.

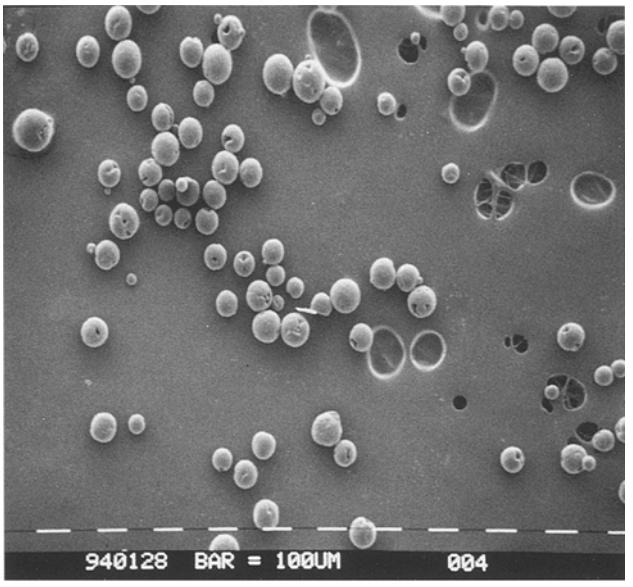

Fig 1 A representative scanning electron micrograph of taxol-loaded PCL microspheres (× 40)

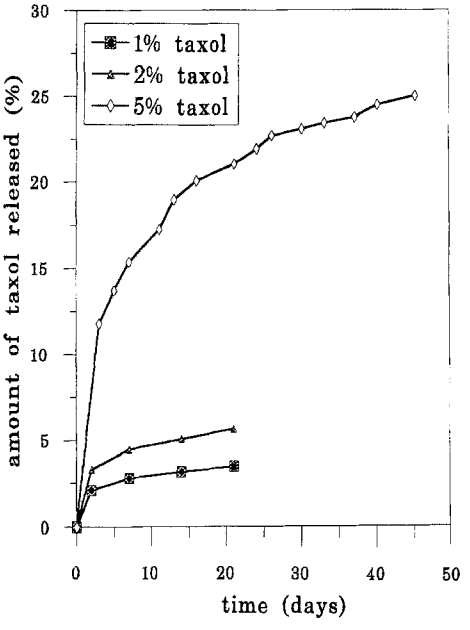

Fig 2 In vitro release rate profiles of taxol from microspheres containing 1%, 2% or 5% taxol in PCL into PBS (pH 7.4) at 37 °C

The time courses of taxol release from 1%, 2% and 5% taxol-loaded PCL microspheres are shown in Fig. 2. The release profiles indicated that over the first

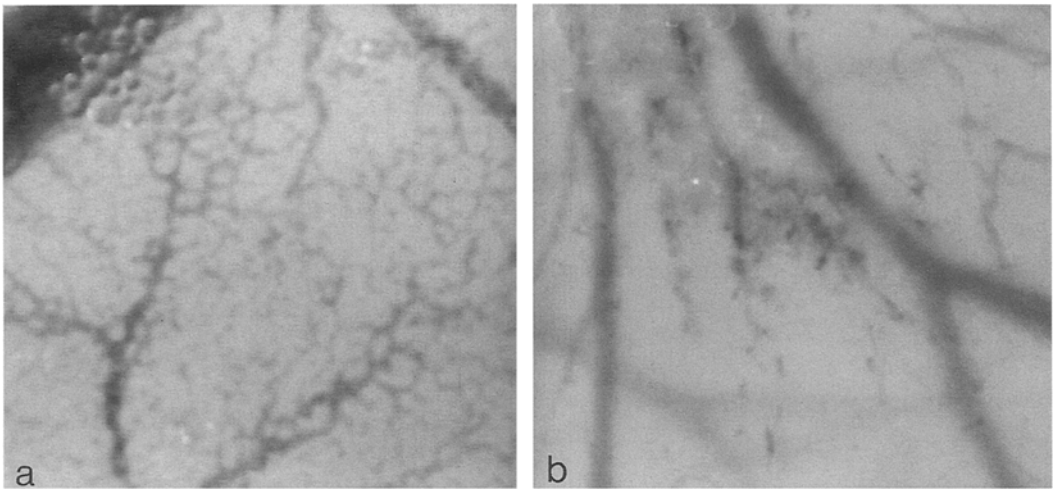

Fig 3a, b Representative stereomicrographs of CAMs treated with (**a**) control and (**b**) 5% taxol-loaded microspheres (seen at the top left corner of the micrographs) showing inhibition of angiogenesis by the taxol-loaded microspheres

10 days, there was a greater amount of taxol released from microspheres at all loading levels than the amount of taxol released over days 10–21. For microspheres containing 1% or 2% taxol, the percent of drug released was approximately 3% and 6%, respectively, after 21 days. At 5% taxol loading, the microspheres had released about 20% of the total drug content after 21 days.

Figure 3 shows CAMs treated with control PCL microspheres (Fig. 3a) and 5% taxol loaded microspheres (Fig. 3b). The microspheres on the surface of the CAMs can be seen in a region at the top left of the micrographs. The CAM with the control microspheres showed a normal capillary network architecture. The CAMs treated with taxol microspheres consistently showed marked vascular regression and zones which were devoid of a capillary network (Fig. 3b).

Discussion

The solvent evaporation method of producing taxol-loaded microspheres resulted in very high taxol encapsulation efficiencies of between 95 and 100%. This was due to the poor water solubility of taxol and its hydrophobic nature favouring partitioning in the organic solvent phase containing the polymer.

PCL is an aliphatic polyester which can be degraded by hydrolysis under physiological conditions, and it is nontoxic and tissue-compatible [2]. The degradation of PCL is significantly slower than that of the extensively investigated polymers and copolymers of lactic and glycolic acids and is therefore suitable for the design of long-term drug delivery systems [2].

Release of drugs from polymeric microspheres in which the drug particles are dispersed in the polymer matrix with no contact between each other occurs by the permeation of solvent through the polymer matrix to the drug particle, dissolution of the drug particle and diffusion of the drug through the polymer to the release medium [16]. At higher drug loadings, where a greater number of drug particles are present in the microspheres, some of these particles are in contact with each other and form a network throughout the polymer matrix. This promotes solvent permeation into the polymer and dissolution and diffusion of the drug through the fluid-filled pores [15, 16]. Release of taxol from PCL microspheres due to degradation or erosion of PCL was thought to be unlikely because studies have shown that under in vitro conditions in water, there was no significant weight loss or surface erosion of PCL over a 7.5-week period [12].

At all taxol loadings, an initial period of faster release rate of taxol from the microspheres was observed. This was thought to be due to dissolution/diffusion of taxol from the superficial regions of the microspheres (close to the microsphere surface), an effect which has been documented for the release of many drugs from polymeric microspheres [4, 15, 16]. Figure 2 also shows that the 5% taxol-loaded PCL microspheres gave disproportionately higher release rates at all time-points compared with the 1% and 2% taxol-loaded microspheres. It is possible that in the 1% and 2% taxol-loaded microspheres, the majority of the taxol particles were discrete and isolated within the polymer matrix and that drug release was limited by the diffusional barrier of the polymer matrix [16]. At 5% loading, many taxol particles may have been in physical contact forming a particle network which connects the inner part of the network to particles located near the surface of the microspheres. This would have allowed penetration of solvent and dissolution and diffusion of taxol from one particle site to another without any limitation

of a polymer barrier, resulting in a greatly enhanced release rate. Similar effects of differing drug loading on release rate profiles of drugs from microspheres have been reported [4, 15, 16].

Another factor which may also contribute to the differences in release rate profiles for the 1%, 2% and 5%-taxol-loaded microspheres is the lower crystallinity of the PCL matrix at higher taxol loadings. Preliminary differential scanning calorimetry studies of the thermal behaviour of taxol-PCL mixtures solidified from DCM solutions have indicated that an increase in taxol loading from 1 to 5% results in a lower crystallinity of the polymer (data not shown). A decrease in PCL crystallinity leads to increased permeability of the polymer to water and solutes [11].

Taxol microspheres at 5% loading were shown to release sufficient drug to produce an avascular zone and extensive inhibition of angiogenesis when placed on the CAM (Fig. 3b). Angiogenesis inhibition is defined as a zone of avascularity 2–6 mm in diameter which is a direct result of vessel occlusion, disruption, degeneration and regression.

We have developed a slow-release polymeric microsphere delivery system for taxol which should have clinical importance as a chemoembolic drug formulation given the antitumor and antiangiogenic properties of taxol.

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