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Albumin Microspheres as Vehicles for the Sustained and Controlled Release of Doxorubicin

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Abstract—Biodegradable albumin microspheres have been prepared with the intention of targeting doxorubicin preferentially to tumour tissue. A high-yielding microsphere manufacturing process has been developed that involved the denaturation of an aqueous protein emulsion by chemical and/or thermal crosslinking methods. Microspheres can be closely sized to a diameter of $25{\cdot}3 \pm 2{\cdot}6$ μm with the aid of microsieves. The in-vitro release of doxorubicin from albumin microspheres was measured using a continuous flow system. Doxorubicin release can be sustained for up to 10 days and the rate of release could be controlled by manipulating protein denaturation conditions between the temperatures 110–135°C in the presence of 0–2% gluturaldehyde. Release of doxorubicin was significantly faster in human plasma compared with isotonic saline.

Albumin microspheres have been used as a carrier for a variety of drugs, as highlighted by Tomlinson (1983) and Gardner (1985). Widder & Senyei (1983) entrapped adriamycin in 1 μm magnetized albumin microspheres that were subsequently tumour-targeted with the application of an extracorporeal magnetic field. Fujimoto et al (1985) have used mitomycin C entrapped in albumin microspheres targeted to hepatic tumours in a clinical trial. Improved patient survival for microsphere treatment compared with conventional chemotherapy was observed.

In the development of a particulate drug carrier, a number of pharmaceutical design criteria need to be met: (i) the carrier should be biodegradable; (ii) carrier degradation products and their metabolites must be clinically non-toxic; (iii) the ability to entrap water-soluble drugs throughout the carrier matrix is required; (iv) it is essential that release of entrapped drug from the carrier be sustained over long periods; (v) this release should be easily controlled and a wide range of drug release profiles be attainable; (vi) ease of carrier preparation is advantageous and a narrow size range of the final product necessary; (vii) carrier sterility is essential for parenteral therapy; (viii) a monodisperse aqueous suspension of the carrier should be readily obtained; (ix) upon introduction into the arterial blood supply of a tumour, carrier particles need to be extensively distributed within the tumour microvasculature.

Materials and Methods

Doxorubicin-containing albumin microspheres were manufactured using a modified phase emulsion polymerization technique first described by Scheffel et al (1972). Crosslinkage of the albumin matrix was attained with the use of chemical and/or thermal denaturation procedures.

Thermally stabilized microspheres

Pure doxorubicin HCl (35 mg) and bovine serum albumin (300 mg) were dissolved in 1 mL of deionized water and added dropwise to 150 mL of purified olive oil contained in a well stirred and baffled 250 mL reaction vessel. The resulting emulsion was stirred for 10 min, the speed of the propeller (43 mm diameter) being kept constant with the aid of an optical tachometer. The emulsion was then gradually heated to a maximum of between 110–135°C, left for 5 min and cooled to room temperature (22°C) with stirring to yield an oil suspension of microspheres.

This was centrifuged at 3000 rev min^{-1} for 5 min, the supernatant discarded and the microsphere pellet collected. Microspheres were resuspended in anhydrous ether and centrifuged at 3000 rev min^{-1} for 3 min. This washing procedure was repeated three times after which microspheres were dried in a vacuum desiccator, affording a deep red freely flowing powder which was stored at 4°C.

Chemically stabilized microspheres

The manufacture of chemically stabilized microspheres was similar to the above procedure with the exception that the original aqueous drug/protein solution also contained glutaraldehyde at varying concentrations, the emulsification of which was carried out at 4°C to slow the chemical denaturation of the microsphere matrix.

Microsphere sizing

To achieve a narrow microsphere size range a sieving technique was employed. 100 mg of microspheres was suspended in ether (100 mL) and sized between microsieves (Endecotts Ltd) with mesh apertures of 32 and 20 μm. Individual fractions were collected and dried.

Size ranges of collected fractions were quantified by light microscopy. Diameters of a random sample of 200 microspheres were measured against a graduated eyepiece graticule.

Microsphere drug content

The doxorubicin content of albumin microspheres was determined using an acid-ethanol extraction method. This method hydrolyses the drug to the anthracycline, the

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concentration of which is determined fluorimetrically by comparison with a standard curve prepared from the drug using the same extraction conditions.

The microspheres (10 mg) were suspended in 100 mL of 70% ethanol–0·45 M HCl with the aid of an ultrasonic bath (Branson model B-12). The suspension was stirred at 4°C for 24 h and then a sample of the supernatant was analysed spectrophotofluorometrically (Perkin-Elmer, model 203:Exc.- 470 nm, Emiss.-542 nm) for fluorescence of adriamycin.

As doxorubicin is a heat-sensitive compound, the supernatant from an aqueous suspension of drug containing microspheres (5 mg mL^{-1}) was collected and analysed for drug decomposition by spotting onto precoated silica gel plates and chromatography in a solvent system (chloroform–methanol–acetic acid–water, 80:20:14:6 v/v). The spot corresponding to doxorubicin (determined from a reference doxorubicin solution) was scraped from the plate and extracted from the silica using 70% ethanol-0·45 M HCl. The rest of the fluorescent material on the plate was pooled and extracted by the same procedure. Acid-ethanol extraction concentrations were then determined spectrophotofluorometrically as previously described and the degree of decomposition determined by comparison of the fluorescence due to the drug with the total fluorescence.

In-vitro drug release

A continuous flow drug release system was developed to measure the in-vitro release of drug from microspheres. Sized albumin microspheres (25 mg) were immobilized between a series of membrane filters and washed with medium at 37°C for 10 days. The medium used was either a 0·9% NaCl solution or freshly collected human plasma. Effluent from sphere washings was collected and assayed for total drug fluorescence using an acid-ethanol extraction technique described by Benjamin et al (1973). Statistical comparisons of the drug release rates in plasma and saline were carried out using an analysis of covariance of regression lines.

Results and Discussion

Albumin microspheres satisfy the pharmaceutical requirements for a particulate drug carrier and the process developed for their manufacture gave yields of up to 69%. The yield obtained within a particular size range was largely determined by the emulsification step. A baffled reaction vessel has been demonstrated as an efficient system for the production of microspheres via a phase emulsion polymerization technique (Sherrington 1984). Baffling maximizes the vertical motion of the reaction fluids and helps to prevent vortexing by breaking up horizontal streaming of emulsion particles. The use of a vertical flow propeller aided this desirable flow pattern.

The mean size of a batch of microspheres was particularly dependent on the speed and duration of emulsion stirring. A linear relation was demonstrated between mean sphere diameter and the log of propeller stirring speed (Fig. 1). This relation was used to optimize the yield of microspheres of a particular size range. Yields of closely sized, large microspheres were generally less than those of small microspheres,

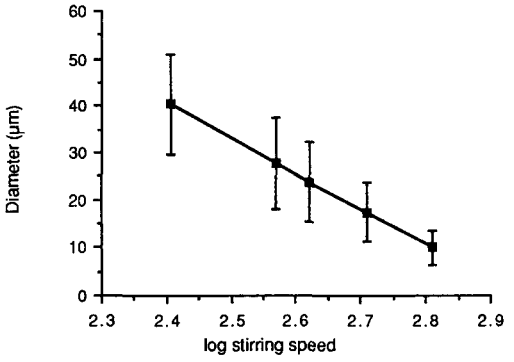

FIG. 1. Effect of manufacture cell stirring speed on mean microspheres diameter. (Determined for microspheres prepared from a 30% albumin solution, emulsified at constant speed for 10 min).

due to the wider size ranges produced using the manufacturing technique (Fig. 1).

It is necessary to have a narrow size range of doxorubicin containing microspheres for in-vivo use. When they are introduced into the arterial blood supply of an organ, large trapped microspheres hinder the progress of smaller spheres to the small branch vasculature, as a result small branch arteries may receive disproportionately fewer spheres (Heymann et al 1977) with a consequent loss in drug concentration. Meade et al (1986) demonstrated that microspheres, 50 μm in diameter, do not embolize in tumour tissue to the same extent as their smaller counterparts. Also smaller microspheres release their drug proportionately faster than large spheres. Therefore, uneven drug release rates are likely for microspheres of widely varying sizes.

To manufacture closely-sized microspheres, a microsieving technique was employed. The results of a typical batch of microspheres, diameters of which were measured by light microscopy, demonstrated that all were between 20 and 33 μm in diameter with a mean sphere size of $25{\cdot}3 \pm 2{\cdot}6$ μm (s.d.).

Denaturation of the aqueous protein emulsion was achieved using two methods. As doxorubicin is moderately heat sensitive, thermal denaturation can only be carried out to temperatures of up to 140°C without significant drug decomposition. Using the thermal crosslinking technique and a temperature of 135°C for 10 min, 6% of the drug present in the microspheres had decomposed whereas microspheres prepared at 150°C for 5 min showed 34% of the total sphere drug content as thermal decomposition products. To achieve a higher degree of protein crosslinkage and hence slower drug release (Gupta et al 1986a, b), without decomposition, a combination of microsphere preparative methods was used. This involved an initial chemical crosslinking of the protein emulsion before thermal denaturation.

The degree of protein crosslinking also determines the biodegradability of the albumin sphere matrix. In general, the greater the extent of protein crosslinkage, the slower the microsphere matrix will degrade. Evans (1972) found that albumin microspheres manufactured by heating at 105°C for 40 min began to dissolve 15 min after intravenous injection. But microspheres hardened at 180°C for 18 h do not begin to biodegrade until six months after injection.

Maximum microsphere drug content attainable using the present manufacture process was approximately 13% by weight. After this value was exceeded the structural integrity of microspheres was affected. Microsphere drug contents also affect release rates. The more doxorubicin in the microspheres the quicker it was released.

Doxorubicin-carrying microspheres have an advantage over some other drug containing vehicles, such as liposomes, in that they can be easily used clinically. Microspheres take the form of a freely flowing powder that can be suspended, without sphere aggregation, in normal saline containing non-toxic concentrations of a surfactant (0·01% Tween 80). A major advantage of microspheres is their long bench life. Those that have been stored in a desiccator at room temperature for six months have shown no detectable drug decomposition.

The system developed to measure the in-vitro release of drug from albumin microspheres consisted of microspheres immobilized in the pores of a membrane filter being washed by a continuous flow of fresh medium. This apparatus was devised to mimic the in-vivo situation where microspheres are embolized in the small vasculature of an organ. However, in-vivo release is more complex and dependent on a number of factors including blood flow and drug concentration gradients around an embolized microsphere. The measurement of in-vitro drug release can only be used as a comparison of drug release profiles of different sphere types.

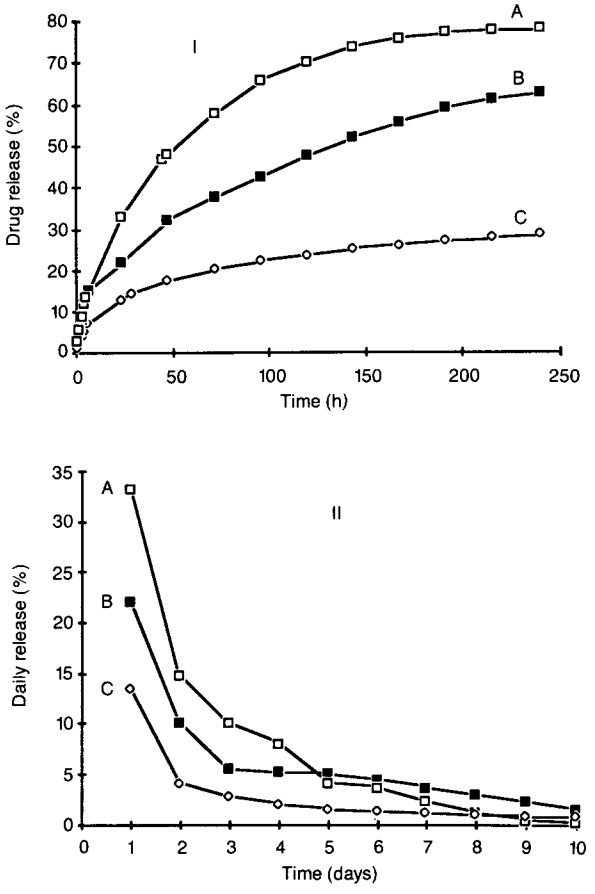

FIG. 2. Release of doxorubicin from microspheres stabilized at (A) 120°C for 10 min, (B) 120°C with 1% glutaraldehyde for 10 min, (C) 135°C with 2% glutaraldehyde for 10 min. I Represents time course of cumulative doxorubicin release, II represents doxorubicin release in terms of the percentage of the total original drug content released per day.

The results in Fig. 2 were obtained from three sets of microspheres manufactured with varying degrees of protein crosslinkage. All three sets had a doxorubicin content of 5% by weight and were washed with 0·9% NaCl solution. The same data are illustrated in release profiles I and II, profile I being the cumulative release of drug against time, profile II showing the percentage of the total sphere drug content release daily.

Release of the drug from albumin microspheres can be sustained for up to 10 days and it is evident from the present results that release rates can be controlled by the manipulation of manufacturing conditions. For microspheres that have undergone only thermal denaturation, release is relatively fast. However, if the same thermal hardening conditions are used in combination with chemical crosslinking (Fig. 2 curve B) release can be significantly slowed. A combination of strong thermal and chemical crosslinking conditions produced a very slow drug release (Fig. 2 curve C). It follows that microspheres with a multitude of other drug release profiles could be produced by altering the degree and extent of protein denaturation.

Release profile II illustrates the biphasic nature of microsphere drug release, there was an initial burst of loosely bound drug followed by a more sustained release over the ensuing days. Zunino et al (1981) have suggested that doxorubicin forms a covalent bond with albumin protein. Its sustained release could be due to gradual hydrolytic cleavage of these bonds, with the initial drug burst being due to doxorubicin that has weak physical attraction to the sphere matrix. Curve C provides evidence for the covalent attraction of doxorubicin to albumin proteins. After a continuous flow of saline for 240 h, the drug release had almost ceased, although 67% of the total original drug content was still associated with the microspheres.

If large numbers of microspheres are to be embolized in an organ, an initial burst of up to 35% of their drug content may be undesirable. If that is the case, the loosely bound drug can be largely removed by suspending the microspheres in

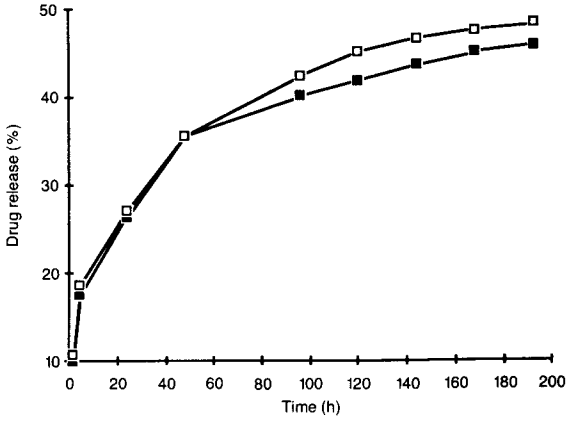

FIG. 3. Cumulative release of doxorubicin from microspheres, stabilized at 110°C in the presence of 1% glutaraldehyde, using fresh human plasma (□) and isotonic saline (■) as release media with a consistent flow rate of 20 mL min^{-1}.

normal saline and placing them in an ultrasonic bath for 10 min.

The release of the drug was tested with freshly collected human plasma and isotonic saline as the washing media (Fig. 3), to examine the effect blood borne enzymes have on the drug release. The release of the physically bound drug was not affected, however, the sustained release of the drug was significantly faster ($P < 0.05$) after plasma washing.

Blood contains several proteolytic enzymes. Morimoto et al (1980) demonstrated that albumin microsphere degradation is faster in the presence of a protease. Therefore doxorubicin-carrying albumin microspheres may have a faster drug release in-vivo compared with the in-vitro situation.

These results demonstrate that the release of doxorubicin from albumin microspheres can be sustained for up to 10 days. In addition microsphere drug release was shown to be easily controlled through the microsphere manufacture process. The pharmaceutical properties of albumin microspheres provide a promising alternative to current chemotherapeutic methods.

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