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Dexamethasone-loaded magnetic albumin microspheres: preparation and in vitro release

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Abstract

Magnetic albumin microspheres containing nearly 13% w/w dexamethasone were prepared. Two separate studies were carried out to investigate: (a) the responsivity of these microspheres in a 8000 G magnetic field in a flow rate (0.5 cm/s) equal to that of the blood flow rate in capillaries; (b) the in vitro release profile of dexamethasone from magnetic albumin microspheres up to 7 h after dispersion in normal saline medium, using a USP dissolution apparatus. The results obtained suggest that the retention of microspheres in the presence of the magnetic field for 15 min was significantly ($P < 0.05$) more than those in the absence of the magnetic field. Drug release in the first hour was found to increase and then reached a maximum. After 7 h, approximately 30% of the total drug content of microspheres was released. A third order equation for the drug release was also calculated. From this study, it is suggested that magnetic albumin microspheres could be retained at their target site in vivo, following the application of a magnetic field, and are capable of releasing their drug content for an extended period of time. This would make them a suitable depot for delivering chemotherapeutic agent(s) in vivo.

Keywords: Magnetic albumin microspheres; Dexamethasone; In vitro release; Magnetite; Drug delivery; Targeting

1. Introduction

In recent years, considerable interest has been shown in the use of albumin microspheres as platforms for active drug targeting as well as producing a sustained and controlled rate of drug release (Morimoto et al., 1981; Widder and Seneyei, 1983; Gallo and Gupta, 1989).

In the previous studies, a phase separation emulsion technique was developed for the preparation of the microspheres. Stabilization of the albumin microspheres matrix was accomplished by either heat denaturation at various temperatures (110–190°C) or crosslinking with carbonyl compounds in an ether phase reaction. The degree of stabilization controls the rate of drug diffusion out of the carrier as well as the extent of carrier degradation (Widder et al., 1980).

Magnetic albumin microspheres are capable of being retained in the capillaries by using extracorporeal magnets. Electron microscopic studies of

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rat tail skin perfused with microspheres in a 8000 G magnetic field of 30 min duration showed that microspheres were internalized by endothelial cells and trapped between the plasma membranes of two adjacent endothelial cells and hence were not cleared by the reticuloendothelial system (Widder et al., 1978). The studies confirm second order drug targeting (targeting to specific organs or tissues (Thies, 1989)) in the target tissue of healthy animals (Gupta et al., 1989).

Targeting by magnetically responsive albumin microspheres has a high efficiency. For example, doxorubicin hydrochloride, entrapped in magnetic albumin microspheres, has been shown to cause enhanced drug concentration in the target tissue compared with the administration of free drug (Gupta and Hung, 1989). In addition, the amount of drug reaching the heart and liver was reduced.

Dexamethasone sodium phosphate is a synthetic glucocorticoid, which could be used in the treatment of lymphocytic tumors and lymphomas. It also has anti-inflammatory effects as well as preventing the cell mediated immune reactions (Haynes, 1991).

In this study, magnetic albumin microspheres containing approximately 13% w/w dexamethasone (from a total microsphere weight of 240 mg, composed of 140 mg human serum albumin, 40 mg magnetite and 40 mg dexamethasone sodium phosphate) were prepared and their in vitro accumulation in the presence and absence of a magnetic field was evaluated. In addition, the in vitro drug release profile up to 7 h was also determined by using the USP dissolution apparatus.

2. Materials and methods

2.1. Materials

Hydrated ferrous sulphate ($FeSO_4.7H_2O$) and hydrated ferric chloride ($FeCl_3.6H_2O$) were obtained from Merck Chemicals Co., Tehran, Iran. Dexamethasone sodium phosphate (DSP) was purchased from Sicor Chemicals, Italy. Human serum albumin (98% purity) was obtained as a gift from the Iranian Blood Transfusion Service (I.B.T.S.), Tehran, Iran, and cottonseed oil was supplied by Pars Rokan Nabati, Tehran, Iran.

2.2. Preparation of the magnetic fluid

A modified method, similar to that described by Shimoiizaka et al., 1976 was used in the preparation of the magnetic fluid. 6N NaOH was added to 100 mL of a solution containing Iron (Fe) in the ratio of 1:2 (Fe^{2+}: Fe^{3+}) and mixed with a 1 M $FeSO_4$ and $FeCl_3$ solution. The resulting mixture (pH = 11) was allowed to digest for 30 min at 60°C to give the colloidal magnetite suspension; 6 g of oleic acid was then mixed with 9.6 mL of a 3 N NaOH solution and 50 mL of distilled water, the mixture was then heated to 60°C. Following the dissolution of sodium oleate, the solution was added to the magnetite suspension and stirred for 30 min at 90°C. After cooling, the pH was lowered to 5.5 with a 1 N HCl solution, resulting in the formation of a flocculated suspension. The flocculated suspension was filtered through a Whatman (No.4) filter paper. The filter cake was broken up in distilled water and refiltered. The resulting cake was then mixed with a 5% w/v sodium dodecyl sulphate solution and stored until use.

2.3. Experimental procedures

2.3.1. Preparation of DSP-Loaded magnetic albumin microspheres

Magnetic albumin microspheres were prepared by a modified method, similar to that described by Widder et al., 1979.

Human serum albumin (150 mg), DSP (40 mg) and magnetic fluid (40 mg) were added to 0.5 mL of distilled water. The resulting dispersion was then added dropwise (20 drops/min) into 100 mL of a continuously stirring cottonseed oil and stirred at a constant rate of 2200 rpm for 30 min using a Heidolph (Type: RZR2-Heidolph-Germany) electric stirrer. The emulsion was then homogenized using an Erweka homogenizer (Erweka-AR400-Erweka-Germany) for 5 min at 25°C. This procedure generated small, non cross-linked microspheres, containing both Fe_3O_4 and the entrapped drug (DSP). The resulting micro-

spheres were then denatured by heating the homogenate at 120°C for exactly 10 min. The suspension was then cooled to 25°C, washed 4 times with anhydrous diethyl ether, stored for 48 h at − 10°C and finally dried in a dessiccator at 4°C. The prepared microspheres were then stored until use. The percentage of dexamethasone (1.3 mg DSP equivalent to 1 mg dexamethasone) in the prepared microspheres would therefore be 13% (30.8/240).

2.3.2. Particle size analysis

The size of the microspheres prepared were determined by using a particle size analyser (Hiac/Royco, 4100-Pacific Scientific). Samples (10 mL) containing microspheres dispersed in 0.1% w/v Tween 80 in normal saline was examined and the total number of particles within the samples was determined. This procedure was repeated three times.

2.3.3. Magnetic responsivity of the DSP-loaded magnetic microspheres

The apparatus shown in Fig. 1 was designed for this study.

The apparatus consists of a Millipore pump (model XX5522050-USA) which pumped air into the erlenmeyer flask containing normal saline. This resulted in the flow of normal saline through the glass tube (30 cm length and 3.5 mm internal diameter) which was exposed to an electromagnet

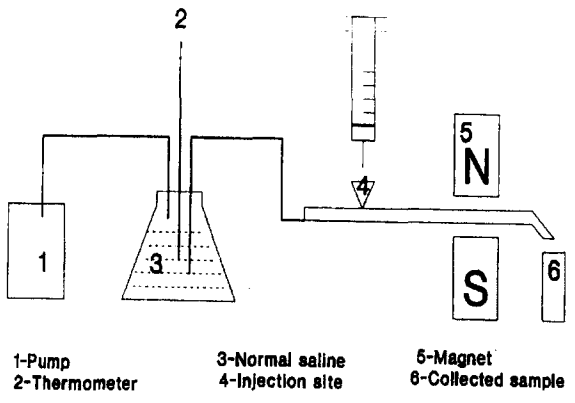

Fig. 1. The apparatus used for determining the microspheres response to the magnetic field.

(Varian 7400-Varian-USA) with a pole diameters of 15 cm and a pole distance of 5 cm. The homogenesity of the magnet was one part in 10^5. By regulating the pump pressure, the flow rate within the glass tube was controlled at 3 mL/min (0.5 cm/s). The experiment was carried out at room temperature (21°C).

Prior to injection, microspheres (25 mg/mL) were dispersed in normal saline containing 0.1% w/v Tween 80 and a stock solution was prepared. A flow of 0.5 cm/s of normal saline, resembling the blood flow rate passing through the capillaries (Widder et al., 1978), was established. A 1 mL aliquot of the microspheres suspension in the test vehicle was then injected into the injection site. The 8000 G magnetic field was established for 15 min and one sample was collected every minute. The magnetic field was then removed and samples collected for a further 5 min. Microsphere content of the collected samples was then evaluated by Turbidimetery, using a Shimadzu 160A UV-Vis. Spectrophotometer, (Shimadzu Ltd.,Japan) at 550 nm wavelength.

As a control, 1 mL of the microspheres suspension from the same stock sample was injected into the injection site and samples were collected and their microsphere content evaluated in the absence of the magnetic field for a period of 20 min.

2.3.4. In vitro drug release from magnetic albumin microspheres

Two series of microspheres were prepared: drug-loaded microspheres (I) and drug-free microspheres (II) which were prepared in the same manner to that of the drug containing microspheres except for omitting the drug.In order to keep the weight of the carrier constant, 170 mg (I) containing 30 mg DSP (23 mg dexamethasone) and 140 mg (II) were dispersed in 400 mL of normal saline in two beakers of the USP dissolution apparatus 2 (Erweka-DT6R-Erweka-Germany), according to the USP XXII specification. The temperature was kept at 37 ± 0.5°C throughout the experiment and the stirring rate of the paddles was set at 90 ± 5 rpm. The experiment was run for a total period of 7 h and 5 mL samples were removed from the test beakers at set intervals up to 7 h. After the removal of each

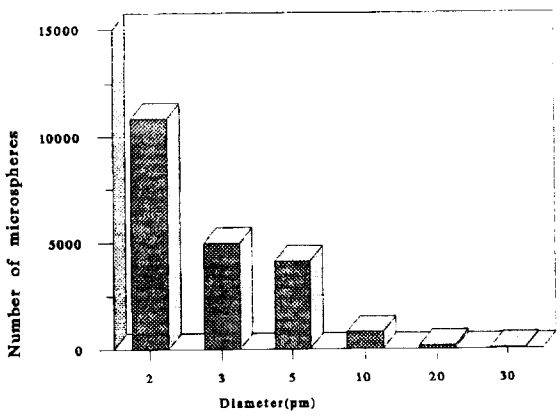

Fig. 2. Diameter distribution of the magnetic albumin microspheres.

sample, the resulting suspension was centrifuged (Hettich Universal-Germany) at 2000 × *g* for 15 min, 4 mL of the supernatant layer was then removed from the centrifuge tube and replaced with 4 mL of normal saline to keep the volume constant and placed back in the dissolution beakers. The drug concentration of the supernatant layer was then determined spectrophotometerically at 242 nm, using a Shimadzu 160A UV-Vis. spectrophotometer. The absorption of each sample was read against it's blank. In order to ensure that all the microspheres sedimented to the bottom of the centrifuge tubes, the resulting supernatant layer from centrifugation was evaluated spectrophotometerically. After 15 min centrifugation, the spectrum of the supernatant layer showed no absorption in the visible area of the spectrum, suggesting the absence of the microspheres in the supernatant layer.

3. Results and discussion

3.1. Measurment of the size distribution of the magnetic albumin microspheres

Fig. 2 shows the size distribution of the albumin microspheres prepared in this study; 95.3% and 75.6% of microspheres were respectively less than 5 and 3 μm in diameter. The standard errors (S.E.) of the data obtained are too small to be seen in the histogram.

The particle size analyser used for determining the size distribution of the microspheres was only able to measure diameters more than 2 μm. In previous studies, magnetic albumin microspheres which were prepared had an average diameter of 1 μm (Widder et al., 1979). However, the size range of the microspheres necessary for penetration into the capillaries is not clear. The average diameter of the microspheres obtained with the particle size analyser used in this study was about 3 μm. Further studies, using electron microscopy, are needed to determine the average size of the microspheres more accurately.

The mean size of a batch of the microspheres is particularly dependent on the speed and duration of the emulsion stirring (Jones et al., 1989). Homogenesity in size distribution is an important factor for successful targeting, and allows the carrier to penetrate the target site at the capillary level (Widder et al., 1978).

3.2. Magnetic responsiveness of the magnetic albumin microspheres

In Fig. 3, the percentage of the microspheres failed to remain in the glass tube in the absence and presence of the magnetic field (8000 G) has been compared. In the absence of a magnetic field, unretained microspheres were significantly ($P < 0.05$, paired *t*-test) more than those in the presence of the magnetic field. Retention of the

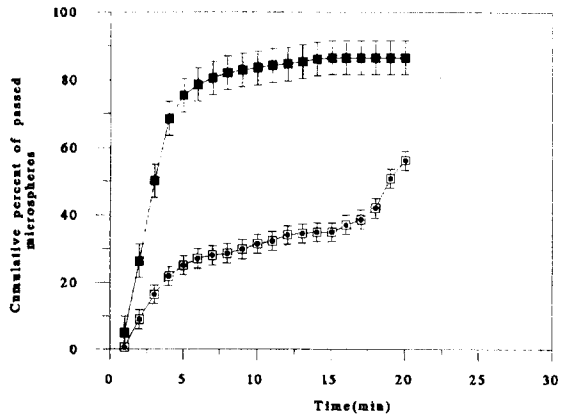

Fig. 3. Transport of microspheres through the tube in the presence and absence of the magnetic field (n = 3, S.E. bars). Symbols: ⊢□⊣ with magnetic field; ⊢■⊣ without magnetic field.

microspheres in a 8000 G magnetic field (the overall percentage of the exiting microspheres after removal of the magnetic field and those microspheres which remained in the glass tube) were 65% against 13.4% in the absence of the field.

Based on the results obtained, the majority of the microspheres with approximately 17% w/w magnetite in a 8000 G magnetic field and in a flow rate equal to 0.5 cm/s were retained and did not exit the glass tube. Therefore, it is predicted that the microspheres prepared by this method can accumulate in the capillaries following in vivo administration. Nevertheless, it is important to note that the experimental conditions encountered in this study, e.g. temperature was lower than the body temperature, were not exactly the same as those seen in vivo. Hence, extensive in vivo studies are needed to be carried out in order to determine the actual percentage of the microspheres which will be retained in the capillaries.

Magnetic targeting of the microspheres was developed to overcome the two major problems encountered in drug targeting, namely the reticuloendothelial clearance (reticuloendothelial system readily takes up a variety of microparticles including liposomes, microspheres, as well as other colloidal particles (Juliano, 1987)) and target site specificity. Microspheres are infused into an artery supplying a given in vivo target site. A magnet of sufficient field strength to retard the microspheres solely at the capillary level vasculature is placed externally over the target area. Restriction of the microsphere at the microvascular level can be achieved by taking advantage of the physiological differences between the linear flow velocity of blood in large arteries (approximately 30 cm/s) against that of the capillaries (0.5 cm/s). A greater field is necessary to retard the microspheres in faster moving arterial system as opposed to intra-capillary retention (Widder and Seneyei, 1983).

To date, the lowest limit of magnetic field strength for the effective retention of a magnetic delivery device has not been determined. This study has shown that a 8000 G magnetic field is sufficient for retaining the majority of the microspheres.

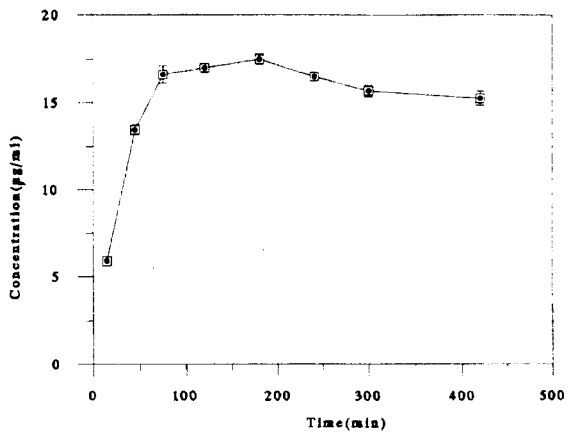

Fig. 4. Release of dexamethasone from magnetic albumin microspheres up to 7 h after dispersion (n = 3, S.E. bars).

Gupta and Hung, 1990 had shown that the use of a 1000 G magnetic field increased maximum concentration of drug at the target site and also reduced the delivery of drug to the non-target tissue. Based on the experiment conducted, it was suggested that the 1000 G magnetic field was only partially able to control the distribution of drug delivered via magnetic albumin microspheres. Therefore, in order to enhance the efficiency of drug targeting, a magnetic field of higher strength should be employed.

3.3. In vitro DSP release from magnetic albumin microspheres

Fig. 4 shows the release profile of dexamethasone from microspheres until 7 h after dispersion. In the first hour, the concentration of the drug released from the microspheres increased and reached a maximum. The total amount of dexamethasone present within the 170 mg of the microspheres used is equal to 23 mg. As seen in Fig. 4, the maximum concentration of dexamethasone released is 17.5 μg/mL. Since the overall volume of normal saline present within the dissolution apparatus beakers is 400 mL, the total amount of the dexamethasone released will be equal to 7 mg (400 mL $\times$ 17.5 μg/mL). Therefore, if the binding of dexamethasone to microspheres would be 100%, then the maximum quantity of drug released after 7 h will be 30% (7 mg/23 mg).

Evaluation of drug released from microspheres by this method was simple and reproducible. However, this method like other in vitro methods is not exactly precise. Centrifugation for 15 min can alter natural release of drug from the microspheres and could introduce error into the results obtained.

Based on the 'Least squares' method (Kreyzig, 1979), an equation for the release of dexamethasone from the albumin microspheres was also calculated (Eq. 1).

$$C = 4.69 + 0.198T - (8.84 \times 10^{-4})T^2 + (1.129 \times 10^{-6})T^3 \quad (T < 420) \qquad (1)$$

In this equation, C represents the concentration of the drug released (mg/mL) and T is time in minutes.

The release of water-soluble drugs from albumin microspheres is usually characterized by an initial rapid release (burst effect), followed by a slower release of the remaining drug (Natsume et al., 1991). DSP release from magnetic albumin microspheres also obeys this pattern.

Natsume et al., 1991 suggested that the mitomycin C release from albumin microspheres with various diameters can be evaluated by a set of first order release kinetics. Drug diffusion rate from the spheres to the sink solution is very low in the albumin microspheres and thus the drug in the core and peripheral regions of the spheres release at the same rate. The results obtained also suggest that the initially rapid drug release could mainly be due to the smaller microspheres.

It was suggested by Okada et al., 1991 that the release kinetics is first-order, and the derived first-order release rate constant is a function of the following parameters: radius of core particle, radius of the microspheres, solubility of the core substance in the dissolution media, density of the core particle, and the permeability constant of the core substance in the swollen matrix.

The permeability constant varies depending on the preparation conditions, and the reason for variation is shown clearly to be the difference in the degree of swelling of the microsphere (Okada et al., 1992).

The volume within the beakers of the dissolution apparatus is constant and it is therefore predicted that the concentration of the released drug following the initial burst effect should be increasing at a constant rate. In contrary, the results obtained showed no increase in the dexamethasone concentration within the beakers following the initial rapid release. This may be due to the slow release of the remaining drug in the microspheres, as well as numerous sampling through the experiment. In addition, British Pharmacopeia, 1993 states that dexamethasone is sensitive to light and could degrade gradually over an extended period of time if exposed to direct light. Throughout our experimental studies, the dexamethasone powder and samples were well-protected from light. It is, however, possible that the dexamethasone released into the dissolution beaker (unprotected from light) could gradually degrade with time, resulting in a small decrease observed in Fig. 4 after 3 h.

Another factor which affects the drug content within the microspheres is the partition coefficient of the drug. An increase in the partition coefficient causes a decrease in the drug content within the microspheres prepared, due to the drug migration towards the outer organic phase, during preparation. The drug partition coefficient affects the release in such a way that drugs with higher partition coefficients are released faster (and to a greater extent) than those with lower partition coefficients (Filipovic-Grcic and Jalsenjk, 1993). The partition coefficient of DSP is very low and the slow release of it from the microspheres could be due to the high solubility of DSP in water.

Because of the therapeutic uses of glucocorticoids (Haynes, 1991), magnetic albumin microspheres containing DSP can be used for targeted treatment of lymphocytic tumors, and could also due to its nature reduce the immunologic responses of the host to the carrier.

In conclusion, it is suggested that magnetically responsive albumin microspheres offer an alternative approach in achieving drug targeting. The results obtained from this study clearly suggest that magnetic albumin microspheres containing DSP are retained at the target site, in the presence of a 8000 G magnetic field, and are capable of

releasing their drug for an extended period of time. Hence, it is predicted that these microspheres could be retained on the target tissue in vivo and release their drug for prolonged periods of time. However, further studies need to be carried out to determine the effects of various factors such as the strength of the magnetic field, concentration and size of the magnetite in the microspheres, size and density of microspheres and experimental conditions.

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