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MAGNETIC MICROSPHERES AND MICROCAPSULES AS CARRIERS FOR INTRAVASCULAR ADMINISTRATION OF METRONIDAZOLE

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ABSTRACT

In this study gelatin microspheres of metronidazole, containing magnetite, with and without ethylcellulose coating have been prepared. The preparation is based on dispersion of a gelatin-metronidazole-magnetite aueous suspension in liquid paraffin, followed by drying with isopropanol treatment. Coating of gelatin microspheres with ethylcellulose was obtain by organic phase separation method. The magnetic responsiveness and the rheological properties of the microcapsule suspensions in physiological saline with 6% dextran, suggests that the microcapsules can be localized at a target site in vivo by meaus of an externally applied magnetic field. The release of metronidazole from microspheres declined at an apparent first-order rate, but from ethylcellulose conted microcapsules followed an apparent zero-order rate. Gelatin microcapsules offer promise for attaining target site specificity.

INTRODUCTION

Metronidazole is an efficient sensitizer of mammalian hypoxic celles to the effects of radiation, but a

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high serum level of the drug is necessary for the radiosensitizing efficiency, requiring the administration of relatively large amounts of 6 g/m^2 or more /1/. By limiting drug delivery to a specific region or structure in the body, therapeutic drug levels may be achieved at the local site. The selective delivery and retention of drugs at specific target sites within the body pose a substantial problem in drug design and formulation. A number of approaches have been suggested /2/. Among this proposals was the concept of using magnetically guided albumin microspheres as a directed drug delivery system /3/.

In this study gelatin microspheres of metronidazol, containing magnetite, with and without ethylcellulose coating have been prepared. The retention of these microspheres and microcapsules by an external magnetic field was measured in an in vitro model, and the in vitro release properties of the drug from the drug delivery systems were evaluated.

EXPERIMENTAL

Preparation of Microspheres

The procedure is a drying method in solution /5,6/. Sixty four g of distilled water were added to 19 g of gelatin (Gelatin weiss, Merck). After being allowed to stand at room temperature for 30 minutes, the gelatin had swollen and was dissolved on a water bath at 55-60°C, with stirring. The solution was placed in 1000 ml beaker and 6 g metronidazole (F.R.IX grade /4/) and 10 g magnetite were added. Then 185 ml of mineral oil (F.R.IX grade /4/) containing 0,5 g sorbitan trioleate (Schuchard, München) previously heated to 50-60°C was added and the mixture was agitated for 5 minutes with a stirring rate of 300 rpm. Then the glass vessel was placed in ice water and cooled quickly to 5°C. The stirring at 300 rpm was continued for 30 minutes, and 75 ml isopropanol was added. After 10 minutes the gelatin microspheres were separated by filtration and washed three times with isopropanol. The suspension of microspheres

in isopropanol was sieved through wire mesh (aperture size 60 um). The large microspheres were again separated from the suspension by centrifugation at 500 rpm were removed, and the supernatant was centrifuged at 3000 rpm. The microspheres obtained dried at ambient temperature, were spherical free-flowing grains, having a mean diameter of 30 um.

Preparation of Microcapsules

Gelatin microspheres were coated with ethylcellulose (U.S.P.XVI grade /7/), using an organic phase separation method /8,9/. Five g of microspheres to be coated were poured into 50 ml cyclohexane solution of 150 mg ethylcellulose and 300 mg polyethylene, heated at 50°C. Stirring speed was maintained at 300 rpm for 20 minutes, then heating was discontinued and the system allowed to cool to room temperature with gentle stirring. The ethylcellulose microcapsules were rinsed several times with n-hexane containing 1% hydrogenated sunflower oil, and air dried for 24 hours.

Microspheres Metronidazole Content

A 0,25 g sample was accurately weighed, 100 ml 5% hydrochloric acid was added and the suspension was allowed to stand at 60°C for 48 hours. The solution was diluted and metronidazole was assayed spectrophotometrically, at 277 nm.

The Magnetic Responsiveness of the Microspheres and Microcapsules

The suspension of 0,5 % (w/v) microparticles in 6% (w/v) dextran 70 (M.W. 70.000) solution in physiological saline (0,9 % NaCl) was used as the flow phase which was pumped with a laminar flow rate in various glass capillary tubes (0,5 ; 1 ; 1,3 mm in diameter) horizontally mounted and passed over a unipolar magnetic field of 0,03 Tesla at some point. Percent of microparticles retained was evaluated as a function of 0,002, 0,01 and 0,02 Reynolds (R) numbers of blood flow for capillaries and small arteries in humans.

$$R = Dvd/n$$

where D is the diameter of the capillary ; v is the rate of settling ; d is the density of dispersion medium and n is the viscosity of the medium /10/.

The Viscosity of the Microcapsule Suspensions

The flow curve of the microcapsules in physiological saline was measured with a Rheotest viscometer (VEB Prufgerate-Werk Medingen/Dresden). The relation between shear rate, D, and shear stress, , was measured.

In Vitro Release Studies

The dissolution medium (1000 ml of 0.1 N hydrocloric acid) was introduced into a beaker and the stirring rate was 60 rpm.The temperature of the water bath wasmaintained at $37^{\circ}C \pm 0.5^{\circ}$.An accurately weighed amountof powder, microspheres or microcapsules,corresponding to 0.05 g metronidazole was gently spread over the surface of dissolution medium.At appropiate intervals 5 ml samples were withdrawn by using a needle and syringe.The samples were filtered,diluted and the absorbance was measured at 277 nm.

RESULTS AND DISCUSSION

It is known that magnetically responsive microspheres bearing drugs and magnetite,could be selectively localized in vivo,resulting in release of significant local concentrations of drug in a defined area.By using appropriate magnetic parameters,more than 50% of theinjected carriers could be targeted. We evaluated the magnetic responsiveness of the microspheres and microcapsules at known Reynolds number in intravascular flow (fig.1).

It is seen that full retention of the flowing microspheres or microcapsules suspension in vitro was acnieved in the applied magnetic field, for the Reynolds numbers of blood flow in humans for capillaries and small arteries. The rheological behavior of the suspensions of microbeads containing metronidazole and magnetite is shown in fig.2.

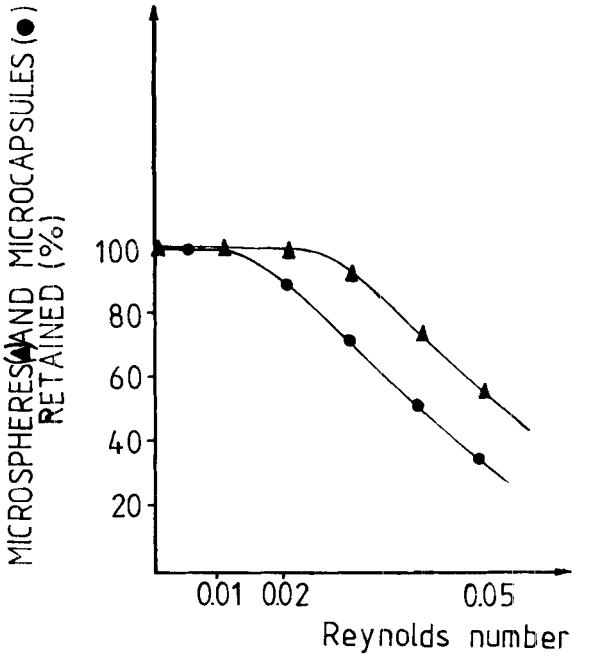

Fig.1 Retention of Microspheres Flowing in the Magnetic Field Applied

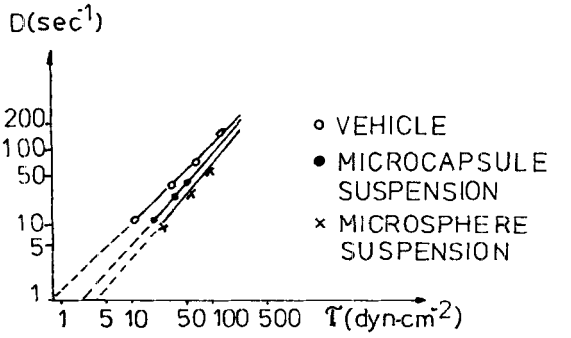

Fig.2 Shear Rate versus Shear Stress Curves of Microsphere and Microcapsule Suspensions in Physiological Saline with 6% Dextran 70.

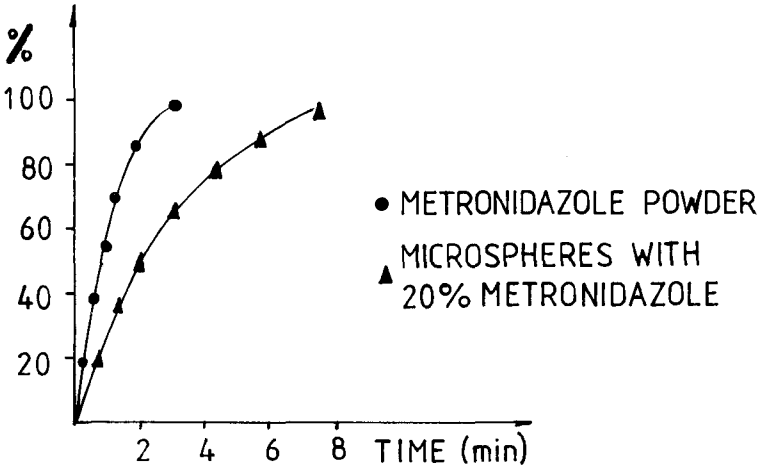

Fig.3 Plot of % Drug Released from Microspheres with Time.

The microsphere and microcapsule suspensions possesses a structural viscosity at a lower shear rate, which is quite similar to that of red blood cell suspension/5/. The flow curve of the suspensions at the shear rate greater than 500/s is Newtonian. This behavior is favorable when a microcapsule suspension is intravascularly injected.

We have attempted to prepare microbeads with a relatively slow release of metronidazole.
The rate of in vitro drug release was very different according to the microbead type (fig.3, fig.4).

For the microspheres, experimental release data were tested according to a first order release of the embeded drug. $A=A_0.e^{-k.t}$ $\ln(A_0-A)=\ln A_0-k.t$ where A= the mass of the released drug ; A_0= initial drug concentration ; k= first order rate constant.
The experimental data fitted the first order release pattern. The calculated rate constant, k, for metronidazole microspheres was 0,397 (min^{-1}) comparatively with 1,719 min^{-1} for the Metronidazole powder (correlation coefficients were, respectively, 0,999 and 0,979).

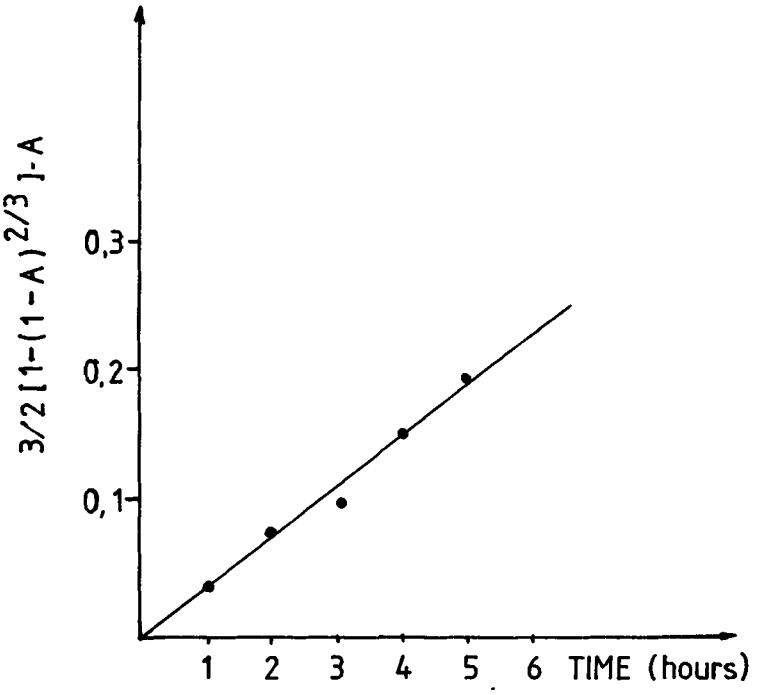

Fig.4 Plot of % Drug Diffused Through the Microcapsule Membrane with Time.

On the other part, the release kinetics of metronidazole from microcapsules complied to the dissolution model for a spherical matrix proposed by Baker and Lonsdale /11/. $\frac{3}{2}/1-(1-A)^{2/3}/-A = kt$ where A= the fraction of drug released up to time t.

CONCLUSIONS

The retention of these microbeads by an magnetic field in vitro and the rheological properties of the microcapsule suspensions in physiological saline with 6% Dextran, suggests that microcapsules injected intravascularly can be localized at a target site in vivo by means of an externally applied magnetic field, and normal blood flow should not be altered at the site of carrier localization. The in vitro release of metronidazole from

microspheres was significantly reduced as compared to the metronidazole powder. The release declined at an apparent first order rate. The release of metronidazole from ethylcellulose-coated microspheres was largerly controlled by the permeability of metronidazole through the coating.

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