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RELEASE OF METHOTREXATE FROM MICROSPHERE-IN-OIL-IN-WATER EMULSIONS

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ABSTRACT

The in vivo and in vitro release characteristics of methotrexate from the microsphere-in-oil-in-water emulsions were studied. The results demonstrated a rapid and slow biphasic release profiles for the emulsions. This may be due to the release of methotrexate from the external aqueous phase of the emulsion for the rapid release phase and from the internal microsphere for the slow release phase. The addition of phosphatidylcholine in the emulsions resulted in a slower release of the methotrexate which

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may be caused by the formation of phospholipid layers on the surface of the emulsion particles to hinder the release of the drug from the emulsions.

INTRODUCTION

Several preparations such as microspheres, emulsions and liposomes are used as carriers for anticancer drugs in cancer chemotherapy to control the drug delivery and enhance the drug concentration in the tumor site but limit the drug concentration in the normal tissues to reduce the side effects of anticancer drugs (1-5).

This study attempted to formulate a system with microspheres and emulsions for anticancer drug delivery. The microspheres were made from bovine serum albumin and prepared to form a microsphere-in-oil-in-water (s/o/w) type emulsion. Phosphatidylcholine was incoporated into the emulsion to form a system called s/o/w/l emulsion. Methotrexate as a frequently used cancer chemotherapeutic agent was found in our preliminary study to be quite stable in the process of encapsulation in microspheres by the heating method. Therefore, it was used for encapsulation in the s/o/w and s/o/w/l emulsions. The in vivo and in vitro release characteristics of the methotrexate from these preparations were investigated.

MATERIALS AND METHODS

Methotrexate (Kingdom Pharmaceutical Co., R.O.C.), methotrexate injection (25 mg/ml, Lederle, U.S.A.), bovine serum albumin (fraction V, Sigma Chemical Co., U.S.A.), silicon oil (100 cps, Tokyo Kasei, Japan), corn oil (Mazola, CPC International, U.S.A.), sesame oil (Sigma chemical Co., U.S.A.), pluronic F68 (Fluka, Switzerland), phosphatidylcholine (from fresh egg yolk, Type XI-E, Sigma Chemical Co., U.S.A.), anhydrous ether (reagent grade, Wako Chemicals, Japan), ketamine injection (Parke-Davis Pharmaceutical Co., R.O.C.) and methotrexate reagent pack (no. 522-20) for TDx analyzer (Abbott, U.S.A.) were used as received without further purification. General chemicals used were of analytical grade.

Preparation of Methotrexate-Loaded Albumin Microspheres

0.5 ml of methotrexate solution (pH 8.5) was mixed well with 0.5 ml of bovine serum albumin solution (312.5 mg/ml). The mixture was added to 60 ml of cooled corn oil (4°C) and homogenized in an ice bath by a homogenizer (Ystral, Germany) for 10 minutes. The emulsion was sonicated for 6 minutes at 125 W (Sonicator, model w-220, Heat system-Ultrasonics, U.S.A.), and then added to 100 ml of preheated corn oil (135°C) dropwise by stirring at 1500 rpm. Both heating and stirring were maintained for 10 minutes to enable

the formation of the microspheres. The suspension of microspheres was allowed to cool in an ice bath to 20°C and then washed with 200 ml of anhyrous ether. The microspheres were centrifuged at 10,000 g for 30 minutes and the supernatant was discarded. After three washes, the microspheres were resuspended in anhydrous ether again and evaporated in a stream of nitrogen gas. The resultant microspheres were further dried under vacuum overnight and stored at -20 °C for ready use.

Determination of Methotrexate in Methotrexate-Loaded Albumin Microspheres

A known amount of microspheres was sonicated with deionized water at 125 w in an ice bath for 1 hour. The homogenate was centrifuged at 10,000 g for 30 minutes and the supernatant was analyzed by HPLC to determine the concentration of methotrexate.

Preparation of Emulsions

50 mg of the microspheres was dispersed in 3 ml of anhydrous ether and mixed well with 1 g of the sesame oil. The dispersion was placed under a stream of nitrogen gas in a water bath at the temperature of 70 °C for 24 hours to evaporate the ether. 1.5 g of the 0.9% sodium chloride solution with 5% pluronic F68 as emulsifier was added to the s/o dispersion and emulsified by a homogenizer (Ystral, Germany) for 3 minutes. The resultant s/o/w emulsion was used for the

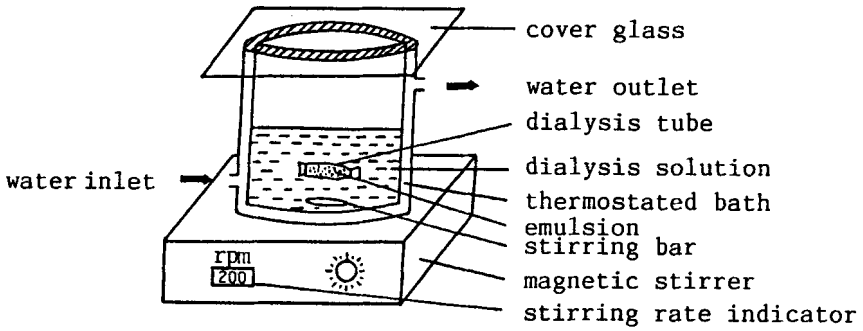

FIGURE 1

Apparatus for the determination of the release of methotrexate from emulsions.

experiment immediately after the preparation. For the preparation of s/o/w/l emulsions, the phosphatidylcholine was dissolved in the microsphere-ether dispersion and mixed well with sesame oil. The rest procedures for the preparation of s/o/w/l emulsions were the same as the above described. The concentrations of the phosphatidylcholine used were 1, 2 and 4 mg/g emulsion respectively.

In Vitro Release Study

2 g of the emulsion was transfered to the dialysis tubing 2 X 2.5 X 6 cm in volume (Spectrapor 2, MW cutoff: 12,000-14,000, Spectrum, U.S.A.) and dialysed against 200 ml of 0.9% sodium chloride solution at 37°C (Figure 1). The dialysis solution was stirred by a magnetic stirrer at the rate of 200 rpm (MS203, Fargo Stirrer, R.O.C.). At appropriate intervals, 5 ml of the

dialysis solution was sampled and 5 ml of the 0.9% sodium chloride solution was refilled back to maintain the original volume of the dialysis solution. The concentration of the methotrexate in the dialysis solution was determined spectrophotometrically (UV650 Jasco, Japan) at 303 nm.

In Vivo Release Study

The male Sprague-Dawley rats weighing 300 - 350 g were anesthetized with ketamine and injected with 0.15 ml of the emulsions at a dose of 1.4 mg/kg of methotrexate into the jugular vein. At the time intervals of 5, 15, 30, 60, 20, 180 and 360 minutes after injection, 1 ml of blood was taken via cardiac puncture. For each determination, at least 5 rats were used.

The methotrexate concentration in blood was determined using a TDx analyzer (Abbott Lab, U.S.A.). The serum for analysis was obtained by centrifugation of the whole blood at 3,000 rpm for 5 minutes after clot retraction. The relationship between the data obtained from the spiked human serum and rat serum was determined as a factor for the rat serum effect. This is to see the suitability of using the TDx analyzer to determine the methotrexate concentration in rat serum, since this analyzer has been designed only for the determination of drug concentration in human serum.

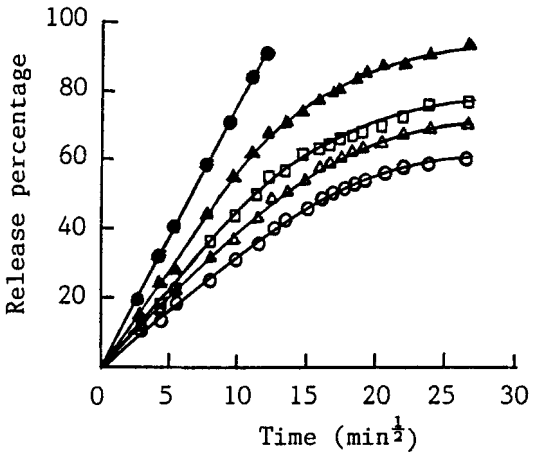

FIGURE 2

In vitro release profiles of methotrexate from aqueous solution (●), s/o/w emulsion (▲) and s/o/w/1 emulsions with phosphatidylcholine concentration of 4 mg/g emulsion (○), 2 mg/g emulsion (△) and 1 mg/g emulsion (□).

RESULTS AND DISCUSSION

Figure 2 shows the release patterns of methotrexate plotted with the percentage of release against the square root of time for the s/o/w and s/o/w/1 emulsions and the aqueous methotrexate solution. The release of methotrexate from the dialysis tubing demonstrated a straight line. Similar release profile was obtained from the external phase of the o/w type emulsion in which the methotrexate was spiked (the result is not shown here). These results indicated that the methotrexate transported across the dialysis membrane was through a diffusion mechanism (6). Also,

the presence of the dialysis membrane did not influence the release of methotrexate from the emulsion. The release rates of methotrexate from the s/o/w emulsion were slower than that from the aqueous solution. The release of methotrexate from the s/o/w emulsion showed a rapid and slow biphasic pattern in the time range studied. During the preparation of s/o/w emulsion, the homogenization process caused the rupture of a small portion of the microspheres in which the entrapped methotrexate leaked into the external aqueous phase of the emulsion. Therefore, the first phase of release of methotrexate may result from the release of the drug from the external aqueous phase of the emulsion which would give a rapid release rate. The second phase of release was probably due to the release of the drug from the microspheres that would lead to a slow release rate (7,8). The s/o/w/l systems demonstrated a biphasic release pattern similar to that of the s/o/w emulsion, but the former presented a slower rate than the latter. Possibly, the phosphatidylcholine coated on the microsphere-in-oil particles to form a barrier for the release of methotrexate to the aqueous phase and result in a slower release rate for the s/o/w/l systems. The increase of concentration of phosphatidylcholine seemed to induce a decrease of the release rate of the s/o/w/l systems (Figure 2). It is probably that more phospholipid layers would form on

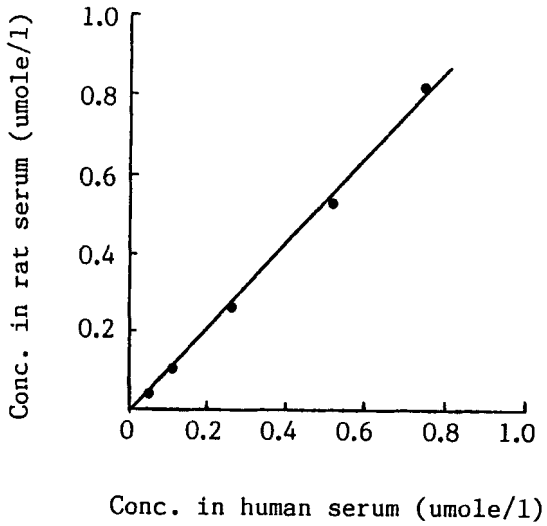

FIGURE 3

Relationship between spiked methotrexate concentration in human serum and rat serum.

the particles of the emulsion to hinder the release of the drug.

Figure 3 shows the results of concentrations of methotrexate spiked in human serum versus rat serum as determined by the TDx analyzer, which indicate a straight line with a correlation coefficient of 0.99985 and a slope of 1.058. The TDx analyzer appeared therefore to be suitable for the analysis of the methotrexate concentration not only in human serum but also in rat serum. The factor for the correction of the examination of human serum to rat serum was 0.058 based on the assumption that identical values with a slope of 1 were obtained for the methotrexate concentrations

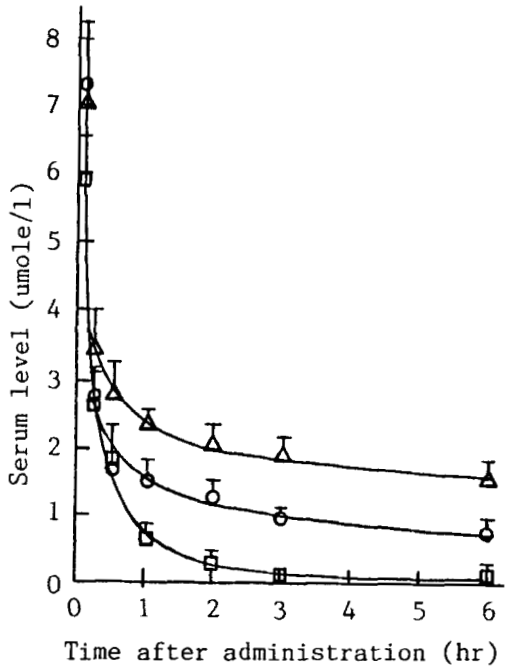

FIGURE 4

In vivo release profiles of methotrexate from aqueous solution (□), s/o/w emulsion (O) and s/o/w/1 emulsion with phosphatidylcholine concentration of 4 mg/g emulsion (Δ).

in human serum and rat serum. This factor was recalibrated for each carousel of the samples.

The leakage of methotrexate in blood from the s/o/w emulsion, s/o/w/1 systems and methotrexate solution after i.v. injection is shown in Figure 4, plotted with the methotrexate concentration in the serum against time after administration. In the early stage of release, the s/o/w emulsion and s/o/w/1 systems demonstrated a similarly rapid release of methotrexate in blood as methotrexate solution did.

After 30 minutes of administration, the methotrexate released more slowly from s/o/w/ emulsion and s/o/w/l systems in blood than from the drug solution and the magnitude of the drug concentrations for s/o/w/l systems was higher than that for s/o/w emulsion. In the early stage of release, the drug concentration in blood may result from the release of methotrexate from the external aqueous phase of the emulsions. Therefore, it demonstrated a result similar to that of the methotrexate solution. The drug in the internal phase of the emulsions would release slowly. Also, the presence of phospholipid layers on the emulsions may be a factor that hindered the release of the drug in blood. As a result, a second stage of slow release was shown.

ACKNOWLEDGMENTS

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