RESEARCH PAPER

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Albumin Microsphere Containing Methotrexate: A Lung specific Delivery System

S.A. DHANARAJ*, K. GOWTHAMARAJAN, K. SHANTHI AND B. SURESH
Department of Pharmaceutics, J.S.S. Center for Research and Post Graduate Studies, J.S.S. College of Pharmacy, Ootacamund-643 001.

Albumin microspheres containing methotrexate were prepared in the size range of 12.7 to 19.5 mm, by heat stabilization technique at various concentration of drug and different speeds of agitation.They were evaluated by scanning electron microscope (SEM).Through the dissolution study on various batches of drug-loaded microspheres, the batch with optimum drug loading and satisfactory release profile was selected as ideal batch (20 mg drug loaded microspheres prepared by using 500 RPM). *In vivo* evaluation was done on all the batches using albino mice.The ideal batch showed significant enhancement in drug localization particularly in lungs in comparsion with free drug.

Drug targeting is a specific form of drug delivery where the pharmacological agent is directed selectively to visit its site of action. It not only reduce the dose of drug reaching to the effective biological sites, but also resullts in reduced toxicity. Various attempts have been made in the field of targetting but in the past few years pharmacists have focusd their research on colloidal drug delivery systems like liposomes, microspheres and nanoparticles, as targeting carriers, which have given more selective targeting.The drug-targeting techniques are classified into two types - active and passive. In passive targeting, the carrier bearing the drug reaches the site according to its particle size via natural phenomena. In active targeting, the carrier bearing the drug reaches to a specific site on the basis of the modificaion made on its surface, rather than the particle size. In the present study, passive targeting has been selected to deliver the drug to lungs.

Methotrexate, a folic acid analogue, is widely used in the treatment of cancer. It is an antimetabolite and immunosuppressive agent and is used in the treatment of rheumatoid arthritis also[2]. Since methotrexate is used in the therapy of lung cancer[2]; it was found appropriate to select it is as a drug candidate to be loaded into albumin microsphers for site specific delivery to lungs. Therefore, our studies have been planned to investigate the process variables in the formulation of methotrexate-loaded albumin microspheres and to evaluate its *in vitro* and *in vivo* characteristics.

*For correspondence

EXPERIMENTAL

Methotrextrate, BP was obtained from Bio-Chem Pharmaceuticals, Mumbai, Egg albumin flakes were purchased from Loba Chemicals, Mumbai, Linseed oil, toluene and diethylether, all of AR grade, were procured from SD Fine Chemicals Ltd, Mumbai. Scanning electron microscope (Kamarajar University, Madurai), UV spectrometer (Shimadzu Japan, 160A model) were the instruments used.

Preparation of Albumin Microspheres containing Methotrexate:

Microspheres were prepared by heat stabilization technique, in which 2ml of 25% w/v albumin solution was added into a mixture of 15ml of linseed oil and 10ml of toluene, preheated to 30-50° and this was stirred at 500rpm. A w/o emulsion was formed. Stirring was continued by raising the temperature of the oil bath to

110° until the microspheres were dehydrated completely (approximately 30 min). Then the microspheres were filtered and washed five times with 5 ml volumes of diethylether and dried at room temperature. A fine yellow free flowing powder was obtained. Average particles size was determined[3] by using a calibrated stage micrometer and the surface characters were analyzed by scanning electron microscope (SEM).

To study the effect of process variables on drug loading, the following variables were considered.

Effect of Drug to Albumin Ratio:

Six batches of drug-loaded microspheres were prepared containing 5, 10, 20, 30, 40 and 50 mg in 2 ml of 25% w/v of albumin solution. These batches were designated as batch A, B, C, D, E and F. Other variables that kept constant are continuous phase:linseed oil 15ml, speed of agitation:500 rpm.

Effect of Speed of Agitation:

Six batches of drug-loaded albumin microspheres each containing 20 mg of methotrexate were prepared with various selected speeds of agitation such as 300, 400, 450, 550 and 600 rpm and were labelled as batch G, H, I, J and K, respectively. Other variables, kept constant were-albumin concentration:2 ml of 25% w/v of egg-albumin, continuous phase:15 ml of linseed oil and the amount of drug:20 mg.

Estimation of the amount of drug incorporated into albumin microspheres:

One milligram portion of the drug-loaded albumin microspheres was incubated with 15 ml of 5% of HCl in absolute ethanol at 4° for 24 h of incubation, the mocrospheres were separated by high-speed centrifugation at 4000 rpm and the drug content was analysed in the supernatant by UV spectrophotometry[4].

***In vitro* release:**

Fifty milligrams of drug-loaded microspheres were taken from each batch into a 250 ml conical flask and 100 ml of pH 7.4 phosphate buffer was added, the flask was kept in a shaker cum incubator and the shaker was adjusted to 80 horizontal strokes per minute at 37°. Five milliliters of drug releasing media were withdrawn at various time intervals of 0, 5, 30 min, 1, 2, 8, 16 and 24 h. The samples withdrawn were filtered through a membrane filter of pore size 0.4 m under vacuum. The drug was estimated in the clear filtrate using an UV spectrophotometer at 303 nm[5].

***In vivo* studies:**

To estimate the amount of drug targeted to various organs such as the kidney, the liver and the spleen, an *in vivo* study was designed. Seventeen groups of mice, each containing 6 animals were selected. The weight of animals was ranging between 20-30 g. The animals were fasted for 12 h and a constant day and night cycle was maintained. The temperature of the animal room was kept at 25° throughout the experiment.

Group I to group VI received drug-loaded microspheres of batches A, B, C, D, E, and F, respectively. The dose was kept at 11.25 mg/kg body weight. Group VII to XI received drug loaded microspheres of batches G, H, I, J and K, respectively. Here also the dose was kept at 11.25 mg/kg. Group XII and XIII received methotrexate loaded microspheres equivalent to 11.25 mg/kg of drug, which were selected as an ideal batch (batch C) from our first phase study (*in vitro*). Group XIV and XV were treated as positive control and received 11.25 mg/kg of pure drug in normal saline. Group XVI and XVII were kept as solvent control and received only saline.

Microspheres were administered to the mice in normal saline intravenously through the tail vein with the help of a syringe attached with hypodermic needle No. 26. After 12 h, all animals from group I to XVI were sacrificed except XIII, XV and XVII, which were sacrificed after 36 h. Lungs, liver, kidney and spleen were isolated and homogenized in 5 ml of 0.2 N NaOH and centrifuged (4000 rpm) for 1 h. The supernatant liquid was collected and analysed for the drug content spectrophotometrically. The same procedure was followed for the groups XVI and XVII and the supernatent was used as blank for spectrometric determination.

RESULTS AND DISCUSSION

From our studies we have observed that the size of microspheres decreased with the increase in the speed of agitation, but the amount of drug loaded was found to increase. Increase in the drug ratio increased the loading but only slight variation was observed in the mean diameter

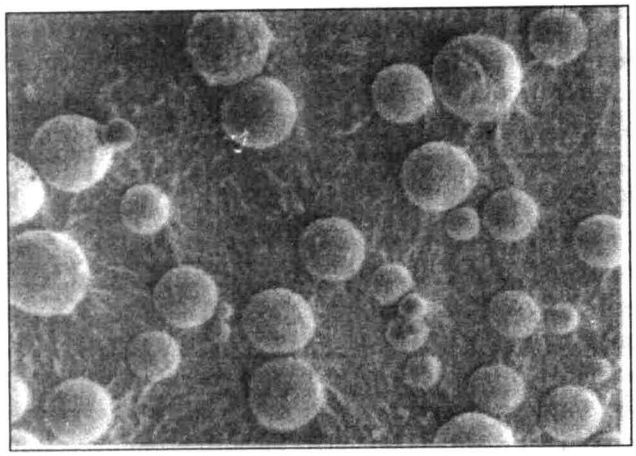

Plate 1: Scanning Electron Mircograph of Ideal Batch (Batch - C)

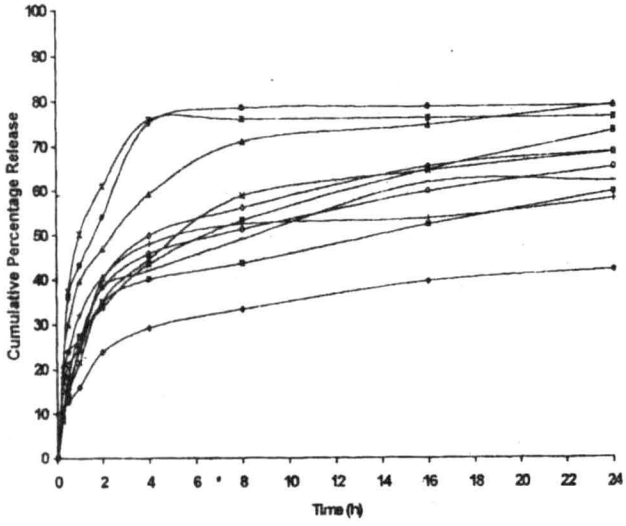

Fig. 1: Albumin microspheres containing methotrexate from various batches such as A(–◆–), B(–■–), C(–▲–), D(–X–), E(–✱–), F(–●–), G(–+–), H(–○–), I(–▬–), J(–◊–), and K (–■–) were subjected to *in* vitro drug release studies over a period of 24 h and the cumulative percentage of drug release was calculated

of the albumin microspheres. In the size distribution analysis, maximum percentage frequency in the size range of 12-17 mm was obtained in the batch prepared with 500 rpm and 20 mg drug loading (Plate-1).

The *in vitro* release was found to be biphasic, that is, an initial burst phase within 2 h and a gradual slow release after 2 h to 25 h. The biphasic release pattern was independent of the amount of drug loaded into the microspheres. The release was maximum in the initial

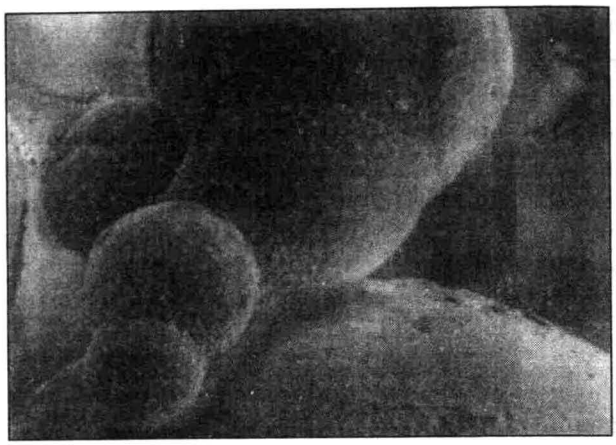

Plate 2: Scanning Electron Mircograph showing Surface Characters of Ideal Batch

burst phase in 40 mg and 50 mg drug-loaded microspheres. This may be because of non-uniform size distribution and agglomeration, as observed by SEM. The *in vitro* release pattern is shown in Fig 1.

Even though the initial burst phase at 2 h was almost 47% in the batch prepared with 500 rpm and 20 mg drug loading (batch C), gradual sustained release was obtained upto 24 h and the cumulative percentage release was also higher when compared with other batches. This may be (78%) because of uniform size distribution and smooth surface without agglomeration, as observed by SEM (Plate-2).

The *in vivo* evaluation was done in albino mice and the drug concentration in various organs such as lungs, kidney, spleen and liver were determined and tabulated in Table 1. Intravenously injected methotrexate in microspheres was found to be better and effective in drug targeting particularly to lungs than the i.v. injection of free drug. The amount of drug targeted using free drug after 12 h was only 11.36% in case of lungs, 9.77%, 6.36% and 20% in the case of kidneys, spleen and liver respectively. Whereas, the drug targeting was 47% in lungs, and 16.8%, 10.5% in kidneys, spleen and livers, respectively, in case of microspheres prepared with 500 rpm and 20 mg drug (batch C). Here we observed that in liver, kidney and spleen there is no much difference in percentage of drug targeted, even in the case of liver the percentage is less than that of free drug. This clearly proves that the drug-loaded microspheres avoid the drug accumulation in liver. The microspheres with a size range of 12-17 mm showed drastic increase in drug localization

TABLE 1: AMOUNT OF DRUG TARGETED TO VARIOUS ORGANS FROM MICROSPHERES AND FREE DRUG

Batch Code	Percentage of drug detected in target organ			
	Liver	Lung	Kidney	Spleen
A	12.5	22.5	18	8.13
B	17	34.1	14.2	4.4
C	15	47	16.8	10.5
D	19.13	46.01	15.33	7
E	13.7	40.03	16	6.2
F	14.1	45.44	9.17	12.5
G	13.04	28.3	25.04	4.1
H	18.6	31.52	24.6	11.81
I	12.51	13.02	24.31	11.82
J	11.2	45.3	9.8	8
K	24	36.5	13.9	3.18
Free drug	20	11.36	9.77	6.36

A = batch prepared with 5 mg drug and 500 rpm speed, B = batch prepared with 10 mg drug and 500 rpm speed, C = batch prepared with 20 mg drug and 500 rpm speed, D = batch prepared with 30 mg drug and 500 rpm speed, E = batch prepared with 40 mg drug and 500 rpm speed, F = batch prepared with 50 mg drug and 500 rpm speed, G = batch prepared with 20 mg drug and 300 rpm speed, H = batch prepared with 20 mg drug and 400 rpm speed, I = batch prepared with 20 mg drug and 450 rpm speed, J = batch prepared with 20 mg drug and 550 rpm speed, K = batch prepared with 20 mg drug and 600 rpm speed.

in lungs. The results of our ideal batch (20 mg/500 rpm, batch C) in mice model showed a maximum percentage of drug targeting (47%) to the lungs. In case of free drug, it was only 11.36%. In our *in vivo* evaluation of ideal batch (batch C), it showed only low percentage (15.8%) drug localization after 36 h. This may be explained in terms of rapid elimination before 36 h. In the conclusion, the results suggest that the albumin microspheres of methotrexate may reduce the degree of side effects keeping its optimum therapeutic efficacy and thus can be better alternative over conventional dosage forms.

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