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RESEARCH PAPER

Prolonged Release of Tegafur from S/O/W Multiple Emulsion

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ABSTRACT

To develop a prolonged and sustained release preparation, we prepared an albumin microsphere-in-oil-in-water emulsion (S/O/W) and examined sustained release from it in comparison with other control preparations such as water-in-oil (W/O) emulsions and microspheres in vitro and in vivo, respectively. Tegafur was used as a model drug. A microsphere-in-oil emulsion was prepared by adding albumin microspheres to soybean oil containing 20% Span 80. To prepare an S/O/W emulsion, the microsphere-in-oil emulsion was added into an aqueous solution of hydroxypropyl methylcellulose containing Pluronic F68. The mean particle size of the albumin microspheres was 3 μm, and the ratio of entrapment of tegafur into albumin microspheres was about 25%. In an in vitro release test, the t_{75} of the S/O/W emulsion was fourfold greater and in an in vivo release test the mean residence time of tegafur from the S/O/W emulsion was more than twofold that from a W/O emulsion or microsphere system. The mean residence time of 5-fluorouracil (5-FU) from an S/O/W emulsion was also greater than with other dosage forms. These results suggest the possible usefulness of an S/O/W emulsion for the sustained and prolonged release of tegafur.

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INTRODUCTION

Multiple emulsions have been used in the fields of prolonged drug-delivery systems, drug-overdose treatment, cosmetics, foods, and carriers for lymphatic drug delivery (1–8). In water-in-oil-in-water (W/O/W) multiple emulsions, the internal and external aqueous phases are separated by an oil layer. W/O/W emulsions require two stabilizing surfactants for their formation and stability, one having a low hydrophilic-lipophilic balance (HLB) to form the primary water-in-oil emulsion, and the other having a higher HLB to achieve secondary emulsification. A microsphere-in-oil-in-water (S/O/W) emulsion, in which the inner phase is replaced by a microsphere, is the other multiple emulsion (9,10). Microspheres are perceived as important drug carriers for targeting and controlled release. Albumin microspheres are biodegradable colloidal particles that have been used for controlled drug delivery and drug targeting (11).

We conducted a study in which we attempted to formulate a system with microspheres and emulsions for anticancer drug delivery. For effective cancer chemotherapy, it is necessary to deliver a sufficiently high concentration of anticancer agent to a tumor site for a required period. Moreover, the lymphatic delivery of anticancer agents is important for preventing the metastasis of cancer cells along the pathways of lymph flow. In previous papers (12,13), we reported that after administration of a W/O or O/W emulsion, the lymph concentration of tegafur, a precursor of 5-fluorouracil (5-FU), was significantly higher than that with an aqueous solution. Tegafur has shown significant activity in gastrointestinal and breast carcinomas, with less myelotoxicity than that of 5-FU (14). In the study described here we prepared an albumin microsphere-in-oil-in-water (S/O/W) multiple emulsion and examined its potential as a sustained and slow release preparation. We examined the release of tegafur as a model drug, and compared the S/O/W type emulsions with other, control preparations such as W/O emulsions and microspheres in vitro and in vivo.

MATERIALS AND METHODS

Materials

Tegafur was obtained from Yuhan Pharm. Ind. Co. (Seoul, Korea). Hydrogenated castor oil was obtained from Dong-A Pharm. Ind. Co. (Seoul, Korea). Soybean oil, 5-fluorouracil (5-FU), bovine serum albumin (BSA; fraction V), proteinase K, and Pluronic F68 were purchased from Sigma Chemical Co. (St. Louis, Mo.). All other materials were of reagent grade and were used without purification.

Preparation of Albumin Microspheres Containing Tegafur

An aqueous solution of tegafur equivalent to 20 mg/ml was mixed well with BSA solution. The mixture of albumin and drug solution was added to soybean oil to make a W/O emulsion, and was then homogenized for 10 min with an Ultra-Turrax stirrer (Janke & Kunkel, T25, Staufen, Germany) and sonicated for 6 min with a sonicator (Sonic & Materials Inc., VC600, Danbury, CT). The W/O emulsion was added dropwise into 60 ml of prestirred soybean oil. A predetermined amount of purified glutaraldehyde was then added to make the hardened albumin microsphere containing tegafur. The cross-linking reaction was allowed to proceed for 3 hr. Subsequently, 200 ml of ether was added to remove the oil, and the microspheres were isolated by centrifugation at 3000 rpm for 15 min. The supernatant was then decanted and the microspheres were resuspended in ether. This washing procedure was repeated three times. The microspheres were collected on a Petri dish, and were then air-dried for 24 hr, vacuum-dried for an additional 24 hr, and stored frozen.

Preparation of S/O/W Emulsion

To make a sphere-in-oil (S/O) emulsion, a predetermined amount of microspheres was dispersed in 1 ml of soybean oil containing 200 μl of Span 80 as a primary emulsifier. One-and-one-half milliliter of 0.9% (w/v) Nacl solution containing 5% (w/v) Pluronic F 68 as a second emulsifier and 1% (w/v) hydroxypropyl methylcellulose as a viscosity enhancer was added to the S/O dispersion and emulsified by vortex mixing for 5 min. The microspheres were dispersed into water containing 0.1% Tween 80, and were then sonicated for 1 min. The particle size was measured with a laser-light-scattering particle-size analyzer (Malvern, Master Sizer E, Worcestershire, England). As a reference material, we prepared a W/O emulsion containing tegafur. A mixture of aqueous tegafur solution and soybean oil containing hydrogenated castor oil was homogenized and sonicated to make the W/O emulsion.

Determination of Drug Content in Albumin Microsphere

A known amount of microspheres was dispersed into a mixture of 4.5 ml water and 0.5 ml proteinase K solu-

tion. The final concentration of proteinase K was adjusted to 100 μg/ml. After incubation with shaking for 4 hr at 45°C, this sample was sonicated for 20 min. The supernatant of the sample dispersion was centrifuged at 12,000 rpm for 5 min and analyzed by UV spectrophotometry (Hewlett Packard, Diode Array Spec. 8452A, Waldbronn, Germany).

Release of Tegafur In Vitro

A test sample (2 ml) was introduced into a-dialysis tube and dialyzed in a volume of 50 ml of 0.9% NaCl solution at 37°C. The dialysis solution was agitated by a magnetic stirrer at about 150 rpm. At appropriate intervals, 3 ml of the dialysis solution was withdrawn and 3 ml of the 0.9% NaCl solution was added back to maintain the original volume of the dialysis solution. The concentration of drug was analyzed by UV spectrophotometry at 270 nm.

Release of Tegafur In Vivo

Male Sprague–Dawley rats weighing 130–150g was obtained from Taehan Experimental Animal Center (Seoul, Korea). Water and feed (Jeil Co., Korea) were freely supplied for more than 2 weeks at 20–25°C, and the rats were housed at 50–60% relative humidity. Rats weighing 250–300g were used. Under light ether anesthesia, the femoral vein and artery were cannulated with polyethylene tubing (PE 50, Clay Adams, Parsippany, NJ) for drug administration and blood sampling, respectively. Ordinarily, animals awoke in about 15–20 min. Test sample was then orally administered through stomach tube as a tegafur concentration of 4 mg/kg. At various times after tegafur administration, arterial blood samples (0.3 ml) were collected from the femoral artery into heparin-locked polyethylene tubing and immediately centrifuged for 2 min at 12,000 rpm. Following this, 100 μl of plasma was frozen at less than −20°C until assay. Tegafur and 5-FU in the blood samples were analyzed by the HPLC method of Wu et al. (15). Briefly, 100 μl of plasma sample was mixed with 100 μl of water containing 100 μg/ml of theophylline as an internal standard, and the solution was adjusted with water to a total volume of 1 ml, after which 0.1 ml of 0.5 M sodium phosphate solution and 8 ml of ethyl acetate were added. After extraction and centrifugation, the organic layer was taken and evaporated with a centrifugal vaporizer. The residue was redissolved in 100 μl chromatographic mobile phase, and 20 μl of this solution was injected on the HPLC column. Since 5-FU has been reported to be adsorbed on glass surfaces, polypropylene tubes were used in all procedures. We used the noncompartment method for the determination of pharmacokinetic parameters. All calculated values were expressed as mean ± SE. Statistical differences were assumed to be significant when $p < 0.05$ (Student's t-test). The total area under the plasma drug level-versus-time curve (AUC_{0-t}) was obtained by summing each individual area between two consecutive time intervals, using the trapezoidal rule.

RESULTS AND DISCUSSION

Preparation of Microspheres and Emulsions

Figure 1(a) shows the uniform, smooth spherical shape of an albumin microsphere prepared by the solvent-extraction method. A photomicrograph of the S/O/W emulsion was shown in Fig. 1(b). We found multiple emulsion droplets containing many small microspheres dispersed within them. The particle-size distribu-

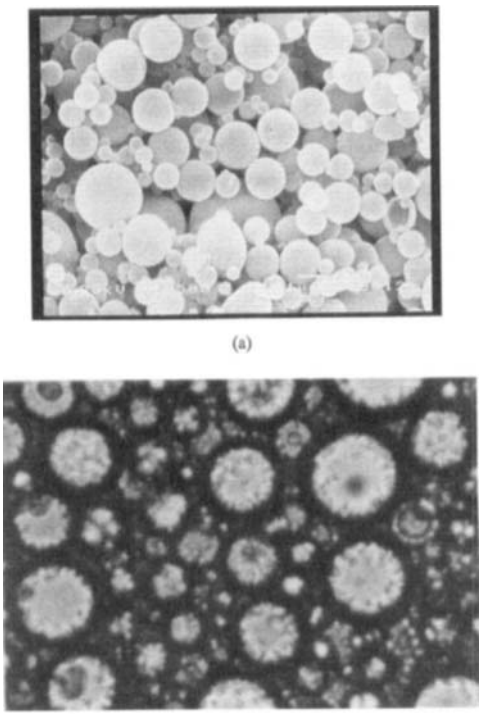
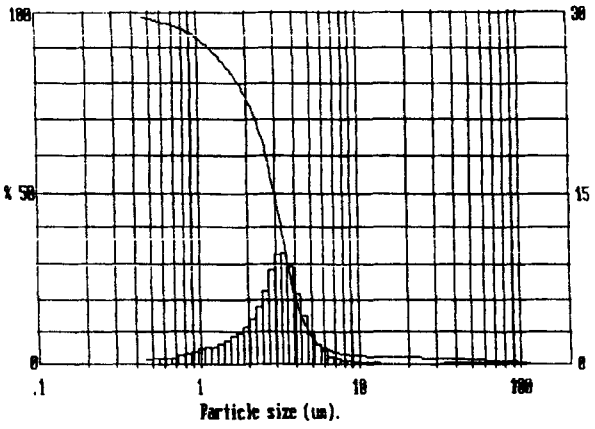
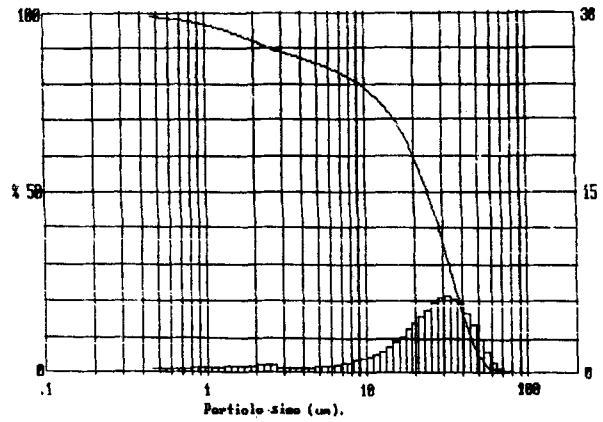


Figure 1. (a) Scanning electron micrograph of albumin microspheres-(b) microphotograph of S/O/W emulsion.



(a)



(b)

Figure 2. Particle-size distribution of (a) albumin microspheres and (b) S/O/W emulsion.

tion of the albumin microspheres as shown in Fig. 2 was determined by laser-light-scattering particle-size analysis. In Figure 2, it is seen that the particle size range (diameter) was 0.5–15 μm and the mean size of microspheres was 3 μm. The particle-size distribution of the S/O/W emulsion was in the range of 0.5–80 μm, and the average particle size was about 24 μm [Fig. 2(b)]. The percent entrapment of tegafur into albumin microspheres was 25 ± 5%.

In Vitro Release

The release of tegafur from S/O/W emulsion was plotted as the percentage of release vs. time, as shown

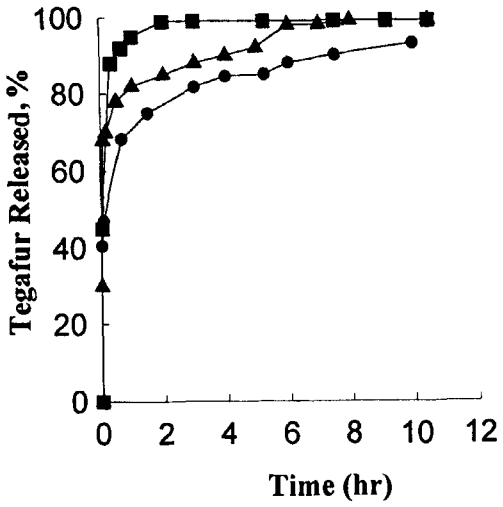


Figure 3. In vitro release profiles of tegafur at 37°C. (●) S/O/W emulsion; (▲) microspheres; (■) W/O emulsion.

in Fig. 3. Release of tegafur from albumin microspheres and W/O emulsion was quantitated. In this release study, W/O emulsion and microspheres were used as a control group for comparing the release profile of S/O/W emulsions. The result showed that the release rate of tegafur from S/O/W emulsion was slower than that from other control preparations, such as W/O emulsion and microspheres. In the in vitro release test, the time to reach 75% release, (t_{75}) with the S/O/W emulsion was fourfold greater than with the W/O emulsion or microspheres. The release rate of tegafur from S/O/W emulsion was faster in the initial stage and slower in the later stage. According to Law and Lin (10), the rapid and slow biphasic patterns in the time range of release may result from release of the drug from the external phase and from the microspheres in the S/O/W emulsion, respectively. This result suggests that transport of tegafur through the oil phase was the rate-limiting step in release.

In Vivo Release

The tegafur concentration in blood after oral administration of S/O/W emulsion and other control groups in shown in Fig. 4, plotted as the tegafur concentration in the plasma vs. time after administration. The procedure was quantified by the internal standard method, using peak area ratios. Compared with the results for control groups, S/O/W emulsion showed a delay in the appearance of tegafur in the plasma and a more gradual decrease in the plasma level of the drug. The maximum plasma

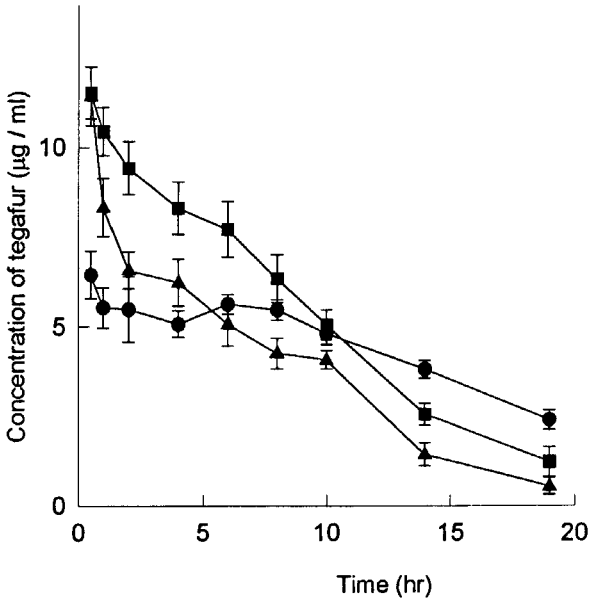


Figure 4. Plasma concentration of tegafur after oral administration to rats. (●) S/O/W emulsion; (▲) microspheres; (■) W/O emulsion.

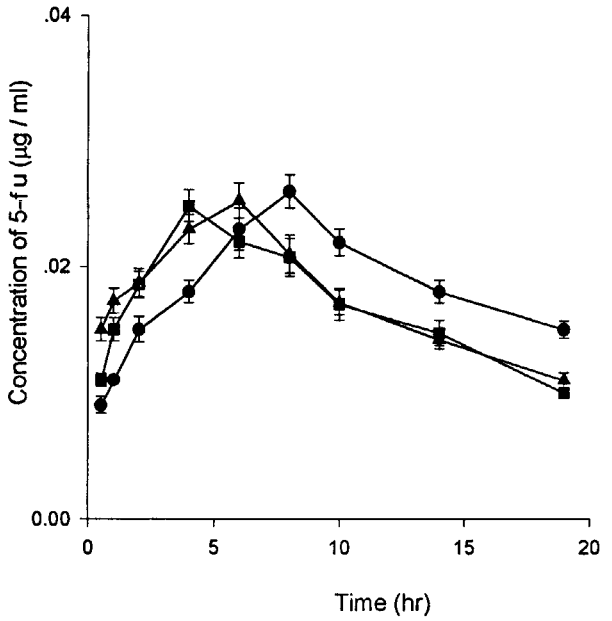


Figure 5. Plasma concentration of 5-FU after oral administration of tegafur to rats. (●) S/O/W emulsion; (▲) microspheres; (■) W/O emulsion.

levels of tegafur were attained in 30 min after oral administration of microspheres and W/O emulsion. When S/O/W emulsion was administered, tegafur took 2 hr to reach peak plasma levels, and the release of tegafur from S/O/W emulsion was sustained in comparison with other formulations. From the concentration-vs.-time curves, we obtained the pharmacokinetic parameters using the noncompartment method. Some pharmacokinetic parameters are shown in Table 1. Here, AUC, AUMC, and MRT are the area under curve, the area under the moment curve, and mean residence time, respectively. In comparison with other control groups, the MRT for tegafur in S/O/W emulsion was significantly ($p < 0.05$) increased. The MRT of a drug provides a useful estimate of its persistence time in the body. This increase in MRT with S/O/W emulsion may result from the slow release of tegafur from S/O/W emulsion. Figure 5 shows the release profiles of 5-FU, and Table 2 shows some pharmacokinetic parameters obtained from these data. As shown in these results, there was a similar tendency of release of 5-FU with tegafur. These results, indicate that S/O/W emulsion is useful for the sustained release of tegafur.

Table 2

Pharmacokinetic Parameters of 5-FU

Type	AUC	AUMC	MRT
S/O/W	0.72 ± 0.14	18.2 ± 0.31	25.3 ± 3.5
Microsphere	0.58 ± 0.12	12.5 ± 0.28	21.6 ± 2.8
W/O	0.54 ± 0.08	10.3 ± 0.25	18.7 ± 2.6

Table 1

Pharmacokinetic Parameters of Tegafur

Type	AUC	AUMC	MRT	CL/F
S/O/W	122.4 ± 8.3	1892 ± 415	15.5 ± 2.8	0.0099 ± 0.0010
Microsphere	86.5 ± 14.4	746 ± 266	8.1 ± 1.5	0.0151 ± 0.0024
W/O	118.3 ± 12.2	1096 ± 365	9.2 ± 2.1	0.0114 ± 0.0016

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