

MODIFIED SUSTAINED RELEASE CHITOSAN-COATED ZIDOVUDINE MICROSPHERES

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ABSTRACT

Zidovudine microspheres (MS) were prepared by dry-in-oil method using ethylcellulose (EC) as a matrix polymer, Further, the microspheres modified by addition of polyethylene glycol (PEG) and hydroxypropyl cellulose (HPC), called MS-P and MS-H, respectively, were prepared. The *in-vitro* release from MS, MS-P and MS-H were examined in phosphate buffer, pH 6.8, at 37°C and 60 rpm. Chitosan-coated zidovudine microspheres (Chi-MP) were prepared by the precipitation of droplets of chitosan solution containing MS, and their adhesion to the goat small intestinal mucosa was tested. The plasma concentrations after duodenal administration were investigated for zidovudine powder suspension, MS, and Chi-MP. It is observed that the particle size was raised with the increase in amount of zidovudine added. The drug content and addition of PEG or HPC affected the drug release rate. The microspheres with moderate drug content, prepared by addition of modest amount of PEG, exhibited better gradual drug release. Chi-MP showed a good mucoadhesion. The maximum plasma concentration of zidovudine for Chi-MP was less than one third of that for zidovudine powder suspension. Chi-MP tended to show the higher and steadier plasma levels than MS.

Keywords: Zidovudine, Chitosan, Microspheres, Incorporation efficiency.

INTRODUCTION

Microencapsulation is defined as the application of a thin coating to individual core materials that have an arbitrary particle size range from 5 to 5000 nm. It is used to modify and retard drug release^{1,2}. Microencapsulation may improve the absorption of a drug and reduce side effects such as irritation of the gastrointestinal mucosa. Zidovudine is widely used for the treatment of Acquired Immuno Deficiency Syndromes (AIDS) and related conditions, either alone or in combination with other antiviral agents. This virustatic drug has low oral bioavailability (60%) due to considerable first-pass metabolism, thus necessitating frequent administration of large doses (200 mg every 4-6 h) to maintain therapeutic drug level^{3,4}. However, patients receiving zidovudine frequently develops anemia and leucopenia. The side effects of zidovudine are dose dependent and a reduction of the total administered dose reduces the severity of the toxicity. Thus the short half-life of 1 h and frequent dosing of large doses due to low oral bioavailability makes zidovudine a good candidate for microencapsulation^{5,6}.

Thus, in the present study, the sustained release microspheres of zidovudine have been developed using ethylcellulose (EC), which is often, utilized as a matrix for preparation of prolonged release dosage forms. Since localization and retention of the drug to the absorption site are known to influence the absorption, chitosan coating, which was reported to enhance the accessibility and localization to the absorptive membrane via bioadhesion, has been further applied^{7,8}. The drug absorption after duodenal administration has been compared among zidovudine suspension, simple microspheres and Chi-MP.

MATERIALS

Zidovudine was procured from Auspure Biotechnology Co.Ltd, Ethylcellulose (EC; 49% ethoxy, 100 cp grade) and polyethyleneglycol (PEG; MW 20,000) were obtained from Wako Pure Chemical Industries, Ltd. Chitosan was procured from Qingdao Jiaonan Bright Moon Seaweed Industrial Co. Ltd as a free gift sample. Hydroxypropylcellulose (HPC; type H) was purchased from Nippon Soda Co., Ltd. Sorbitan tristearate (SS-30) and sorbitan sesquiolate (SO-15) were purchased from Nikko Chemicals Co., Ltd. All other chemicals were of reagent grade.

METHOD OF PREPARATIONS

Zidovudine microspheres with EC (MS) were prepared as follows: EC (1.5 g) and zidovudine (0.9, 1.5 or 2.25 g) were dissolved in 25 ml of acetone. The solution was added drop-wise to 250 ml of liquid paraffin containing SS-30 at 2% (w/v) and stirred at 600 rpm and 20°C. The emulsion was stirred at room temperature

for 1 h, then at 35°C for 5 h and finally at 57°C for 1 h. Approx one hundred ml of n-hexane warmed at 55°C was added to the mixture, and filtered using a membrane with a pore diameter of 0.45 µm. The residue was washed with n-hexane warmed at 55°C to yield MS.

Zidovudine microspheres with PEG (MS-P) were prepared as follows

PEG (0.075, 0.15 or 0.3 g) was dissolved in 25 ml of acetone warmed at 35°C. After cooling the solution at 20°C, 1.5 g of zidovudine and 1.5 g of EC were added. The subsequent procedures were the same as those described for MS.

Zidovudine microspheres with HPC (MS-H) were produced as follows: HPC (0.075, 0.15 or 0.3 g) was added to 25 ml of acetone at 20°C, and stirred vigorously at 14000 rpm for 1 min. Then, 1.5 g of zidovudine and 1.5 g of EC were added to the suspension, and dissolved. The subsequent procedures were the same as described for MS.

Coating of Chitosan on Zidovudine loaded microspheres

Chitosan-coated zidovudine microparticles (Chi-MP) were prepared by coating MS with Chitosan. MS prepared from the solution of EC (1.5 g) and zidovudine (1.5 g) in 25 ml of acetone was used. Chitosan (100 mg) was dissolved in 5 ml of 2% (v/v) acetic acid aqueous solution. MS (100 mg) was suspended in the solution, and added drop-wise to 20 ml of liquid paraffin containing SO-15 at 1% (w/v) and stirred at 600 rpm. The suspension was added drop-wise to 500 ml of double solvent layers of n-hexane/1 M NaOH (2 : 3, v/v) and stirred at 300 rpm. One min later after end of dropping, the particles precipitated in 1 M NaOH aqueous layer were collected by filtration using mesh (opening 150 µm), washed with 500ml of water, and dried in a desiccator under vacuum at room temperature to produce Chi-MP.

RESULTS AND DISCUSSION

Particle Characteristics and Bioadhesion of Chi-MP:

The microspheres (MS, MS-P, MS-H and Chi-MP) were characterized such as particle size, true density, tapped density, compressibility index and flow properties. Dried samples of microspheres were mounted onto stubs using double sided adhesive tape and vacuum coated with gold film using a polar vapour coater unit^{9,10}. Then all samples were examined for micro structural observation in Scanning Electron Microscope (Model HITACHI S.415A) Fig.1. Optical microscopy was also used to evaluate the quality of the coating obtained under the various conditions used and the mean particle size was calculated by measuring 200–300 particles with the help of a calibrated ocular micrometer¹¹.

Table 1: Particle characteristics of MS, MS-P, MS-H and Chi-MP prepared by various formulations

Particle type	EC (g)	Zidovudine (g)	PEG (g)	HPC (g)	Mean particle diameter (µm)	Drug content (% w/w)	Incorporation efficiency (% w/w)	Formulation No.
MS	1.5	0.9	-	-	12.2	7.90	21.1	1
	1.5	1.5	-	-	17.4	14.3	28.3	2
	1.5	2.25	-	-	18.3	33.1	36.7	3
MS-P	1.5	1.5	0.075	-	18.6	12.4	25.4	4
	1.5	1.5	0.150	-	18.7	14.7	30.8	5
	1.5	1.5	0.300	-	19.2	13.6	29.9	6
MS-H	1.5	1.5	-	0.075	18.8	15.3	31.2	7
	1.5	1.5	-	0.150	20.9	16.7	35.3	8
	1.5	1.5	-	0.300	20.4	17.1	37.6	9

For Chi-MP, the drug content was 11% (w/w), the content of FTC-Chi was 30% (w/w). Therefore, zidovudine was hardly lost in the coating process. The particle size was approximately 200 µm, and ten or more MS were contained in one particle of Chi-MP. The adhesive process of Chi-MP to the goat intestinal mucosa is shown in

Fig. 2. Chi-MP exhibited quick adhesion to the mucosa. The decrease in the ratio of adhering Chi-MP at 50 min after the start of the test was considered due to dissolution and exfoliation of part of the mucosa. This suggested that Chi-MP should have a fairly good adhesion to the intestinal mucosa.

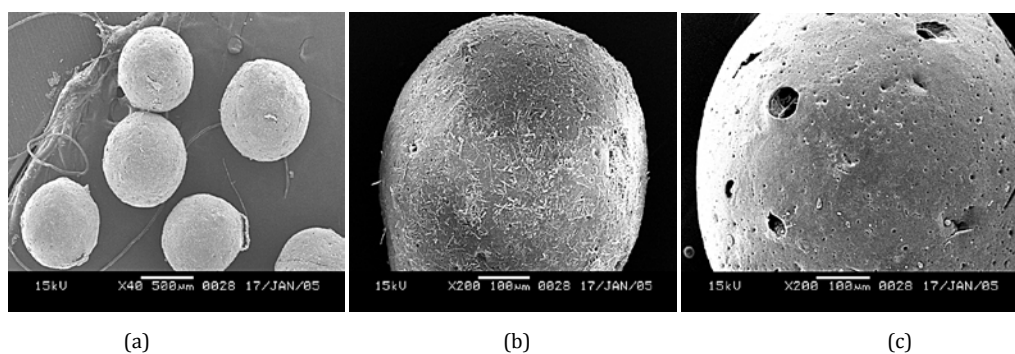


Fig. 1: SEM Photograph: (a) Chi-MP blank microspheres. (b) Zidovudine loaded Chi-MP microspheres and (c) Zidovudine loaded Chi-MP microspheres after dissolution.

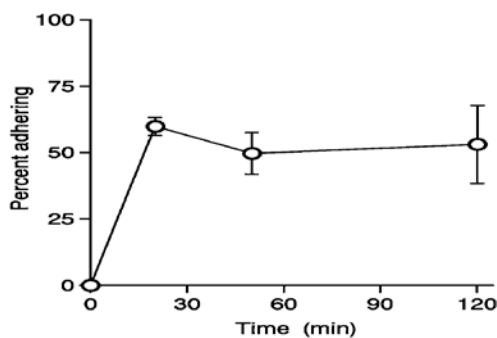


Fig. 2: In Vitro Bioadhesion of Chi-MP to the goat Intestinal Mucosa

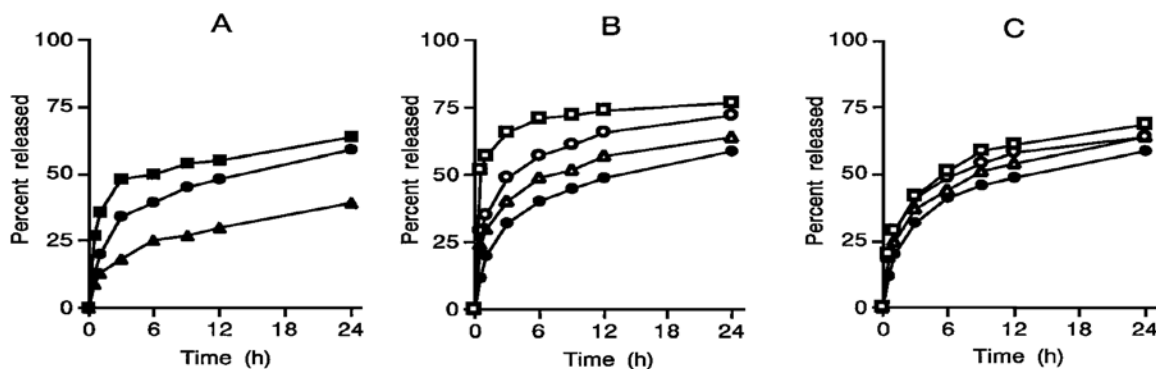


Fig. 3: Release Profiles of Zidovudine from MS (A), MS-P (B) and MS-H (C) Prepared at Different Formulations in the JP XIII Second Fluid at 37°C

[The results are for the formulations described in Fig. 3: Formulations 1 (▲), 2 (●) and 3 (■) in Fig. 3.A, Formulations 2 (●), 4 (△), 5 (○) and 6 (□) in Fig. 3.B, Formulations 2 (●), 7 (△), 8 (○) and 9 (□) in Fig. 3.C.]

Drug release characteristics

The drug release from MS, MS-P and MS-H was shown in Fig. 3. For MS, the percentage released was raised with increase in the drug content^{12,13}. Addition of PEG increased the percentage release for 24 h; especially, the initial release was enhanced by PEG. Since PEG is water-soluble, zidovudine incorporated near PEG was considered to be released fast following hydration or dissolution of PEG. The addition of HPC tended to raise an overall release, but its influence was not marked. This observation was probably because hydration and subsequent dissolution of HPC took more time than those of PEG.

CONCLUSION

Zidovudine microspheres were prepared by the dry-in-oil method using ethylcellulose as a matrix polymer and allowed prolonged drug release. The release rate could be modified by addition of polyethylene glycol or hydroxypropyl cellulose. Zidovudine microspheres could be coated easily with chitosan by the precipitation technique using double solvent layers of n-hexane-alkaline aqueous solution. The obtained chitosan-coated zidovudine microparticles showed good intestinal adhesion in vitro and steady maintenance of plasma concentration in in-vivo test. Ethylcellulose induced sustained release and bioadhesion by chitosan might be useful to facilitate sustained action of zidovudine. It has been reported that chitosan coating shows the bioadhesion to the intestinal mucosa, which allows strong and close contact with mucosal membrane and localization of particles at sites showing good absorption, resulting in enhancement of drug absorption. These properties may facilitate zidovudine absorption in Chi-MP; the good bioadhesive property was observed in vitro (Fig.3). However, it will be required for more detailed evaluation to further examine how chitosan coating modifies absorption of the drug.

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