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Research Paper

Preparation, characterization and taste-masking properties of microspheres containing azithromycin

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Abstract

Objectives The aim of this study was to prepare a microsphere formulation in order to mask the bitter taste of azithromycin.

Methods Microspheres of azithromycin with ethyl cellulose were prepared by the modified solvent diffusion method. The microspheres were mixed with other excipients to form orally dry suspensions and the sensory test for taste masking was evaluated.

Key findings Results demonstrated that the suspension could significantly mask the bitter taste of azithromycin and the relative bioavailability of suspensions to reference preparations was 102.7%.

Conclusions The results indicate that the microsphere formulation can be a promising drug carrier for masking the bitter taste of azithromycin.

Keywords azithromycin; microspheres; solvent diffusion method; taste masking

Introduction

Azithromycin (AZI) is a semisynthetic macrolide antibiotic with a 15-membered azalactone ring. It binds to 50S ribosomal subunits of susceptible bacteria and suppresses protein synthesis.[1,2] It is effective against a variety of Gram-positive and Gram-negative bacteria, but has an unpleasant taste.[3,4] This bitter taste greatly restricts the further development of oral preparations of azithromycin and hence its clinical applications, as well as decreasing patient compliance, especially where infants, children and the elderly are concerned.

Microencapsulation techniques have widely been used for taste masking. The materials used to coat the drug particles create a physical barrier and enhance the stability of the particles. There are many masking techniques described in the literature,[5,6] such as adding flavours or sweeteners, coating with polymers by spray techniques,[7–9] absorption to ion-exchange resins,[10,11] microencapsulation with various polymers,[12–15] and inclusion complexes with cyclodextrins.[16,17]

In this study, in order to mask the bitter taste of azithromycin, the drug microspheres were prepared with ethyl cellulose as a taste-masking material using the modified solvent diffusion method. The drug loading, particle size encapsulation efficiency, and drug release from the microspheres were investigated.

Materials and Methods

Materials

Crude azithromycin (AZI) was supplied by the JiangSu Hai Guang Company Ltd. (JiangSu, China). AZI suspensions were purchased from Ouyi Pharmaceutical Co Ltd (ShiJiaZhuang, China). Its standard was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Ethyl cellulose (EC, 20 cp) was kindly supplied by Colorcon Ltd. (Shanghai, China). Ethyl acetate was obtained from Tianjin Xintong Fine Chemicals Ltd (Tianjin, China) and was used as the polymer solvent. Sodium dodecyl sulphate (SDS) was supplied from Shentai Chemical Reagent Co. Ltd (Tianjin, China). All the solvent and chemicals were of analytical grade.

Preparation of microspheres

All microspheres were obtained by the solvent diffusion method. The optimized composition of the formulation was in the ratio of AZI : EC : ethyl acetate (1 g : 0.5 g : 10 ml, m/m/v).

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At first, the drug and EC were codissolved in ethyl acetate as the oil phase. 0.5 g SDS was dissolved in 100 ml distilled water, which was presatured by ethyl acetate as the aqueous phase. The oil phase was slowly injected, via a syringe, into the water phase while agitating. The system was stirred continuously for about 15 min, and the droplets gradually solidified and formed microspheres. Then, the system was filtered to separate the microspheres from the aqueous phase. The resultant product was washed with distilled water and dried in an oven at 37°C for 2 h. The microspheres were obtained using the optimized process parameters with an expected drug release percentage. The whole process was carried out at room temperature.

Microsphere characterization

Particle size

Particle size of the AZI microspheres was determined by an AccuSizer780/DPS dry power particle size analyser (PSS Ltd, Santa Barbara, CA, USA) before they were dispersed in deionized water.

X-ray analysis

An X-ray diffractometer (DX-2500, DanDong FangYuan Equipment Company Limited, China) was employed to study the crystallinity of the drug in the microspheres. The X-ray copper target tube was operated at 40 kV and 250 mA. The scan time was 6°/min and the scan scope was 0–50°.

Drug loading and encapsulation efficiency

The samples were assayed by a HPLC method. The HPLC method was performed on an Agilent 1100 (Palo Alto, CA USA) system with a UV detector, A Diamonsil C18 column (250 mm × 4.6 mm i.d., 5 μm) was used. The mobile phase comprised 30 mM potassium dihydrogen phosphate (pH 7.0) and acetonitrile (70 : 30, v/v) at a flow rate of 1 ml/min. The detector wavelength was 210 nm.

The drug loading and the encapsulation efficiency of the microspheres were determined using the HPLC method described above, and calculated using Equations 1 and 2 respectively. For each batch of the microspheres, a quantity of 30 g was taken and ground to fine powder. About 300 mg powder was then accurately weighed and added to a 100 ml volumetric flask containing 70 ml of mobile phase. After 30 min of ultrasonic extraction, the solution was diluted with mobile phase to 100 ml and then filtered through a 0.45 μm membrane. Twenty microlitres was injected for drug content analysis.

$$\text{Drug loading} = \frac{\text{weight of drug in microspheres}}{\text{weight of microspheres}} \times 100\% \quad (1)$$

$$\text{Encapsulation efficiency} = \frac{\text{weight of drug in microspheres}}{\text{theoretical drug content}} \times 100\% \quad (2)$$

In-vitro drug release studies

The drug release of the microspheres was performed in triplicate using the Chinese Pharmacopeia 2005 apparatus type II (paddle method). The microspheres were incubated in 900 ml of phosphate buffer (pH 6.0) and at 37 ± 0.5°C and the rotation speed was 100 rev/min. The quantity of the microspheres was chosen to be equivalent to 100 mg azithromycin. Five millilitres of medium was withdrawn and filtered at each time interval, and then the same volume of fresh dissolution medium was added. Then 5 ml H_2SO_4 solution (75 ml H_2SO_4 diluted to a volume of 100 ml with distilled water) was added to the sample solution as the colour-developing reagent. After 30 min of coloration, the solution was assayed by UV spectrophotometry at a wavelength of 482 nm.

Preparation of the azithromycin suspension

The AZI and excipients for 100 bags were weighted according to the ingredients listed in the following: AZI microspheres 15.3 g (equvalant to 10 g AZI), sucrose 175.7 g, HPMC 5.0 g, citric acid 1.5 g, gum acacia 1 g, silicon dioxide 1 g and orange flavour 1 g. The materials mentioned above were screened by 100# mesh and blended to uniformity. An exactly weighed quantity of the powder mixture was filled into a pouch and packaged.

Taste-masking evaluation

A single-blind study was designed for a taste-masking test in the buccal cavity. The experiment was approved by the Medical Ethics Committee of the Affiliated Hospital of Hebei University, China. Self-made suspensions (F-1), AZI reference suspensions (F-2) and AZI drug powder (F-3) were taken by 10 volunteers. Each volunteer held a quantity of the product equivalent to 100 mg AZI in the mouth for 30 s and then the mouth was thoroughly rinsed with distilled water and the bitterness was evaluated. They were asked to rate the bitter taste of the three formulations using a scale of 1–4. When the score was 2 or less, the taste was considered as acceptable. If the score was higher than 2, the bitterness of the formulation was not acceptable.[18]

Bioavailability study

Twelve healthy adult male volunteers between 21 and 23 years old (mean, 22.1 years; SD, 0.7 years) and weighing from 57 to 71 kg (mean, 62 kg; SD 6.2 kg) participated in the bioavailability study after providing written informed consent. The Medical Ethics Committee of the Affilited Hospital of Hebei University, China approved the study protocols. The study was performed according to Good Clinical Practice and International Conference on Harmonization guidelines. The volunteers were judged to be healthy and were not receiving any medication during the study period. Volunteers were given information on the drug and nature of the study in advance of the trial. The study was conducted according to a single-dose, randomized, two-way crossover design and the washout period was 1 week between treatments. In this design, the volunteers were randomly selected to receive a single-dose of self-made suspensions (F-1) or commercial AZI reference suspensions (F-2) (both equivalent to 100 mg AZI) in the morning and after overnight fasting (10 h) with 240 ml of water. Blood samples (4 ml) were collected into

vacutainers (containing sodium heparin as an anticoagulant) at 0, 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 24 h after dosing. An indwelling cannula placed in the forearm was used for drawing blood during the first 12 h. The last sample was taken by direct venipuncture. Following centrifugation, the plasma was pipetted into polypropylene tubes, frozen immediately and stored at –20°C until analysis.

Plasma samples were analysed using a validated liquid chromatography/mass spectrometry (LC/MS) method. AZI was extracted from plasma by liquid–liquid extraction. A 200 μl aliquot of erythromycin (internal standard, 250 ng/ml) in 50% methanol (v/v) was added to 200 μl of plasma. The sample was alkalinized with 100 μl of 0.1 M sodium carbonate solution and a 4.0 ml aliquot of diisopropyl ether was then added. The samples were centrifuged and the organic phase (upper layer) was transferred to a new tube, evaporated under nitrogen in a 30°C water bath, reconstituted in 100 μl of reconstituting solution (90% 0.02 M acetic acid/10% methanol) and vortexed, followed by centrifugation. Fifty microlitres was injected into a LC/MS system with a C_8 Rapid Resolution (4.6 × 50 mm, 3.5 μm) column. The mass spectrometer was operated in SIM mode and monitored positive ions for AZI (*m/z* 749.40 and 750.40). The range of the plasma assay was 10.0–1000 ng/ml.

Statistical analysis

All data were expressed as mean ± SD. The data from the taste-masking study were statistically analysed using the Chi squared test. The effect of formulation and time on drug release was statistically analysed using ANOVA. A significance level of $P < 0.05$ denoted significance in all cases.

Results and Discussion

The particle size distribution and mean particle size are shown in Table 1. The mean diameter was about 26 μm. Drug loading ranged from 55.7 to 56.8% and encapsulation efficiency was about 83%. The high content of AZI in the microspheres was due to the poor solubility of the drug in water. This suggested that the present method is suitable for the preparation of microspheres of AZI.

The result of X-ray diffraction showed that the pure drug and a mixture of the drug and EC (Figure 1A and B) exhibited crystalline properties, while EC and the microspheres (Figure 1D and C) displayed an amorphous pattern. The peaks of azithromycin were absent or weak in the case of the microspheres. This proved that the drug was changed in part into an amorphous form in the microspheres.

Table 1 Mean diameter, incorporation efficiency and drug loading of microspheres

Batch number	Mean diameter (μm)	Drug loading (%)	Encapsulation efficiency (%)
1	26.5 ± 1.2	55.7 ± 1.0	82.6 ± 2.3
2	25.9 ± 0.9	56.2 ± 1.1	82.3 ± 2.9
3	26.3 ± 1.3	56.8 ± 0.9	83.4 ± 2.6

Values are mean ± SD ($n = 5$).

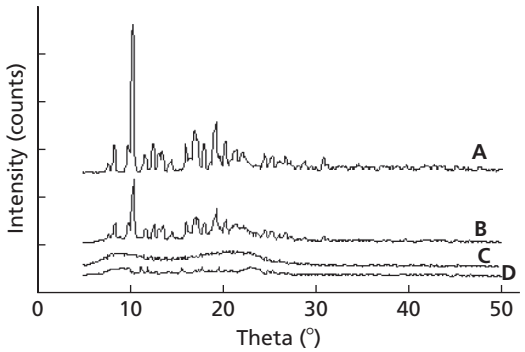

Figure 1 X-ray diffractograms. A, azithromycin; B, mixture of azithromycin and ethyl cellulose; C, azithromycin microsphere; D, ethyl cellulose.

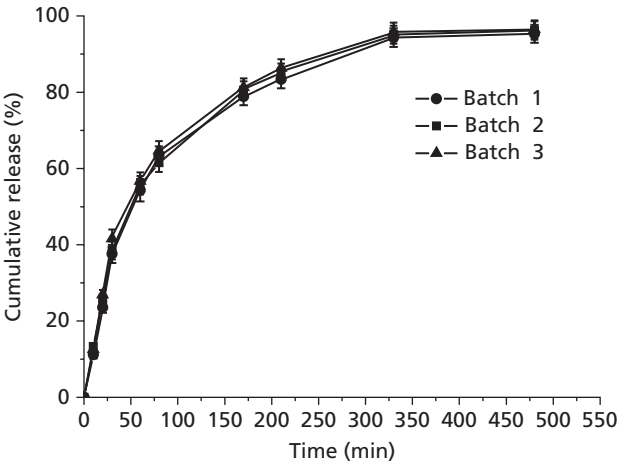

Figure 2 In-vitro release profiles from microspheres produced by optimal formula. Values are mean ± SD ($n = 3$).

In-vitro drug release of three batches of microspheres prepared by the optimal formula is shown in Figure 2. Drug release was complete and the cumulative amount of drug released was above 90% after 8 h.

The mechanism of AZI release from the microspheres was studied by fitting the dissolution data obtained to distinct models: (1) zero-order, (2) first-order, (3) Higuchi and (4) Korsmeyer–Peppas. Goodness-of-fit analysis applied to release data showed that the release mechanism was described by the Higuchi model, indicating that the release was consistent with a diffusion-controlled mechanism.

The results of the taste-masking test are shown in Table 2. The results indicate that self-made suspensions (F-1) can mask the bitter taste of AZI completely ($P < 0.05$). AZI reference suspensions (F-2), made by adding flavours or sweeteners, cannot do so. AZI drug powder (F-3) was bitter.

The plasma concentration–time profiles following oral administration of self-made suspensions (F-1) and AZI reference suspensions (F-2) are illustrated in Figure 3. Pharmacokinetic parameters are listed in Table 3.

Table 2 Results of taste test

	Score of taste test					
	F-1		F-2		F-3	
	Number of people	Score	Number of people	Score	Number of people	Score
No bitter taste	2	2	1	1	0	0
Slightly bitter	8	16*	3	6	1	2
Moderately bitter	0	0*	6	18	5	15
Bitter	0	0	0	0	4	16
Mean Score		1.6*		2.5		3.3

*$P < 0.05$, significant difference compared with F-2 ($n = 10$). Score: 1, no bitter taste; 2, slightly bitter; 3, moderately bitter; 4, bitter.

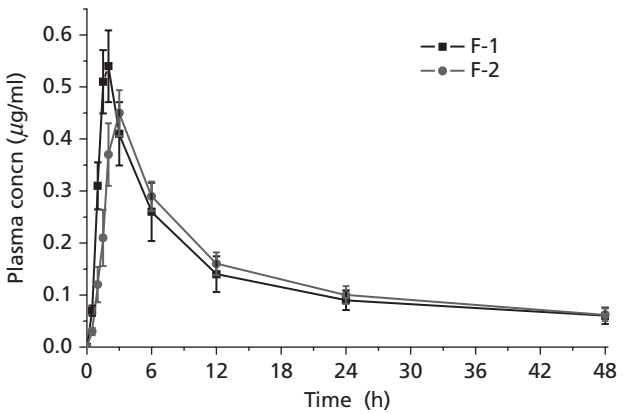

Figure 3 Plasma concentration–time profiles of azithromycin following oral administration. Values are mean ± SD ($n = 12$).

Table 3 Pharmacokinetic parameters of commercial suspension and self-made suspension

Pharmacokinetic parameters	Self-made suspension	Commercial suspension
c_{max} (mg/l)	0.48 ± 0.09	0.56 ± 0.03
t_{max} (h)	2.8 ± 0.39	1.8 ± 0.26
$AUC_{0-48\ h}$ (μg·h/ml)	7.24 ± 1.19	7.10 ± 1.28
$AUC_{0-\infty}$ (μg·h/ml)	7.51 ± 1.31	7.30 ± 1.66
F (%)	102.7 ± 9.5	

Values are mean ± SD ($n = 12$).

The plasma concentration of the reference suspensions rose quickly and the maximum concentration (0.56 mg/l) was reached 1.8 h after administration. There was a marked fall in plasma concentration between 3 and 12 h. For self-made suspensions, the maximum concentration (0.48 mg/l) was reached 2.8 h after administration, but it was lower than that of the reference suspensions. The extended t_{max} might be attributed to the in-vitro sustained release of the drug from the microspheres. Compared with reference suspensions, the relative bioavailability of F-1 was found to be about 102.7%, showing that complete absorption occurred *in vivo*.

Conclusions

Azithromycin microspheres with polymers were prepared successfully by the solvent diffusion method. The encapsulation efficiency was very high for the microspheres obtained. In-vitro release tests showed that the microspheres exhibited sustained release. The X-ray diffractograms showed that the drug existed in part in an amorphous state in the microspheres. The suspension prepared by microspheres can effectively mask the bitter taste of the drug and it had a relative bioavailability of 102.7% compared with the reference preparation.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Acknowledgements

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