Asian Journal of Pharmaceutical Sciences 13 (2018) 343–352

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Original Research Paper

Creation of an assessment system for measuring the bitterness of azithromycin-containing reverse micelles

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ARTICLE INFO

Article history:
Received 13 December 2017
Accepted 22 February 2018
Available online 16 March 2018

Keywords:
Azithromycin
Taste masking
Taste assessment
Pharmacokinetics

ABSTRACT

We aimed to develop a novel method for assessing the bitterness of azithromycin-containing reverse micelles (AM-containing RMs). Azithromycin-containing reverse micelles were prepared by processing Lipoid E80 and medium chain triglycerides via a freeze-drying method. The bitterness threshold of azithromycin was determined by human taste test, and an equation was derived to correlate the azithromycin concentrations and bitterness scores of standard solutions. Simulated salivary fluids and sampling times were fixed based on the drug release profile of AM-containing RMs, with Zithromax® (a commercial formulation of azithromycin) used as the control. The drug release concentrations from stimulated salivary fluids were then used to assess the bitterness of AM-containing RMs and Zithromax®. Afterward, the oral bioavailability of both formulations was evaluated by *in vivo* experiments in male Wistar rats. The results showed that the bitterness threshold of azithromycin standard solutions was between 25.3 μg/ml and 30.4 μg/ml. Thereafter, we calculated that the bitterness scores and the drug release concentrations of the azithromycin-containing reverse micelle formulation were similar to those of Zithromax® at each time point after 10 min of dispersal in simulated salivary fluid. In addition, the AUC_{0-t} after oral administration of AM-containing RMs was 1.75-fold ($P < 0.05$) higher than that of Zithromax®. In conclusions, a system for assessing bitterness was developed using an *in vitro* drug release evaluation method and a human taste test panel. We found that the bitterness

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https://doi.org/10.1016/j.ajps.2018.02.001

of azithromycin was successfully masked by reverse micelles, which also improved the oral bioavailability of azithromycin compared to that of Zithromax®.

1. Introduction

The taste of medicines plays an important role in patient acceptability and compliance, especially in children [1–2]. Thus, various taste-masking techniques and formulations are used to reduce or eliminate bitterness by obscuring the unpleasant taste of drugs or preventing dissolved drugs from interacting with taste receptors [3–7]. These can be roughly divided into physical, chemical, and physiological methods [1–2]. In the physical barrier method, polymeric and lipid coatings, ion exchanges resins, and cyclodextrins are used to create a molecular or physical barrier around/on the active pharmaceutical ingredient or dosage form. This reduces the concentration of bitter substances in the oral cavity by binding the compounds to an excipient or by entrapping them in a particulate to prevent their release [8–10]. The chemical method involves modification of the active pharmaceutical ingredient by changing solubility (using salt, pH, etc.) or creating prodrugs. The physiological approaches include addition of sweeteners/flavoring agents and changes in viscosity to obscure taste, or by using a "taste blockade" to numb the taste buds.

Taste assessment is a key aspect of drug development and quality control for taste-masking preparations [8]. Human taste test panels are the most common method, but they are limited by safety and ethical issues, especially when testing potentially toxic drugs [1]. Thus, non-human tools for evaluating bitterness have been developed, including *in vitro* and *in vivo* taste evaluation methods such as electronic taste sensors, drug release methods, animal behavioral tests, and cell based assays [9,11–13]. To assess the efficacy of taste-masking preparation that use physical barriers, the *in vitro* drug release evaluation method should always be adopted, because it is easy to perform and does not require sophisticated instruments. In addition, solid preparations are suitable for use.

The drug release evaluation method involves measuring the amount of dissolved drug in simulated salivary fluids (SSFs) to analyze the taste of formulations. Although analytical methodologies for evaluating drug release have been used to screen taste-masking formulations [4,12], but mimicking the oral cavity is challenging because of its complex dynamic process (e.g., continuous production of fresh saliva). For instance, the use of larger dissolution media far exceeding normal volume for human saliva has been the most common bias in recent studies [1]. This may cause problems because information about the volume of dissolution medium and sampling time could affect the results of bitterness assessment [1]. On the other hand, *in vivo* drug release studies using human taste test panels have ambiguities regarding appropriate saliva withdrawal times and saliva dilution volumes for determining bitterness. Therefore, a system for assessing the bitterness of taste-masking formulations should combine data from drug release tests in simulated oral cavities and human taste test panels [3,8].

Azithromycin, a well-known highly bitter macrolide antibiotic, is a standard drug for treating pediatric bronchial pneumonia [14]. The use of sweetening and flavoring agents for obscuring its bitterness has not been successful (US 5633006). In this study, we developed a taste-masking formulation using a reverse micelle delivery system that had been used for oral delivery in previous reports [15–17]. The reverse micelles with polar cores and hydrophobic shells were structured by incorporating phospholipids into an oil phase via a freeze-drying method.

To reduce the ambiguities inherent in drug release studies that use human taste test panels, we created a system for assessing bitterness based on *in vitro* and *in vivo* experiments. We demonstrated the method for creating this system by testing the bitterness of the azithromycin-containing reverse micelles (AM-containing RMs), with Zithromax® (100 mg, Pfizer; a commercial product of azithromycin) as a control. Bitterness thresholds and bitterness scores were obtained from a human taste test panel, and drug release evaluations were performed in SSFs. Successful taste-masking was concluded if the drug release concentration was lower than the bitterness threshold of azithromycin.

2. Materials and methods

2.1. Materials

Azithromycin was gifted by Sichuan Jiangchuan Pharmaceutical Co., Ltd. (Sichuan, China). Roxithromycin was obtained from Dalian Meilun Biological Technology Co., Ltd. (Liaoning, China). Medium-chain triglycerides were provided by Tieling North Asia Pharmaceutical Co., Ltd. (Liaoning, China). Egg phosphatidylcholine came from Lipoid E80 (Ludwigshafen, Germany). Distilled water was self-prepared. The solvents used for the mobile phases were high performance liquid chromatography grade. All other chemicals were of analytical grade.

2.2. Preparation of reverse micelles

Azithromycin-containing reverse micelles were prepared using a freeze-drying method. Phospholipid dispersion was achieved by adding Lipoid E80 to water to a make a concentration of 40 mg/ml. The mixture was homogenized with a high pressure homogenizer (MS1001L, PANDA) as follows: it was homogenized twice at 600 bar, then homogenized five times at 1300 bar in an ice/water bath that maintained the temperature under 25 °C. Azithromycin was dissolved in water to give

an initial drug concentration of 3 mg/ml. Then, it was added dropwise to an equivalent volume of phospholipid dispersion solution. Afterward, the mixed solution was placed in a high-speed disperser (T25 digital, IKA) at 3000 rpm for 5 min and immediately transferred into 7 ml freeze drying vials with a fill volume of 2 ml. This was frozen and maintained at below –60 °C for 2 h in a freeze dryer (LGJ-25C, Sihuan).

The frozen samples were lyophilized using the following procedure: primary drying was performed by keeping the blisters at a shelf temperature of 10 °C for 14 h and then 5 °C for 2 h; secondary drying was accomplished by increasing the shelf temperature to 20 °C and drying for 14 h. The chamber pressure was maintained below 10 Pa during the drying process. When the freeze-drying process was completed, the vials were sealed and stored at −20 °C. Azithromycin-containing reverse micelles were obtained after 500 μl of medium chain triglycerides was added to the vial of lyophilisates.

2.3. Morphology of reverse micelles

The morphology of the reverse micelles was elucidated using transmission electron microscopy (JEM-1230, JEOL). Samples were prepared using the following procedure: the freeze-dried powders were diluted by n-Heptane to a concentration of 1:1000. Then, 10 μl of this solution was dropped on a copper grid with carbon film. Afterward, 10 μl of phosphotungstic acid solution was added and the excess liquid was removed with filter paper. The copper grid was parched by air drying and then transferred to the transmission electron microscope for measurement.

2.4. Preparation of different azithromycin solutions

A series of aqueous solutions of azithromycin (5.1, 8.1, 15.2, 25.3, 50.6, 75.9, 101.2, 151.9, 202.5, 253.1, 303.7, and 404.9 μg/ml) were used for determination of the bitterness threshold. They were prepared using a stock solution of azithromycin (1010 μg/ml) as the standard solution. This stock solution was made by adding 101.0 mg azithromycin to 100 ml acetic acid aqueous solution (food grade) in a volumetric flask.

2.5. Determination of bitterness threshold

2.5.1. First phase of taste panel evaluation

The bitterness threshold value of azithromycin was determined by a human taste test panel. After a test for bitterness sensitivity [8], eight healthy adult human volunteers (five females and three males, 20–40 years of age, all nonsmokers) were selected and written consent forms were obtained. The protocol for the taste panel studies was approved by the Institutional Human Ethics Committee.

The test was performed as follows: the volunteers were asked to keep 5 ml of a standard solution on the back of their tongues for 15 s and then immediately record the bitterness score using a bitterness intensity scale of 1–4, where 1–4 indicate no, slight, moderate, and strong bitterness, respectively. Afterward, they thoroughly rinsed their mouths five times with purified water to remove all traces of bitterness. They then waited 5 min before repeating the test for each standard solution. The final results were an average of the scores from all volunteers.

2.5.2. Second phase of taste panel evaluation

Some volunteers sensed the bitterness threshold between 15.2 and 50.6 μg/ml azithromycin. A second phase of panel testing was conducted with a series of lower dilutions containing 10.1, 15.2, 20.2, 25.3, 30.4, and 50.6 μg/ml azithromycin using the same procedure as described in Section 2.5.1.

The bitterness threshold was defined as the lowest concentration of azithromycin that was recorded as being between tasteless and slightly bitter by the volunteers in the taste test panel.

2.6. Human taste test panel for Zithromax® and azithromycin-containing micelle formulations

The Zithromax® and azithromycin-containing reverse micelle formulations were made by dispersing the equivalent of 10 mg azithromycin in 20 ml of purified water. Volunteers evaluated the bitterness of each formulation using the procedure described in Section 2.5.1.

2.7. Correlation of azithromycin concentration to bitterness score

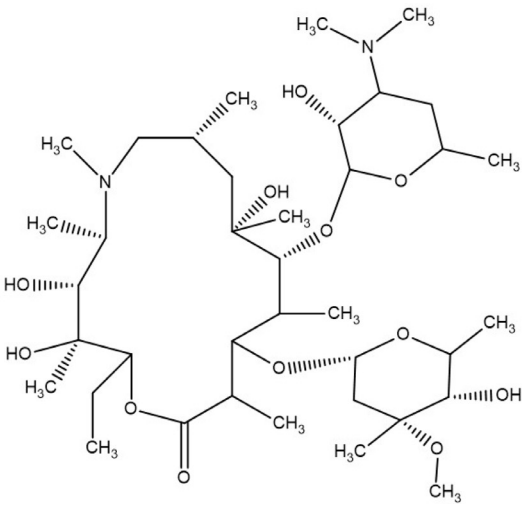
The concentrations and bitterness scores of standard solutions were correlated to establish a fit equation by using OriginPro software (version 8.6) to analyze linear, polynomial, allometric, and logistical fit. The bitterness scores of standard solutions were set as dependent variables, while their concentrations (C) or logistic values ($\log C$) were set as independent variables. An equation with a correlation coefficient approaching 1 was selected as the final equation for calculating the bitterness score of azithromycin concentrations.

2.8. Preparation of simulated salivary fluids

Two different SSFs were prepared to mimic human saliva [1]. The first solution (SSF 1) was prepared by dissolving potassium dihydrogen phosphate (0.534 g) in 120 ml of water and regulating the pH (6.8–7.2) using a hydrochloric acid and sodium hydroxide solution (1 mol/ml). The second solution (SSF 2) was prepared by dissolving 0.244 g of sodium chloride in 100 ml of water and regulating the pH (6.8–7.2) using a phosphoric acid and sodium hydroxide solution (1 mol/ml). Both formulations of SSF had the same osmotic pressure (0.05–0.100 osmol) as natural saliva, and both were sealed and stored at 4 °C while awaiting drug release experiments.

2.9. In vitro drug release studies

In vitro drug release studies were performed in a magnetic stirring apparatus (RCT basic, IKA), in which the release medium was stirred at 100 rpm and maintained at 37 °C. Zithromax® and AM-containing RMs (equivalent to 10 mg of azithromycin) were dispersed into 20 ml of each SSF formulation. The dissolution medium (200 μl) was withdrawn at specified time intervals (0, 5, 10, 15, 20, and 30 min) and centrifuged at 4000 rpm for 10 min. Afterward, 20 μl of supernatant fluid



Molecular formula : $C_{38}H_{72}N_2O_{12}\cdot 2H_2O$

Molecular mass : 785.0

Fig. 1 – The structure of azithromycin.

was obtained for analysis. These sample solutions were analyzed at 210 nm by high performance liquid chromatography (L-7100, HITACHI). The liquid chromatographic column was Shiseido C18 MGII (150 mm × 2.0 mm, 5 μm). The mobile phase consisted of 0.5 mol/ml dipotassium hydrogen phosphate solution (pH 8.2 regulated by using 20% phosphoric acid solution) and acetonitrile (40:60, v/v). The sample (20 μl) was injected into the column with the flow rate set at 1 ml/min. The column temperature was 30 °C.

2.10. *Bitterness assessment method*

The bitterness assessment method was developed using the fitting equation, bitterness scores, and azithromycin concentrations. The determined azithromycin concentrations (accumulative total value of released drug) in SSF and the calculated azithromycin concentrations from human taste test results were statistically analyzed using SPSS software (version 17.0) to compare their variability through *T* values. Then, according to those results, the optimal sampling time and appropriate SSF were determined.

2.11. *Pharmacokinetic studies*

Male Wistar rats (Animal Center of Academy of Military Medical Sciences, body weight 200 ± 20 g) were used for the animal studies. The experiment was reviewed and approved by the local academy's animal ethics committee. A standard feeding environment with proper diet and water was provided to the animals. They were randomly divided into two groups, with six rats in each group. After 12 h of fasting, each rat in Group 1 was administered 10 mg Zithromax®, and each rat in Group 2 was administered AM-containing RMs containing 10 mg of azithromycin. Blood (0.5 ml) from each rat was collected by puncturing the retro-orbital venous plexus at 0.25, 0.5, 1.0, 1.5, 2, 4, 6, 12, 24, 36, 48, 72, and 96 h after drug administration. The samples were collected in heparin-treated centrifuge tubes and centrifuged at 10 000 rpm for 5 min. Then, 100 μl of plasma from each sample was drawn and stored at −20 °C until analysis. The rat plasma samples were analyzed by liquid chromatography–mass spectrometry/mass spectrometry (API 3000 and Agilent 1100, AB Sciex and Agilent) using positive ion mode electrospray ionization, with roxithromycin as the internal standard. The liquid chromatographic column was Agilent Poroshell 120 C18 (50 mm × 2.0 mm, 2.7 μm). The mobile phase consisted of 0.1% formic acid solution and acetonitrile with the following gradients: 5%–90% acetonitrile for 0–1 min, maintenance at 90% acetonitrile for 1–1.5 min, and finally 5%–5% acetonitrile for 1.6–6.0 min. Plasma (10 μl) was injected into the column with the flow rate set at 0.3 ml/min. The column temperature was 25 °C. Using multi-reaction monitoring, we detected azithromycin and roxithromycin ion peaks were at *m/z* 749.6 to *m/z* 591 and *m/z* 837.8 to *m/z* 679.6, respectively.

The following plasma pharmacokinetic parameters were calculated using WinNonlin software (version 6.3): peak plasma concentration (C_{max}), time to reach C_{max} (T_{max}), the elimination half-life ($T_{1/2}$), area under the plasma concentration-time curve from time 0 until the last measured concentration (AUC_{0-t}), and AUC from time zero to extrapolated infinity ($AUC_{0-\infty}$). The pharmacokinetic parameters of Zithromax® and AM-containing RMs were statistically analyzed; and the parameters for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were analyzed for variance to obtain *F* values for determination of *P* values. The T_{max} was analyzed by nonparametric rank sum test.

3. Results and discussion

3.1. *Azithromycin reverse micelles*

Azithromycin (Fig. 1) is a widely known highly bitter drug, so much so that sweetening and flavoring agents used for obscuring its bitterness are not very effective. Patients such as children, the elderly, and those with difficulty swallowing require liquid oral preparations of azithromycin instead of tablets or capsules [8,18–19]. Although liquid formulations

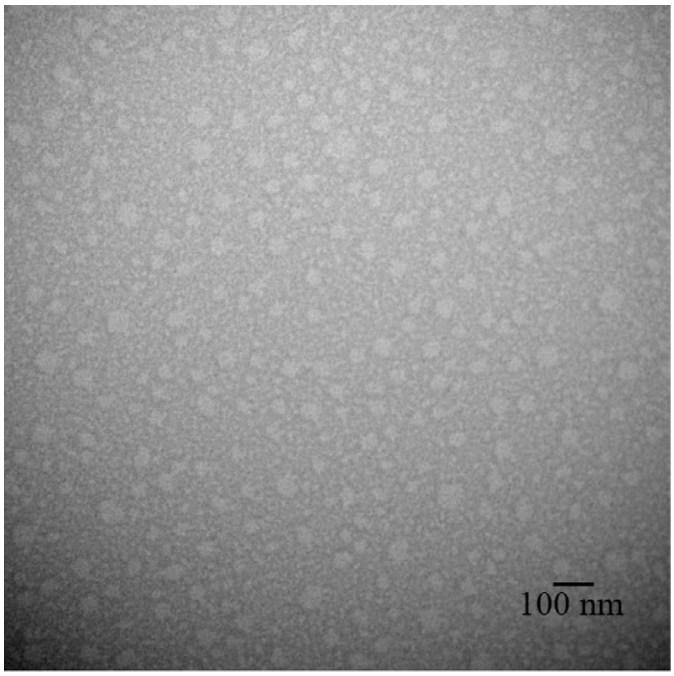


Fig. 2 – Transmission electron microscopy. Photographs of azithromycin reverse micelles.

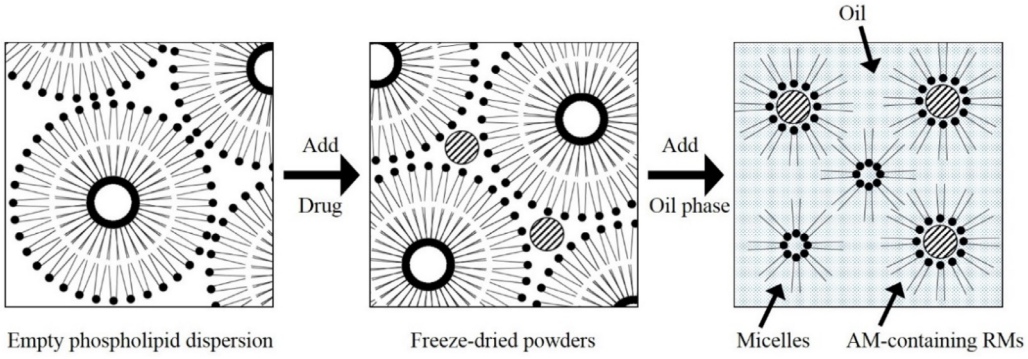


Fig. 3 – Preparation of AM-containing RMs using a freeze-drying method.

enhance dissolution, they also prolong drug contact with the oral cavity, making it more challenging to mask bitterness. One option for blocking bitterness is delaying the release of free drug into the oral cavity. For instance, azithromycin has pH-dependent solubility; it dissolves easily in acidic solutions (US 2005/0123627 A1). The converse is also true: an alkalizer in Zithromax® masks taste by reducing the solubility of azithromycin (US 2005/0123627 A1). However, that method does not effectively eliminate the bitter aftertaste.

In this study, we developed a taste-masking formulation using reverse micelles created by a freeze-drying method and photographs of these reverse micelles are shown in Fig. 2. The reverse micelles were spherically shaped and about 30–40 nm in size. In that preparation, azithromycin molecules were entrapped in the polar cores of reverse micelles that were dispersed in the oil phase. And, a schematic diagram of the procedure for preparing AM-containing RMs is shown in Fig. 3.

3.2. *Bitterness threshold and taste test*

All volunteers reported bitterness scores below 2 for standard solutions containing 25.3 μg/ml azithromycin, whereas the bitterness scores of standard solutions containing 30.4 μg/ml azithromycin were precisely over 2 (Table 1). Therefore, the bitterness threshold of the azithromycin standard solutions was between 25.3 and 30.4 μg/ml.

The taste test results for Zithromax® and AM-containing RMs are summarized in Table 2. None of the volunteers in the human taste test panel tasted any bitterness in either of the formulations.

3.3. *Correlation of azithromycin concentrations to bitterness scores*

The fitting results are shown in Table 3 and Fig. 4. A logistic equation (correlation coefficient R^2 of 0.99067) was derived

Table 1 – Bitterness scores of azithromycin standard solutions ($n = 8$).

Concentration (μg/ml)	Number of volunteers which score as				Mean ± SD
	1	2	3	4	
5.1	8				1.0 ± 0.0
8.1	8				1.0 ± 0.0
10.1	7	1			1.1 ± 0.4
15.2	7	1			1.1 ± 0.4
20.2	6	1	1		1.4 ± 0.7
25.3	5	2	1		1.5 ± 0.8
30.4		6	2		2.2 ± 0.5
50.6		5	2	1	2.5 ± 0.8
75.9			6	2	3.2 ± 0.5
101.2			5	3	3.4 ± 0.5
151.9				8	4.0 ± 0.0
202.5				8	4.0 ± 0.0
253.1				8	4.0 ± 0.0
303.7				8	4.0 ± 0.0
404.9				8	4.0 ± 0.0

Table 2 – Bitterness scores for azithromycin-containing reverse micelle and Zithromax® solutions ($n = 8$).

Preparation	Bitterness scores
AM-containing RMs	1.13 ± 0.35
Zithromax®	1.25 ± 0.46

as the relationship between bitterness scores and log of the azithromycin standard concentrations. This was used to calculate the bitterness scores from the azithromycin drug release concentrations of the Zithromax® and azithromycin-containing reverse micelle formulations.

3.4. *In vitro drug release studies of two different simulated salivary fluids*

Taste assessment is the quality control factor for taste-masking preparations. The procedure for establishing models for evaluating bitterness is shown in Fig. 5. In order to assess the bitterness of Zithromax® and AM-containing RMs formulations, an *in vitro* drug release taste evaluation method was developed using two different SSFs. Drug release profiles may vary with different SSFs, which represent the fluctuating content of saliva as a dynamic process [1]. Some researchers develop various SSFs to simulate pH, viscosity, inorganic ions, and proteins of natural saliva [20]. To mimic oral cavity conditions in our study, we focused on the buffering capacity of SSFs to represent the variable clearance ability of the oral cavity. While the buffering capacities of SSF 1 and SSF 2 differed, the pH and osmotic pressure of the SSFs were controlled at a constant range.

Bitterness is always in direct proportion to the concentration of drug solutions. Thus, our human volunteers recorded more bitterness with higher drug concentrations. In the *in vitro* drug release test, azithromycin was released slowly from AM-containing RMs dispersed in SSF 1 and SSF 2. In our study, the azithromycin in Zithromax® was quickly released in SSF 1 but slowly released in SSF 2 (Fig. 6). Only in SSF 2 were the drug release concentrations of Zithromax® and AM-containing RMs both below the bitterness threshold (25.1 μg/ml and 20.4 μg/ml, respectively) at terminal time. For that reason, we chose SSF 2 as the dissolution medium.

From this result, Zithromax® and AM-containing RMs manifested distinctly different dissolution profiles in the two SSFs. In this reverse micelles, azithromycin molecules were entrapped in the polar cores of reverse micelles that were dispersed in the oil phase. Azithromycin had to pass through two physical barriers (reverse micelles and oil phase) in order to dissolve in the oral cavity. The barriers were not pH-dependent, so the azithromycin-containing reverse micelle formulation did not leave a bitter aftertaste, a fact verified by our taste test volunteers.

Azithromycin released slowly from reverse micelles in both SSFs; whereas Zithromax® released quickly in SSF 1, but slowly in SSF 2. As we discussed earlier in this section, Zithromax® can mask the bitterness of azithromycin because it contains an alkalizer that reduces the solubility of azithromycin before swallowing. The stronger buffering capacity of SSF 1 could have made the alkalizer ineffective, leading to the rapid release of azithromycin. Meanwhile, SSF 2 had a weaker buffering capacity that allowed the alkalizer to slow the release of azithromycin. Contrasting the release profiles of Zithromax® in SSF 1 and SSF 2 led us to choose SSF 2 as the final dissolution medium. In SSF 2, the final drug release concentrations for the Zithromax® and azithromycin-containing reverse micelle formulations were below the bitterness threshold.

3.5. *A system for bitterness assessment*

After a logistic equation was chosen as the correlation equation and SSF 2 was selected as the dissolution medium, we compared variability. We found that after 10 min of dispersion in SSF 2, the determined and calculated concentrations of drugs released at different time points were similar ($P > 0.05$). In addition, the bitterness and drug release profiles of the AM-containing RMs formulation and Zithromax® were similar at

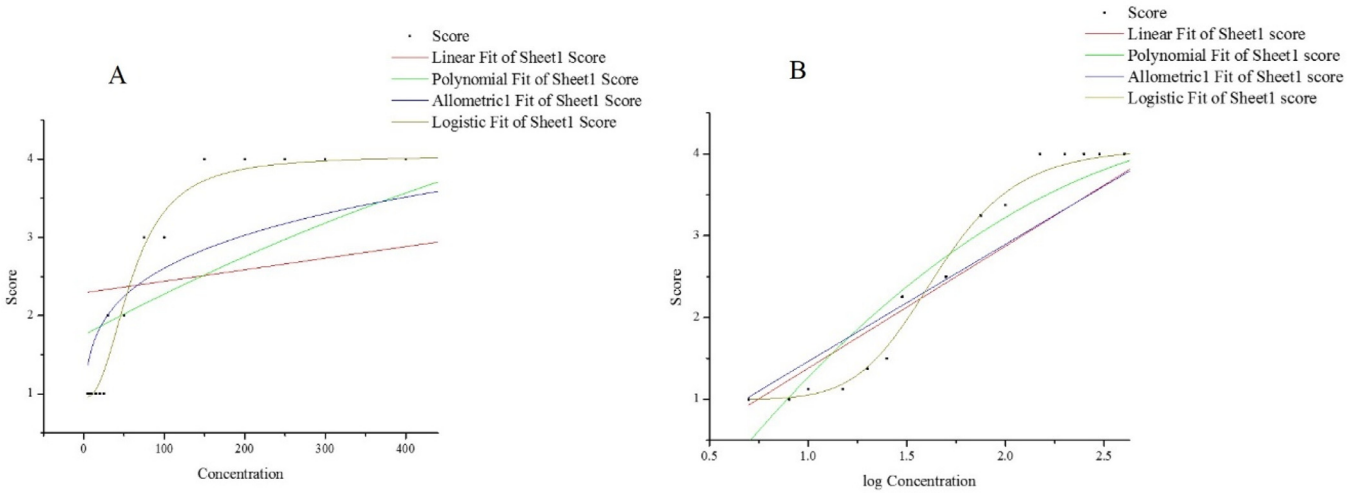


Fig. 4 – Different simulated models for equation fitting: (A) X = C; (B) X = log C.

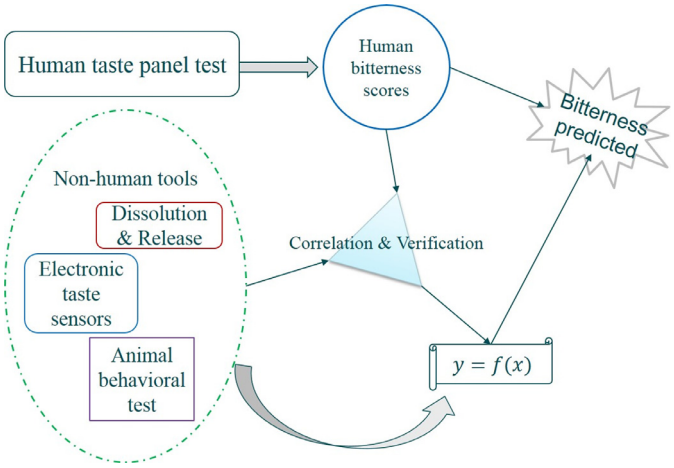


Fig. 5 – Procedure for establishing models for bitterness evaluation.

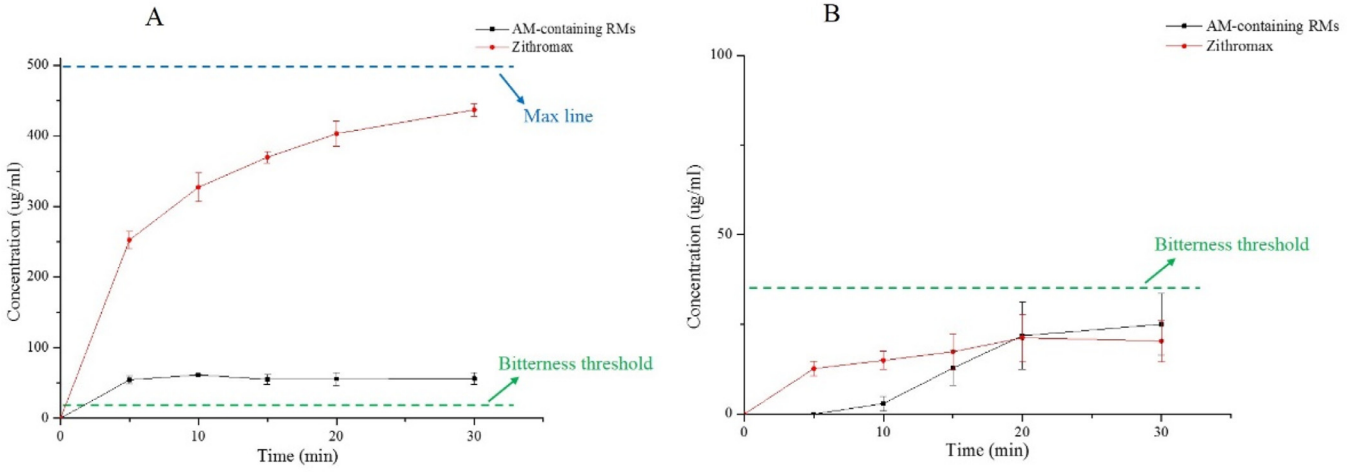


Fig. 6 – Drug release profile of AM-containing RMs and Zithromax® in SSFs: (A) SSF 1; (B) SSF 2 (*n* = 3).

Table 3 – Different simulated models for equation fitting.

X	Pattern	Equation	R-squared
X = C	Linear fit	$y = 2.29128 + 0.00148x$	0.31341
	Polynomial fit	$y = 1.75153 + 0.0548x - 0.000002x^2$	0.59973
	Allometric fit	$y = 0.96543x^{0.21573}$	0.75058
	Logistic fit	$y = 4.04539 + \frac{-3.09578}{1+\left(\frac{x}{60.42985}\right)^{2.38533}}$	0.97837
X = log C	Linear fit	$y = -0.11457 + 1.49382x$	0.88063
	Polynomial fit	$y = -1.73353 + 3.52519x - 0.52318x^2$	0.92916
	Allometric fit	$y = 1.46199x^{0.98608}$	0.92916
	Logistic fit	$y = 4.07544 + \frac{-3.07795}{1+\left(\frac{x}{1.65112}\right)^{7.99406}}$	0.99067

Table 4 – Bitterness scores, and determined and calculated concentrations of AM-containing RMs and Zithromax® ($n = 3$).

Formulations	Bitterness scores	Calculated concentration (μg/ml)	Determined concentration at 15 min (μg/ml)
AM-containing RMs	1.13 ± 0.35	13.20 ± 9.70	12.80 ± 4.90#*
Zithromax®	1.25 ± 0.46	16.60 ± 12.70	17.40 ± 11.60*

Note: #$P > 0.05$, compared with Zithromax®; *$P > 0.05$, compared with calculated concentration. By the student's t test.

Table 5 – Pharmacokinetic parameters of azithromycin in rats after oral administration of AM-containing RMs and Zithromax®.

Parameter	Zithromax®	AM-containing RMs
AUC_{0-t} (μg/l h)	21120.44 ± 5050.04	36894.22 ± 5292.16**
$AUC_{0-\infty}$ (μg/l h)	25151.95 ± 5472.06	43226.70 ± 9141.21**
MRT (h)	27.87 ± 2.26	35.27 ± 4.15**
$t_{1/2}$ (h)	38.36 ± 18.18	31.47 ± 12.38#
T_{max} (h)	3.00 ± 1.30	3.77 ± 0.88#
C_{max} (μg/l)	1062.69 ± 99.68	786.85 ± 123.68**

Note: #$P > 0.05$, *$P < 0.05$, **$P < 0.01$ compared with Zithromax®. By the analysis of variance and nonparametric rank sum test.

each time point after 10 min (Table 4). Therefore, bitterness could be assessed based on drug release concentrations in SSF 2 at 15 min.

Volunteers in the human taste test panel maintained test solutions in their mouths for 15 s to evaluate bitterness, because Zithromax® and the AM-containing RMs formulations easily dispersed and azithromycin was extremely bitter such that it could be fully tasted within a short period of time. Other researchers have found that 5 s [21] or 15 s [22] was sufficient for evaluating extreme bitterness. Therefore, 15 s was deemed appropriate for testing the bitterness of the two formulation solutions in this study. A logistic equation based on the bitterness scores and azithromycin concentrations of standard solutions was developed to correlate bitterness and azithromycin drug release concentrations in SSF 2 at different time points.

For the *in vitro* drug release evaluation, a sampling time point was needed. It might appear that the time should be set to match the residence time of the formulation in the mouth. However, testing the concentrations within 2 min might cause too much deviation. Furthermore, the determined concentration requires the accumulative total value of released drug. Thus, we set the time point by comparing the variability between the determined azithromycin concentrations *in vitro* and the calculated concentrations of Zithromax® and AM-containing RMs. The time chosen was 15 min. At that point, the taste of the formulations could be assessed by using the determined concentrations to calculate bitterness scores.

3.6. *Pharmacokinetic studies and analysis*

Oral bioavailability is the key factor for deciding whether a preparation can be used. The plasma concentration-time profiles of AM-containing RMs and Zithromax® are shown in Fig. 7. Table 5 summarizes the pharmacokinetic parameters obtained using WinNonlin. We noted that the AUC_{0-t} after oral administration of AM-containing RMs was 1.75-fold ($P < 0.05$) higher than that of Zithromax®. In addition, the mean residence time (MRT; 35.27 h) was higher and the C_{max} (786.85 μg/l) was lower for the azithromycin-containing reverse micelle formulation than for Zithromax®.

In our study, azithromycin molecules were entrapped in the polar cores of reverse micelles that were dispersed in an oil phase. Such lipid-based formulations can promote gastrointestinal absorption and improve oral bioavailability by stimulating the release of endogenous amphipathic substances such as phospholipids and bile salts and improving intestinal lymphatic transport [23–28]. In our *in vivo*

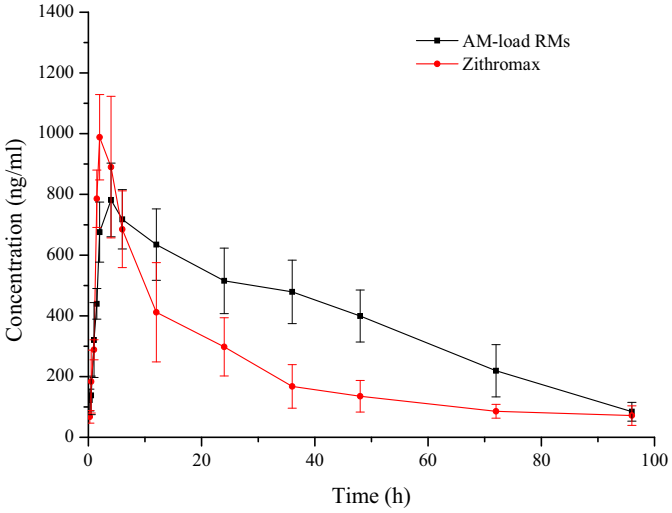


Fig. 7 – Rat plasma concentration-time profiles of azithromycin after oral administration of AM-containing RMs and Zithromax®. ($n = 6$).

pharmacokinetic experiment, the plasma azithromycin concentrations were significantly higher after oral administration of azithromycin-containing reverse micelles than after oral administration of Zithromax®, indicating that azithromycin-containing reverse micelles were sustained-release and could improve oral bioavailability compare to that of Zithromax®.

4. Conclusion

In this study, we first created a new preparation (AM-containing RMs) that successfully masked the bitterness of azithromycin without leaving a bitter aftertaste. Then, a system for assessing bitterness was developed with which we tested AM-containing RMs, using Zithromax® as a control. The bitterness threshold values and bitterness scores of azithromycin formulations were obtained from a human taste test panel. Next, *in vitro* drug release evaluations were performed using different SSFs. This method can be expanded for use with other drugs that require taste-masking.

To summarize, the process we used for establishing a bitterness assessment system followed these steps: (a) An SSF was chosen according drug release experiments and bitterness thresholds. (b) A correlation equation was derived from the bitterness scores and the concentrations of standard solution. (c) A sampling time point for the *in vitro* drug release tests was fixed by comparing the variability between the determined concentrations of drug release and the calculated concentrations. (d) Finally, the calculated bitterness scores were used to assess the bitterness of formulations.

From our results, the bioavailability of AM-containing RMs was unequivocally better than that of Zithromax®. The fact that reverse micelles can block drug release but not decrease bioavailability might be explained by intestinal lymphatic transport or the release of endogenous amphipathic substances.

Conflicts of interest

The authors declare that there is no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Acknowledgments

We would like to express our gratitude to Dr. Fanhua Meng and Master Jinqiu Liu for helping with plasma concentration testing. This work was supported by The National Key Technologies R&D Program for New Drugs of China (2015ZX09J15104-003).

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