Advanced Pharmaceutical Bulletin

Adv Pharm Bull, 2016, 6(3), 455-459
doi: 10.15171/apb.2016.059
http://apb.tbzmed.ac.ir

TUOMS Publishing Group

Short Communication

Anti Pneumococcal Activity of Azithromycin-Eudragit RS100 Nano-Formulations

Khosro Adibkia[1], Golrokh Khorasani[1], Shahriar Payab[1], Farzaneh Lotfipour[2]*

[1] *Drug Applied Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.*
[2] *Food & Drug Safety Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.*

Article info

Article History:
Received: 21 June 2015
Revised: 16 August 2016
Accepted: 31 August 2016
ePublished: 25 September 2016

Keywords:
· Azithromycin
· Anti Pneumococcal Activity
· Nano-Formulation
· Eudragit RS100

Abstract

Propose: Bacterial pneumonia is a common lung infection caused by different types of bacteria. Azithromycin (AZI), an azalide antibiotic, is widely used to manage pneumococcal infections. Studies have shown that antibiotics in nanocarriers may lead to increased antibacterial activity and reduced toxicity. The aim of this work was to valuate in vitro antibacterial performance azithromycin-Eudragit RS100 nano-formulations against *Streptococcus pneumoniae and Staphylococcus aureus*.

Methods: AZI-Eudragit RS100 nanoparticles were prepared via electrospinning technique and the *in* vitro antibacterial performance against *S. pneumoniae and S. aureus were assessed* using agar dilution method.

Results: Nanofibers in the sizes about 100-300 nm in diameter and micro scale in length and nanobeads in the range of 100-500 nm were achieved. The Minimum Inhibitory Concentrations (MIC) showed an enhancement in the antimicrobial effect of AZI-Eudragit RS100 nanofibers (40 µg/ml) compare to untreated AZI solution (>160 µg/ml) against *S. pneumonia*. The MIC value for AZI-Eudragit RS100 nanofibers against *S. aureus* was >128 µg/ml, same as that of the untreated AZI solution.

Conclusion: The enhanced efficiency of AZI in nanofibers could be related to the more adsorption opportunity of nanofibers to *S. pneumonia* capsulated cell wall which provides an antibiotic depot on the bacterial surface compared to *S. aureus*. AZI-Eudragit RS100 nanofibers with enhanced antimicrobial effect against S. *pneumonia* can be considered as a candidate for in vivo evaluations in antibiotic therapy of Pneumococcal infections.

Introduction

Azithromycin (AZI) is a semi-synthetic antibiotic from azalide class (14-membered ring macrolide) which was FDA approved for use in 1994. This antibiotic has been launched to pharmacy market as a result of notable limitations of former 14-membered ring macrolide i.e. erythromycin.[1] AZI achieves high tissue concentrations and reach the sites of infection by direct uptake via phagocytes.[2] Consequently the obtained high tissue concentrations are retained for extended periods resulted in once-daily dosing for 3 or 5 days. AZI like other macrolides inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit of the bacterial 70S ribosome which inhibits peptidyl transferase activity and interferes with amino acid translocation during the translation process (Figure 1).[3] Remarkable microbiological features of make it a good candidate for the management of a wide range of bacterial infections especially those of bacterial pneumonia.[4]

Nevertheless, in line with the global antibiotic resistance trend, significant resistance among the formerly susceptible microorganisms like *Streptococcus pneumoniae* and *Staphylococcus aureus* has been detected. In fact the increasing numbers of reports on the failures of treatment of infections caused by macrolide resistant pneumococcal isolates in the last two decades has been a matter of concern.[4] Three prominent mechanisms for Pneumococcal macrolide resistance can be summarized as the alteration of the ribosomal target site, the production of inactivating enzymes, and the production and utilization of active efflux mechanisms. The first two approach are shown to be more important in the macrolide resistance development.[1]

Several approaches to overcome bacterial resistance issue were developed from which the use of drug carriers such as nanoparticles seems to be one of the promising strategies. Nanoparticles drug delivery vehicles are generally in dimensions of about 5 – 350 nm in diameter. These carriers for pharmaceutical purposes improve therapeutic efficacy of the drug by enhancing drug bioavailability, serum stability and pharmacokinetics.[5-8] Especially nanoparticle based antimicrobial vehicles could interact with microorganisms by fusing with their cell wall or membrane and releasing the load within them and/or adsorbing to cell wall and providing an antibiotic depot there to release drug molecules.[9-12]

***Corresponding author:** Farzaneh Lotfipour, Tel: +98 (41) 33392580, Fax: +98 (41) 33344798, Email: lotfipoor@tbzmed.ac.ir

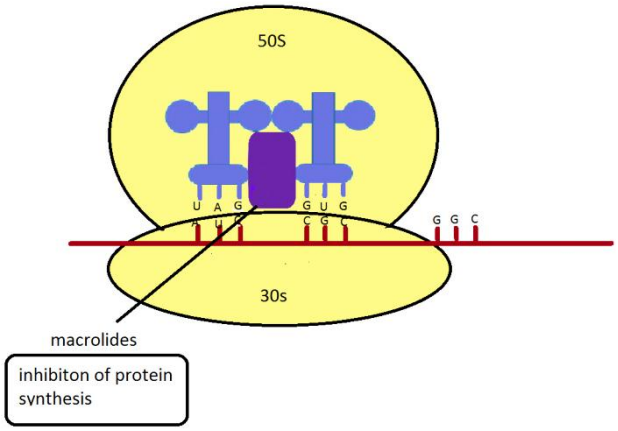

Figure 1. Schematic representation for antibacterial mechanism of macrolides.

Electrospinning is a promising method for nanoparticle production. A polymer-drug solution is injected into a capillary tube while applying electrical charge on the solution to create a liquid jet flying towards a grounded collector screen. By its movement to the collector, the solvent evaporates and a fine network of nanofibers or nanobeads would form on the collector.[13]

AZI loaded Eudragit RS100 nano-formulations were prepared using Electrospinning in our previous published work.[13] Here we aimed to evaluate the activity of the selected nano-formulations against AZI resistant *S. pneumonia* and *S. aureus* strains in comparison with the untreated drug.

Materials and Methods

Materials

AZI powder (Dr Reddy's Pharmaceutical Company, India) Eudragit RS100 (Degussa Darmstadt, Germany), HPLC grade methanol (Concord Technology (Tianjin) Co., Ltd.), Muller Hinton broth and agar media, blood base agar medium (DIFCO, U.K). *S. pneumonia* and *S. aureus* were previously isolated from clinical samples according to standard procedures and stored at -80°C.

Preparation and characterization of the nanobeads and nanofibers

The fabrication of AZI-Eudragit RS100 nanobeads and nanofibers was conducted according to our previously published paper.[13]

Nano-suspension preparation

The stock nano-suspension (containing equivalent AZI of 2.62 mg/ml) was prepared by dispersing 157.2 mg of the AZI-Eudragit RS100 nano-formulations, in 10 ml distilled water by vortexing. In the next step, the vortexed suspension was entered the process of serial dilution in order to obtain a bunch of distinct concentrations to use them within the ongoing agar dilution process. Furthermore, the stock of AZI solution in the same concentration (2.62 mg/ml) was prepared in 5% ethanol in distilled water as negative control.

Antibacterial activity of AZI-Eudragit RS100 nano-formulations

Antibacterial properties of the nano-formulations were investigated against AZI resistant *S. pneumonia* and *S. aureus* standard strains. After activating of the freezed bacteria, the culture was maintained in the blood base agar and nutrient agar media for *S. pneumonia* and *S. aureus* respectively at 4°C and used as stock cultures. A single colony from the stock plate was transferred into the relevant agar media and incubated over night at 37°C. After incubation time the cells were collected from the surface of agar medium, washed twice, and re-suspended in saline solution to provide an optical density equal to 0.5 McFarland or bacterial concentration around 10^8 CFU/ml.

Antibacterial performance against *S. pneumonia* was evaluated by agar dilution method. Firstly, a series of concentrations of antibiotics were prepared by 1:2 diluting trend in order to be added into the agar medium. Two millilitres from each concentration of AZI nano-formulation as well as untreated AZI solution (concentrations ranging from 0.625 to 160 µg/ml) was added to the plate containing 30 ml Haemophillus agar supplemented with 5% sheep blood (temperature=45°C). Therefore, for each concentration from each formulation two plates were obtained. After preparing the plates, they were inoculated with a swab from the adjusted *S. pneumoniae* inoculum suspension and incubated for 2 days at 37°C. The MICs were recorded after 48 h of incubation on the basis of the first concentration that inhibit the bacterial growth.

The antimicrobial activities of prepared nano-formulations against *S. aureus were assessed by* broth macrodilution method according to CLSI protocol. Briefly bacterial inoculum in Muller-Hinton Broth medium, added to serial diluted NPs suspension as well as drug solutions to reach the final concentration of 10^6 CFU/ml. After 24 h incubation at 37 °C , from the content of the tubes streak cultured onto Muller Hinton agar plates. MICs was designated as the first concentration of antibiotic with no sign of bacterial growth.[14]

Results and Discussion

Characteristics of prepared nano-formulations

Nanofibers in the sizes about 100-300 nm in diameter and micro scale in length as well as nanobeads in the range of 100-500 nm in diameter were achieved. As concluded from the SEM results depicted in Figure 2 (a and b), application of higher concentrations of solution resulted in production of nanofibers with smooth surfaces. On the other hand, lower solution concentration resulted in the production of nanobeads with concavities on their surfaces.[13,15-17]

Antibacterial activity of the nanosuspensions

S. pneumonia was cultured in Haemophilus agar containing 5% sheep blood used as an indicator to assess in vitro antibacterial activity of prepared nano-

formulations compared to drug solution. According to the MIC test results, the tested *S. pneumonia* was shown to be *in vitro* resistant to AZI even at higher concentrations. In fact in all the tested concentrations (up to 160µg/ml) *S. pneumonia* colonies were observed on the surface of the cultured plates (Figure 3). While MIC values for sensitive strains were reported to be 0.5 to 2µg/ml in standard CLSI protocol that was significantly different from the MIC value obtained for our test strain. In spite of the obtained in vitro resistance pattern, it should be in vivo studies to extrapolate the data into clinical failures. In fact it has been shown that the host-defense responses lead macrolides and azalides after absorption to the infection site and release them at the infection site. Also, after phagocytosis of the pathogens, expose them to high intracellular drug concentrations.[1,18]

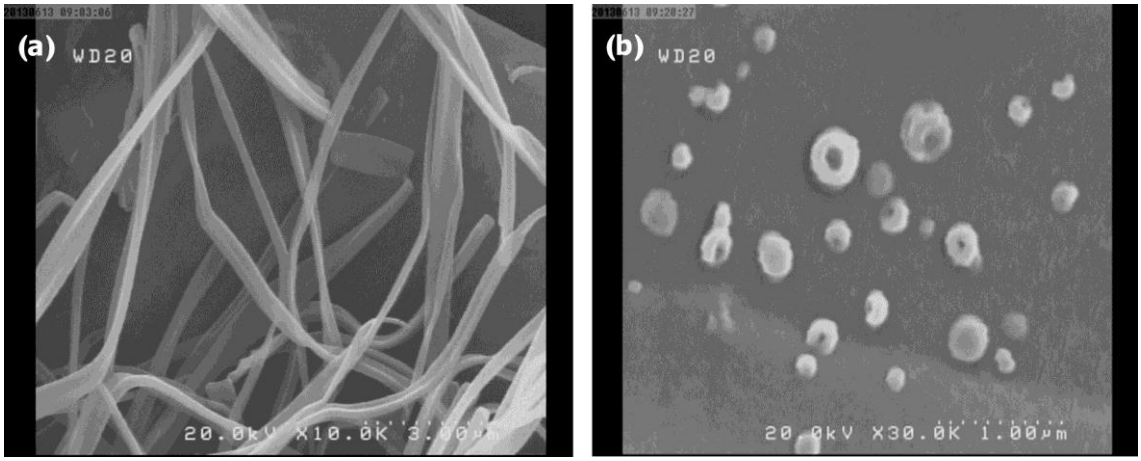

Figure 2. SEM images for AZI-Eudragit RS100 electrospuns. Samples with the drug: polymer ratio of 1:5 and solution concentrations of 20% (a) samples with the drug: polymer ratio of 1:5 and solution concentrations of 10% (b).

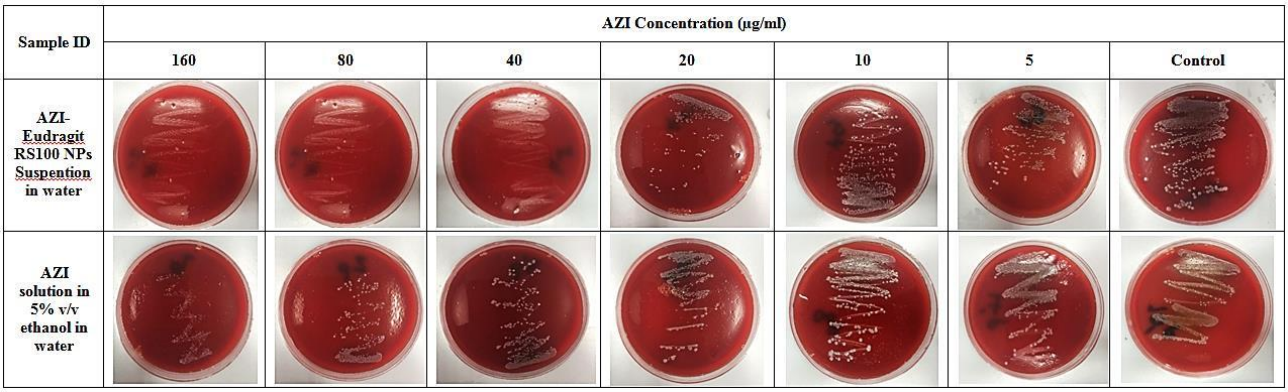

Figure 3. Haemophillus agar plates containing AZI-Eudragit RS100 nanofibers as well as untreated AZI solution with streak cultures of *S. pneumonia.*

On the other hand, the MIC values recorded for AZI-Eudragit RS100 nanofibers were 40 µg/ml, which was significantly lower than the MIC value of the untreated counterpart (Figure 4). This can be presumed as a remarkable improvement in the AZI performance. This improvement in the antibacterial performance of AZI in nano-form can be probably explained by some possible mechanisms. Overall, nanoparticle based antimicrobial delivery systems could interact with microorganisms via two mechanisms and consequently enhance the performance; by fusing with microbial cell wall or membrane and release the load within the cell wall or membrane; via adsorbing to cell wall and provide a antibiotic depot to continuously release drug molecules, which will diffuse into the interior of the microorganisms.[9] Furthermore, it has been reported that antibiotic-loaded NPs can enter host cells and tissues through endocytosis, followed by releasing the loaded drug to eradicate intracellular microorganisms.[19] In the case of AZI-Eudragit RS100 nanofiber the adsorption to the bacteria cell wall to provide an antibiotic depot on the bacterial surface seems to be more feasible due to the large surface area of the nanofibers with more interaction opportunity of the bacterial cell wall. Nevertheless, according to the fact that nano-fibres are nano size just in one dimension, they could not pass through absorption barriers and the fate of formulation in in vivo experiments could be different. Figure 4 shows the cellular structure of *S. pneumonia* which composed of cell wall and a rough polysaccharide capsule. Based on the capsule structure of *S. pneumonia*, it can be predictable that the adsorbing and trapping of the

nanofibers within the complex capsule structure of the bacteria to provide an in situ antibiotic depot would not be farfetched.[20]

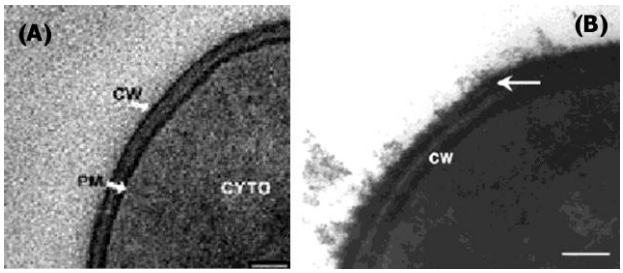

Figure 4. The cell wall structure of (A): *S. pneumonia*[20] with permission and (B): *S. aureus*[21] with permission. CW: cell wall, PM: plasma membrane, Bar = 100µm.

Nevertheless, based on the MIC value obtained for AZI-Eudragit RS100 nanobeads, the observed figures were >160 µg/ml, same as that of the untreated AZI solution indicating that the prepared nanobeads simply maintained the antibacterial efficiency of AZI. Still it would be valuable because of the potential benefits of prepared nanobeads which need to be evaluated in the upcoming in vivo or animal studies.

In vitro antibacterial activity of the prepared nano-suspensions *was* assessed against *S. aureus* by broth macrodilution method and compared to untreated drug solution.

According to the MIC test results, the experimented strain of *S. aureus* was shown to be *in vitro* resistant to AZI. In fact in all the tested concentrations (0.25 to 128µg/ml) *S. aureus* colonies were observed on the surface of the streak cultured plates (MIC >128µg/ml). According to standard CLSI protocol MIC values for susceptible strains were reported to be 2 to 8µg/ml. This finding is in good agreement with the various reports indicating growing in vitro resistance trends described about *S. aureus* against macrolides.

On the other hand, the MIC values recorded for both AZI-Eudragit RS100 nanofibers and nanobeads represented a resistant paradigm as well, with MIC values of >128µg/ml. Indeed, preparation of nano-formulation (nanofibers or nanobeads) could not improve the in vitro antibacterial activity of AZI in comparison with drug solution and just maintained the MIC values of AZI equal to untreated solution. Figure 5 (B) shows a schematic picture of a *S. aureus* cell wall structure. It seems that the adsorption and trapping opportunity of the nano-formulations to the capsule less cell wall structure of *S. aureus* would be lower than capsulated rough *S. pneumonia* cell wall.

Conclusion

In the current in vitro study we assessed the anti bacterial activity of formulated AZI- Eudragit RS100 nanobeads and nanofibers against S. *pneumonia* and *S. aureus;* two common cause of bacterial pneumonia. Herein, it was shown that AZI-Eudragit RS100 nanofibers with enhanced in vitro antimicrobial effect against S. *pneumonia* can be considered as a candidate for *in vivo* evaluations in antibiotic therapy of Pneumococcal infections.

Ethical Issues

Not applicable.

Conflict of Interest

The Authors report no declaration of interest.

References

1. Amsden GW. Pneumococcal macrolide resistance--myth or reality? *J Antimicrob Chemother* 1999;44(1):1-6. doi: 10.1093/jac/44.1.1
2. Lode H, Borner K, Koeppe P, Schaberg T. Azithromycin--review of key chemical, pharmacokinetic and microbiological features. *J Antimicrob Chemother* 1996;37(Suppl C):1-8. doi: 10.1093/jac/37.suppl_c.1
3. Retsema J, Girard A, Schelkly W, Manousos M, Anderson M, Bright G, et al. Spectrum and mode of action of azithromycin (CP-62,993), a new 15-membered-ring macrolide with improved potency against gram-negative organisms. *Antimicrob Agents Chemother* 1987;31(12):1939-47. doi: 10.1128/AAC.31.12.1939
4. Reinert RR, Filimonova OY, Al-Lahham A, Grudinina SA, Ilina EN, Weigel LM, et al. Mechanisms of macrolide resistance among Streptococcus pneumoniae isolates from Russia. *Antimicrob Agents Chemother* 2008;52(6):2260-2. doi: 10.1128/AAC.01270-07
5. Samiei M, Farjami A, Dizaj SM, Lotfipour F. Nanoparticles for antimicrobial purposes in Endodontics: A systematic review of in vitro studies. *Mater Sci Eng C Mater Biol Appl* 2016;58:1269-78. doi: 10.1016/j.msec.2015.08.070
6. Hallaj-Nezhadi S, Dass CR, Lotfipour F. Intraperitoneal delivery of nanoparticles for cancer gene therapy. *Future Oncol* 2013;9(1):59-68. doi: 10.2217/fon.12.171
7. Nahaei M, Valizadeh H, Baradaran B, Nahaei MR, Asgari D, Hallaj-Nezhadi S, et al. Preparation and characterization of chitosan/β-cyclodextrin nanoparticles containing plasmid DNA encoding interleukin-12. *Drug Res (Stuttg)* 2013;63(1):7-12. doi: 10.1055/s-0032-1331165
8. Hallaj-Nezhadi S, Valizadeh H, Baradaran B, Dobakhti F, Lotfipour F. Preparation and characterization of gelatin nanoparticles containing pDNA encoding IL-12 and their expression in CT-26 carcinoma cells. *Future Oncol* 2013;9(8):1195-206. doi: 10.2217/fon.13.82
9. Zhang L, Pornpattananangku D, Hu CM, Huang CM. Development of nanoparticles for antimicrobial drug delivery. *Curr Med Chem* 2010;17(6):585-94. doi: 10.2174/092986710790416290
10. Lotfipour F, Valizadeh H, Milani M, Bahrami N, Ghotaslou R. Study of Antimicrobial Effects of

Clarithromycin Loaded PLGA Nanoparticles against Clinical Strains of Helicobacter pylori. *Drug Res (Stuttg)* 2016;66(1):41-5. doi: 10.1055/s-0035-1548910
11. Azhar SL, Lotfipour F. Magnetic nanoparticles for antimicrobial drug delivery. *Pharmazie* 2012;67(10):817-21.
12. Hallaj-Nezhadi S, Lotfipour F, Dass CR. Delivery of nanoparticulate drug delivery systems via the intravenous route for cancer gene therapy. *Pharmazie* 2010;65(12):855-9.
13. Payab S, Jafari-Aghdam N, Barzegar-Jalali M, Mohammadi G, Lotfipour F, Gholikhani T, et al. Preparation and physicochemical characterization of the azithromycin-Eudragit RS100 nanobeads and nanofibers using electrospinning method. *J Drug Deliv Sci Technol* 2014;24(6):585-90. doi: 10.1016/S1773-2247(14)50123-2
14. Wikler MA. Performance standards for antimicrobial susceptibility testing; 18th informational supplement. CLSI document M100-18 . Wayne PA: Clinical and Laboratory Standards Institute; 2008.
15. Liu H, Hsieh YL. Ultrafine fibrous cellulose membranes from electrospinning of cellulose acetate. *J Polym Sci Part B: Polym Phys* 2002;40(18):2119-29. doi: 10.1002/polb.10261
16. Frey MW, Li L. Electrospinning and porosity measurements of nylon-6/poly (ethylene oxide) blended non-wovens. *J Eng Fibers Fabrics* 2007;2(1):31-7.
17. Jafari-Aghdam N, Adibkia K, Payab S, Barzegar-Jalali M, Parvizpur A, Mohammadi G, et al. Methylprednisolone acetate-Eudragit® RS100 electrospuns: Preparation and physicochemical characterization. *Artif Cells Nanomed Biotechnol* 2016;44(2):497-503. doi: 10.3109/21691401.2014.965309
18. Ballow CH, Amsden GW, Highet VS, Forrest A. Pharmacokinetics of oral azithromycin in serum, urine, polymorphonuclear leucocytes and inflammatory vs non-inflammatory skin blisters in healthy volunteers. *Clin Drug Investig* 1998;15(2):159-67. doi: 10.2165/00044011-199815020-00009
19. Salouti M, Ahangari A. Nanoparticle based Drug Delivery Systems for Treatment of Infectious Diseases. In: Sezer AD, editor. Application of Nanotechnology in Drug Delivery. Vienna, Austria: InTech Publishing House: 2014. P. 155-92.
20. Chang YC, Jong A, Huang S, Zerfas P, Kwon-Chung KJ. CPS1, a homolog of the Streptococcus pneumoniae type 3 polysaccharide synthase gene, is important for the pathobiology of Cryptococcus neoformans. *Infect Immun* 2006;74(7):3930-8. doi: 10.1128/IAI.00089-06
21. Touhami A, Jericho MH, Beveridge TJ. Atomic force microscopy of cell growth and division in staphylococcus aureus. *J Bacteriol* 2004;186(11):3286-95. doi: 10.1128/JB.186.11.3286-3295.2004