Original Article

Examination of the Steps and Attachment Circumstances of the Poly Lactic-co-glycolic Acid (PLGA) Nano-particle to Increase the Effect of Nanomedicine on Vancomycin-Resistant *Enterococcus Faecalis*

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Abstract

Background: Infectious diseases continue to be one of the biggest health challenges around the world, followed by problems caused by antibiotic resistance and excessive use of antibiotics. In general, *Enterococcus faecalis* is the main cause of nosocomial infections and is the most common cause of surgical ulcer infections. This study examines how a vancomycin nanomedicine attaches to poly lactic-co-glycolic acid (PLGA) nanoparticle. Determining the role of vancomycin nanomedicine on reducing the drug resistance of vancomycin in *E. faecalis* (clinical hospital isolates) and determining the cytotoxicity effects of nanomedicine.

Materials and Methods: In this method, first, attachment made through chemical processes such as emulsion between vancomycin antibiotic and a PLGA nanoparticle, and resultant antibiotic tested on vancomycin resistant *E. faecalis*.

Results: The results of this study indicate that the method of nanomedicine attachment to antibiotics was an effective method and it was determined by X-ray Diffraction that the attachment was precisely performed. In the antibiogram method, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the nanomedicine increased in respect to vancomycin antibiotic.

Conclusion: The results showed that produced Nano-antibiotics had a better effect than resistant antibiotics.

Keywords: PLGA, Enterococcus Faecalis, Vancomycin

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Introduction

Infectious diseases continue to be one of the biggest health challenges throughout the world. Antibiotic resistance, excessive consumption of antibiotics in high-doses, high-costs and longtime needs to identify and produce a new and effective antibiotics that it causes extra problems[1,2].

Vancomycin-resistance Enterococcus (VRE) is one of the most common pathogens in hospitals around the world. It was first observed in the 1980s (1986) in Europe and later in the United States. In the United

States, after the observation of the VRE, its prevalence increased dramatically in 1998, and the rate of hospital infection resulting from it between 1989 and 1998 increased 20 times[3,4]. Excessive use of antibiotics (especially over the past 50 years) and the high capacity of Enterococcus for the acquisition and diffusion of antibiotic-resistance agents have made a serious problem in treatment of the related diseases. In Enterococcus, resistance to glycopeptides is a result of a gene cluster consisted of VanA-E and VanG. Among them VanA genotype is more important[3,5].

Recently, unusual agents have been used to eliminate the drug resistance of some antibiotics. These agents are various classes of antimicrobial nanoparticles and carriers in the nanoscale for antibiotic delivery, which have been proven to be effective in laboratory samples and animal models[6,7]. Vancomycin resistant Enterococcus is one of the most common pathogens in the hospitals all over the world causing serious illnesses such as bacterial urogenital infections, endocarditis. One of the new drug therapy methods is drug delivery using nano-polymers such as nanoparticles[8,9]. These carriers are nanometer-size polymers. These carriers can enclose the drug inside them and thereby reduce its toxicity to the body. Meanwhile, these carriers increase the half-life of the drug in the body; therefore, a lower dosage of the drug will be needed for the treatment, which will in turn reduce the adverse effects of the drug in various tissues[4, 10]. The nanoparticles have unique properties. The application pattern of nanoparticles (NPs) or solid particles or dispersed particles is 10 to 1000 nm because of their small size and high reactivity [5,11]. The aim of this study was to Examination of the Steps and attachment Circumstances of the Poly Lactic-co-glycolic Acid (PLGA) Nano-particle to increase the effect of Nanomedicine on Vancomycin-resistant *Enterococcus faecalis*.

Methods

The ready-made PLGA nanoparticles were purchased from the Sigma Company. The PLA to PGA proportion in this experiment was same.

First, 100 mg of PLGA dissolved in 1.3 and 0.7 ml Dichloromethane- acetone. Vancomycin was added to the nanoparticle and Dichloromethane solution on a magnetic rotator. The solution called as an initial water/oil emulsion. The resulting emulsion was sonicated in three steps, each for 10 seconds and 4 ml of polyvinyl alcohol 1% solution was added to this initial emulsion. This emulsion was also sonicated three times as the previous step. After sonication, 4 ml of polyvinyl alcohol 0.5% was added to this secondary emulsion. Then, for three hours, in order to evaporate the Dichloromethane, it was placed on a magnetic rotator (all steps were done on ice). The particles were centrifuged for 40 minutes at 16000 rpm. After removal of the supernatant, the resulted sediment was washed with 10 ml of distilled water in three steps, and the nanoparticles were dried by lyophilization. The PLGA Nano Capsules containing the drug, until the microbial steps, were stored in -20° freezer[19].

The method of measuring the minimum inhibitor concentrations (MIC) by microbroth method was determined using the Mueller Hinton growth medium and antibiotic powders and McFarland Suspension 0.5, in accordance with the International Committee of Clinical Laboratory Standards Committee (NCCLS).

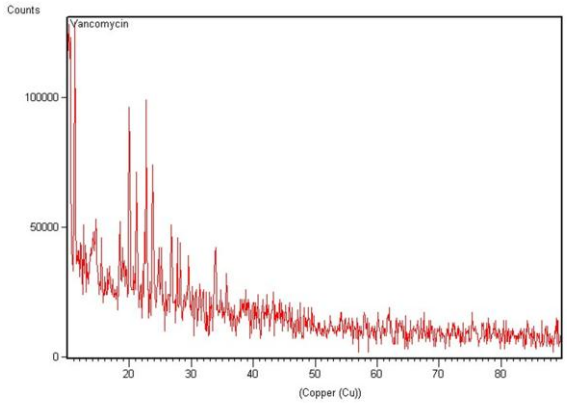
The minimum inhibitor concentration was determined based on the CLSI method and the lowest concentration of antibiotic that inhibited the growth of the bacterium was recorded as MIC (Negative control was bacteria suspension with antibiotic and positive control was bacteria suspension without antibiotic).

Results

The information related to each material existed in the X-ray device, and the name of the material could be declared according to the spectrum obtained. In this case, the PLGA nanomedicine attached to vancomycin was a new material therefore the device could not detect it. In the spectrum 1, vancomycin antibiotic peaks are observed.

When a PLGA nanoparticle attaches to a vancomycin, new peaks were created, previous peaks disappeared, and in some cases, peak intensity was varies. In cases where the preceding material did not disappear, remaining some of the peaks indicated that some of the PLGA nanoparticles had not been attached to the vancomycin. Various peaks in spectrum 3 represented the attachment.

As the results of MIC and the minimum bactericidal



Spectrum 1. XRD of Vancomycin.

concentration (MBC), the effect of produced nanoparticles and the vancomycin in various concentrations on vancomycin-resistant *E. faecalis* has been shown. In this study, MIC was 64µg/ml in the presence of vancomycin and was 16µg/ml in the presence of the vancomycin PLGA nanoparticle.

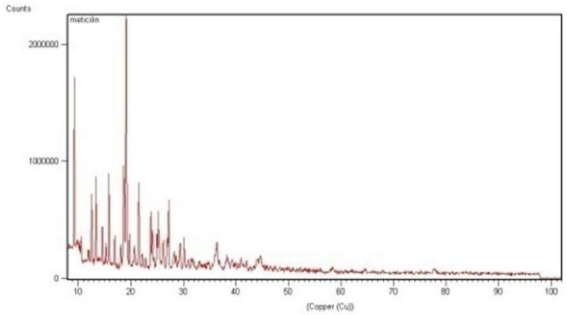
Discussion

Despite the widespread application of antibiotics especially Penicillin and the detection of novel anti-microbial factors, Enterococcus remain the prevalent head of hospital infections especially in ICU[12,13]. The Enterococcusu may create meningitis and bacteremia among children and endocarditis among adults. A primary problem in facing with the Enterococcus is that they can become resistant fast against a variety of antibiotics[12,14].

In the recent research, the PLGA (PLA/PGA=50/50) nanoparticle was used for encapsulating vancomycin. After conducting experiment steps, the effect of encapsulation process and effects of particles on each other through X-ray diffraction (XRD) device investigated.

The results showed that the vancomycin antibiotic successfully was attached to the PLGA nanoparticle and decreased the amount of MIC and MBC *E. faecalis* in the presence of vancomycin than the vancomycin antibiotic which indicates the effectiveness of this method [15,16].

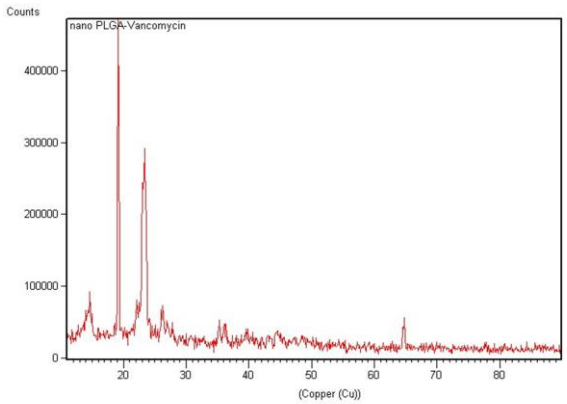
Ansari et al. (2014) separated curcumin from turmeric and synthesized with poly lactic-co-glycolic



Spectrum 2. XRD of PLGA Nano Particle.

acid nanoparticle. Due to the antibacterial effects of curcumin, this material is changes into a nano form and analyzed its effects on methicillin-resistant stuff. It was stated in this study that PLGA covers the intended drug and free a constant amount of drug by means of emission in polymer and polymer matrix degradation. Meanwhile, this nanoparticle is a proper carrier of delivering the drug to the target cell; and these nanoparticles can be prescribed into capillaries systematically and without accumulation problem[17].

Afsharnejad et al. (2012) studied on cyanoacrylate composite for dental applications and conducted the cell toxicity test on L929 cell lines through MTT method. In 24-hour and 48-hour tests, more than 70% of cells survived. However, the 30% cells survived after 72 hours by neighboring the nanocomposite on the L929 cells. The lower the amount of concentration of this nanocomposite, the lower amount of cell



Spectrum 3. XRD of Vancomycin PLGA Nano-Particle.

toxicity will be[18].

Yang et al. (2014) studied on the PLGA nanoparticles that possess significant absorption and were little molecules with low potential. By means of this nanoparticle, a drug was created with low solubility and permeability into the cell although it increases the negative load of cell surface. Recently, PLGA-NPs oral nanomedicine has been created that in this research the aim was introduction and evaluation of the physio-chemistry of above-mentioned nanomedicine, which has covered the positive load of chitosan. The thein orphan as a medicine was added to the model. Ca-Co-2 was also added to the above-mentioned nanomedicine, which significantly increases the cell absorption. The disease is about smart and targeted medicines[19].

Stevanovic et al. (2013) used PLGA nanomedicines, silver and ascorbic acid in order to evaluate the effect of these nanoparticles on the positive and negative gram bacteria, *E. coli* and penicillin-resistant *Staphylococcus aureus*, which created a nanoparticle by this material; and analyzed it according to morphology and osteogenic markers' expression on osteoblast MC3T3-E1 cells in the laboratory environment[20, 21].

Conclusion

It was shown that this nanoparticle was effective on these bacteria.

Acknowledgment

None.

Conflict of Interest

The authors declare that there is no conflict of interests.

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