Development of Gentamicin-loaded Solid lipid Nanoparticles: Evaluation of Drug Release Kinetic and Antibacterial activity against Staphylococcus aureus

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ABSTRACT

Context: The attention to solid lipid nanoparticles (SLNs) particularly in the field of drug delivery has increased during the recent years.

Objective: This research was designed to load gentamicin onto SLNs to produce a drug delivery system with controlled release.

Materials and methods: Ultrasonication/highspeed homogenization technique was carried out to prepare gentamicin-loaded SLNs. The tests including: size, zeta potential, drug release kinetics, stability and scanning electron microscopy were performed to evaluate in vitro characterization of the new nano-drug. Agar well diffusion and macrodilution methods were used to study the antibacterial activity of gentamicin-loaded SLNs against Staphylococcus aureus.

Results: The average particle size of gentamicin-loaded SLNs was 282.3 nm with zeta potential of +8.16 mV. The drug-loading efficiency was found 40%. The drug release study showed a gradual gentamicin release up to 96 h without significant burst effect.

Conclusion: Gentamicin-loaded SLNs is a good drug delivery system with desirable release characteristics.

Key words: Drug Delivery system; Solid lipid nanoparticles; Release Kinetic; Gentamicin; Staphylococcus aureus

INTRODUCTION

Even though the therapeutic efficacy of numerous antimicrobial drugs has been well established, inefficient drug delivery can result in the inadequate therapeutic index (1). Gentamicin sulphate is a water-soluble antibiotic of the aminoglycoside group, derived from Micromonospora purpurea, an actinomycete (2). Inhabitation of protein synthesis and induction of a significant increase in misreading of messenger RNA is the mechanism of gentamicin action. Although the gentamicin is the first choice aminoglycoside for the treatment of serious infections but because of its important side effects, mostly related to nephrotoxicity and ototoxicity, its usage is restricted in the recent years (3, 4). The structure of gentamicin was shown in figure 1.

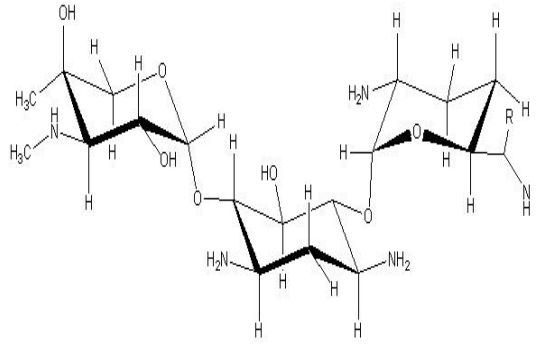

Figure 1. The structure of gentamicin molecule.

Improvement in gentamicin pharmacokinetic profile with the aim to reduce the harmful effects and to increase the effectiveness of the drug via achievement and maintenance of a safe and efficacious drug level have been the ideal goals in all the therapeutic strategies concerning gentamicin. Many studies have previously demonstrated that nanoparticles such as liposomes, polymeric nanoparticles, solid lipid nanoparticles, dendrimers and particularly metal nanoparticles were able to reduce the harmful effects of drugs (5). Solid lipid nanoparticles introduced in 1991 represent an alternative carrier system to traditional colloidal carriers such as emulsions, liposomes and polymeric nanoparticles. SLNs are constructed from synthetic/natural lipids such as glyceryl behenate (compritol), stearic triglyceride (6), cetylpalmitate and glycerol tripalmitate (tripalmitin) with the size range of 50-1000 nm (7, 8). Because of many advantages such as good biocompatibility, low toxicity, good tolerability, site-specific targeting (7,

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9) and their great promise for improving bioavailability of poorly water-soluble drugs (10), SLNs are at the fore-front of the rapidly developing field of nanotechnology with several potential applications in drug delivery (6). In association with using of SLNs in biological systems, their great potential have been considered for the delivery of drugs into the CNS. Also aqueous dispersions of SLN can be converted into dry, reconsitutable powders by spray-drying (11). Aforementioned characteristics make SLN an interesting carrier system for optimized delivery of drugs. In recent years many studies have been reported on SLNs encapsulated antimicrobial agents such as rifampicin, isoniazid, pyrazinamide (12), ciprofloxacin hydrochloride (13) and tobramycin (14). Also many techniques have been developed to prepare SLNs, such as High shear homogenization, solvent emulsification or evaporation, ultrasonication (high speed homogenization) and solvent diffusion method (15). In this study, in order to design a drug delivery system and examine the possibility of overcoming some of the side effects of gentamicin that are dependent on the drug release profile, gentamicin was loaded onto SLNs using the ultrasonication technique and *in vitro* characterization of the new drug delivery system was studied.

MATERIALS AND METHODS

Materials

Cholesterol, ethanol, hydrochloric acid, acetone, nutrient agar and nutrient broth media were purchased from Merck, Germany. Tween 80, Gentamicin sulphate and DO405 dialysis tube 23×15 mm with 10–12 kD cut-off were purchased from Sigma-Aldrich, USA. *Staphylococcus aureus* (ATCC 12600) was obtained from microbial bank of institute Pasteur, Tehran, Iran.

Preparation of gentamicin-loaded SLNs

Loading of gentamicin onto SLNs was performed in 3 steps including:

1) The solution of deionized water and tween 80 (as a surfactant) was prepared (1% w/w). 20 mg of gentamicin was added to the solution and the pH was adjusted to 2 using hydrochloric acid (1 N). Then, the mixture was homogenized for 15 min at 15000 rpm using a high-speed blender to prepare the aqueous phase.

2) 39.5 mg of cholesterol was added to the solution containing 2 mL of acetone and 3 mL of ethanol. Then, the solution was heated in a water bath at 80°C for 10 min to prepare the lipid phase.

3) The hot lipid phase was added to the aqueous phase in a homogenizer at 17000 rpm for 15 min at room temperature. Then, the mixture was sonicated at 50°C for 20 min using a bath-sonicator system. Finally the temperature of the mixture was brought down to the room temperature.

Particle size and zeta potential analysis

After the gentamicin-loaded SLNs preparation, the samples were centrifuged at 20000 rpm for 30 min at 4°C. Then the supernatant was decanted and the sediment re-dispersed in deionized water. Next, the particle size and surface potential (the zeta potential) of nanoparticles was determined using a zeta sizer (Nano ZS3000; Malvern Instruments, Malvern, UK) at the LASER wavelength of 633 nm.

Morphology study

Morphological feature of the gentamicin-loaded SLNs was studied using scanning electron microscopy (Philips XL30; Philips, Almelo, The Netherlands). For this purpose the specimen was mounted on an aluminum stub, sputter-coated with a thin layer of gold-palladium. After the sample was allowed to be dried, it was examined using the SEM.

Quantification of drug entrapment efficiency

The gentamicin-loaded SLNs solution was centrifuged at 11000 rpm and the supernatant was collected. Then, the quantity of gentamicin in the supernatant was measured by ultra violet spectroscopy at 256 nm. The entrapment efficiency was calculated using the following equation:

$$\text{Entrapment efficiency } \% = \frac{\mathbf{Drug}_{total} - \mathbf{Drug}_{supernatant}}{\mathbf{Drug}_{total}} \times 100$$

In vitro gentamicin-release study

Evaluation of the gentamicin release profile was determined using the dialysis method. 30 mL of gentamicin-loaded SLNs was centrifuged at 11000 rpm for 30 min. The supernatant was decanted and the sediment re-dispersed in phosphate buffer solution (pH 7.4). Then, the solution was placed in DO405 dialysis tube and immersed in 40 mL of PBS (pH 7.4). 1 ml samples were withdrawn from the solution at suitable intervals until 96 h and analyzed for the gentamicin content using the UV-vis spectrophotometry.

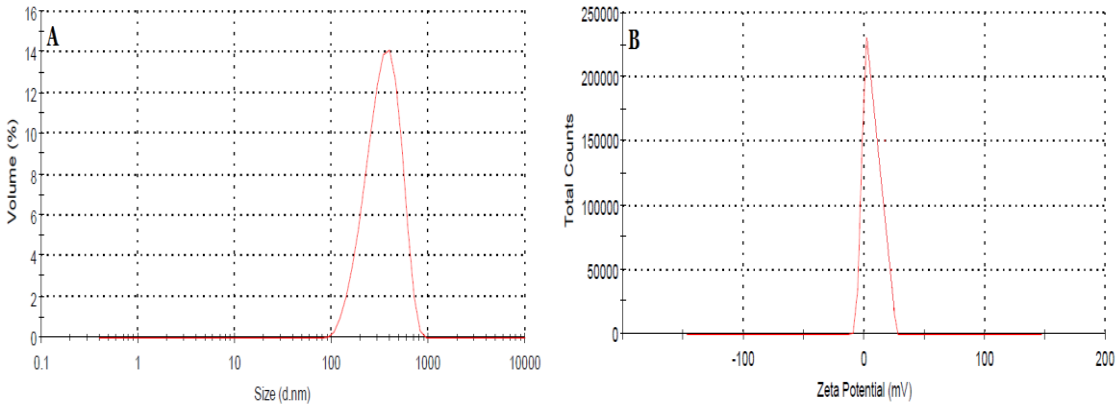

Figure 2. Particle size (A) and zeta potential (B) diagram of gentamicin-loaded SLNs. The average size is 282.3 nm approximately and zeta potential is 8.16 mV.

Stability study

After preparation of gentamicin-loaded SLNs, it was divided into the two portions: one portion was kept at room temperature and another was refrigerated at 2-8ºC. The particles size and zeta potential were evaluated at different time intervals up to 72 h.

Antibacterial activity

In order to evaluate the antibacterial activity of gentamicin-loaded SLNs, the agar well diffusion and macro dilution methods were carried out against *Staphylococcus aureus* (ATCC 12600).

Agar well diffusion method

The bacterial suspensions with a cell density equivalent to 0.5 McFarland (1.5×10^8 CFU/mL) were transferred individually onto the surface of Muller–Hinton agar plates using the sterile cotton swabs. The wells with 8 mm diameters were prepared by punching a sterile cork borer onto the agar plates and removing the agar to form a well. Then, 100 µl of the gentamicin-loaded SLNs, free gentamicin, unloaded SLNs, mixture of gentamicin and SLN, and normal saline solution (as a negative control) were poured into the wells, separately. After the incubation at 35°C–37°C for 24–48 h, the zones of inhibition around the wells were measured in mm using a caliper.

Macro dilution method

The solution of gentamicin-loaded SLNs (the equivalent of gentamicin concentration was calculated based on the loading ratio) was prepared and serially diluted in 5 ml of Muller–Hinton broth to reach the concentration range of 0.25-500 µg/ml. Then, 50 µl of *S. aureus* inoculum was added to each tube to reach the final concentration of 5×10^6 CFU/mL. After 48 h incubation at 35°C–37°C, the test tubes was examined and the MIC and MBC were determined. Simultaneously all of the above mentioned steps were performed for a stock solution of free gentamicin for MIC and MBC determination via the serial dilution method for comparison study.

Data analysis and statistics

All the experiments were repeated 3 times for each test and the results were presented as mean ± SD and verified using the analysis of variance (p value>0.05).

Results and Discussion

Size and zeta potential of gentamicin-loaded SLNs

The mean particle size and zeta potential of gentamicin-loaded SLNs were determined 282.3 nm and +8.16 mV, respectively (Figure 2). The particle size was found relatively small with a poly dispersity index (PdI) of 0.255 that was quite desirable (PdI, is an index for describing the monodispersity and polydispersity of nanoparticles). PdI less than 0.3 indicate excellent homogeneity and its high value indicates the high level of non-uniformity (16, 17). The zeta potential was acceptable in terms of the *in vivo* interaction of the nanoparticles with the immunological clearance system of the host body. In this study the SLNs were stabilized with tween 80 to have lower particle size and higher storage stability.

Morphology study

SEM micrograph of the produced gentamicin-loaded SLNs was shown in Figure 3. The gentamicin-loaded

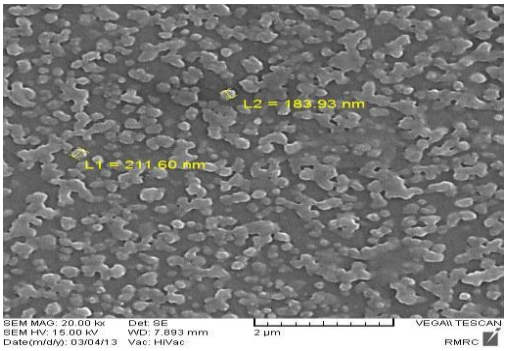

Figure 3. SEM micrograph of gentamicin-loaded SLNs with the size range of 180-280.

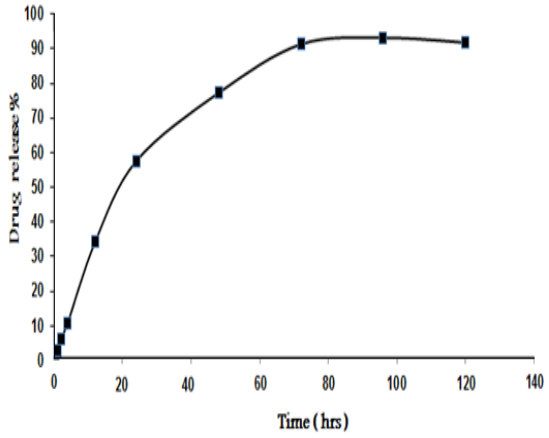

Figure 4. The release profile of gentamicin from SLNs.

SLNs particles are relatively spherical and regular in shape according to the SEM image. There was seen a little amount of agglomeration of particles too. Agglomeration is a phenomenon which arises from the gluing of particles together one by one. One reason for this phenomenon could be due to incomplete drying process. Owing mainly, gentamicin has a hydrophilic nature thus; it is unavoidable that traces of water remain inside the particle or near its surface. The size bar in the image shows that the particles size are between 180 and 280 nm that is in accordance with the results obtained by zeta sizer.

Entrapment efficiency and release study

The percent of gentamicin entrapment was found 40% that is approximately desirable for gentamicin-loaded SLNs. Entrapment efficiency depends on the amount of lipid, solubility of drug in lipid, process temperature and presence of Tween 80 as the surfactant (17). The release profile of gentamicin

Table1. Particle size of gentamicin-loaded SLNs during stability test.

	Refrigerated condition		Room temprature	
Time(h)	Size(nm)	Zeta potential (mV)	Size(nm)	Zeta potential (mV)
0	282.3	8.16	281.7	8.21
1	282.3	8.16	284.2	8.06
2	283	8.55	282.9	8.65
4	283.6	8.41	280.7	7.21
8	284.4	8.32	284.2	8.92
12	284	9.2	285.1	7.82
24	282	7.93	283.3	8.93
48	282.5	7.93	282.7	7.93
72	283	8.0	282.9	8.6

analysed by UV-spectrophotometry was shown in Figure 4. As it can be seen during the first 24 h, a quick release is observed (about 60 percent of the total amount of entrapped gentamicin is released within this time). Then, in continuing a sustained and gradual release profile is prolonged for 72 h (about 94% of the total loaded gentamicin is released during this time). This is relatively a reasonable model for drug releasing from nanoparticles, and it can be inferred that solid lipid nanoparticles are good carriers for stable gentamicin release.

Stability study

The most principal characteristics used for evaluation of nanoparticles stability are the particle size distribution and zeta potential. According to the results, the nanoparticles size remained unchanged for 72 h and there was not seen the swell ability of the particles in aqueous media. This feature is very important with respect to long-circulating therapeutic targets (Table 1).

Antimicrobial activity

The agar well diffusion test was performed on Mueller-Hinton agar. According to the results, gentamicin-loaded SLNs possess antimicrobial activity against *Staphylococcus aureus*, but there was no significant difference between gentamicin-loaded SLNs, free gentamicin and the blend of gentamicin and SLNs (Non-loaded) in their antibacterial activities (table 2). Free SLNs showed no antibacterial activity too. The results for MIC and MBC determination were shown in table 3. As it can be seen, there is no observable difference between the MIC and MBC of

Table 2. Antibacterial susceptibility test by measurement of bacterial inhibition zones around the wells.

Trial groups	Zones of inhibition around the wells in mm
gentamicin-loaded SLNs	26.33±0.57
Free gentamicin	26.66±1.52
Unloaded SLNs	0
Blend of gentamicin and SLNs (Non-loaded)	26.50±0.47
Normal saline solution as negative control	0

Table 3. MIC and MBC for free gentamicin and SLNs loaded by gentamicin.

	free gentamicin (µg/mL)	Gentamicin-loaded SLNs(µg/mL)
MIC	6	6
MBC	7	7

free gentamicin and SLN-loaded gentamicin. This means that, loading of gentamicin on SLNs does not lead to an enhancement in the antimicrobial function of gentamicin. In other words, SLNs has no antimicrobial activity itself.

Conclusion

Based on the findings; SLNs are good vehicles for gentamicin drug delivery with desirable release characteristics and without any effect on antibacterial activity of gentamicin.

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