Journal of Biochemistry and Cell Biology

Solid Lipid Nanoparticles of Guggul Lipid after Pulmonary Delivery

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Abstract

Using guggul lipid as the primary lipid component, diclofenac sodium-loaded solid lipid nanoparticles (SLNs) were created and then their physical characteristics, permeation profiles, and anti-inflammatory action were examined. The SLNs were made utilising the melt-emulsion sonication/low temperature-solidification process, and they were then evaluated for physical characteristics, in vitro drug release, and accelerated stability experiments before being formed into gel. Regarding drug absorption in vivo and ex vivo as well as anti-inflammatory effectiveness, various gels were compared with a commercial emulgel (CEG) and a simple carbopol gel containing drug (CG). Maximum physical parameters kept the SLNs steady. The two particles with the highest in vitro drug release were GMS nanoparticle 1 (GMN-1) and stearic acid nanoparticle 1 (SAN-1). The receptor fluid drug concentration of Guggul lipid nanoparticle gel 3 (GLNG-3) was 104.68 times higher than that of CEG. With regard to CG, GLNG-3's enhancement ratio was 39.43. At 4 hours, GLNG-3 revealed to be around 8.12 times greater than CEG. AUC for GLNG-3 was 15.28 times greater than AUC for CEG. In the first hour, edoema inhibition in GLNG-3 reached 69.47%. The features of SLN are controlled by the primary lipid component's physicochemical characteristics. The physicochemical characteristics and stability of SLN derived from guggul lipid were good. Additionally, it demonstrated a favourable penetration profile as well as a regulated drug release profile. The primary goal of this research was to investigate the biodistribution of amikacin solid lipid nanoparticles (SLNs) after pulmonary delivery to raise its concentration in the lungs for treating cystic fibrosis lung infections and to offer a fresh approach to amikacin therapeutic application. In order to achieve this goal, 99mTc-labeled amikacin was loaded in cholesterol SLNs. Following in vitro optimization, the appropriate SLNs and free drug were delivered through the pulmonary and routes to male rats, and quality and biodistribution experiments were carried out. The outcomings demonstrated that pulmonary delivery of SLNs of amikacin by microsprayer caused higher drug concentration in lungs than kidneys while i.v. administration of free medication generated reverse conditions.

Keywords: Amikacin; Pulmonary; Nanoparticles

Introduction

The ethyl acetate extract of guggul resin, known as guggul lipid, is recognised by the Indian Pharmacopoeia as coming from the plant Commiphora wightii (family: Burseraceae). The substance that makes guggul lipid active is guggulsterone (4, 17(20)-pregnadiene-3, 16-dione), which ranges in concentration from 4.0 to 6.0%. A combination of guggulsterone's E and Z stereoisomers can be found in guggul lipid [1]. A powerful antilipidemic among them is Z-isomer. By altering the active component's release, lipid-based formulations are a significant category that can be utilised to affect how quickly an active ingredient is absorbed. Lipid-based carriers are desirable pharmaceutical formulation possibilities due to their biocompatibility. Solid lipid nanoparticles (SLNs) were created in the early 1990s and have since been seen as promising drug delivery methods, particularly when it comes to giving the active ingredient they contain a sustained-release profile [2]. The key benefits of SLNs over other conventional drug carriers include superior biocompatibility, reduced cytotoxicity, drug targeting, modulated drug release, and the potential for large-scale industrial production. The selective permeability of skin poses the main obstacle to these efforts, despite the fact that using the skin as a route of administration offers benefits such ease of access, avoidance of first pass metabolism, and gastrointestinal problems [3]. The epidermis and dermis make up the skin. Stratum corneum, the top layer of dead cells in the epidermis, is present. The cell envelope that surrounds corneocytes in SC is made up of cross-linked proteins and a lipid envelope that is covalently bound. The lipid lamellar regions that the corneocytes are embedded in are also oriented parallel to the corneocytes' surface [4]. The SC lipids are crucial in structuring and maintaining the lipid barrier, which protects against outside aggressors and water loss through the skin and is what causes the skin's selective permeability [5].

There are a number of techniques that have been developed to increase the transdermal drug absorption, including physical ones like iontophoresis, electroporation, ultrasound, and ablation, as well as chemical ones like alcohols, terpenes, and azones. In the current work, we created an SLN formulation utilising diclofenac as a model medication and Guggul lipid as the primary lipid component, and we assessed it for physical characteristics, stability, and anti-inflammatory effect [6]. The created formulations were compared with an established, commercial transdermal emulgel containing diclofenac diethylammonium. A carbopol gel formulation containing free diclofenac sodiumwas also developed and assessed for release.

In the Caucasian community, cystic fibrosis (CF) is the most prevalent deadly hereditary condition with autosomal recessive heredity. Exocrine glands in the airways produce secretions with an exceptionally high viscosity, which define this illness. Mucus' greater viscosity causes a reduction in the removal of microorganisms from the respiratory tract as well as a long-term bacterial infection of the airways [7]. Cystic fibrosis infections are treated with the aminoglycoside amikacin, which is effective against the majority of gram-negative bacteria. Due to the significant nephrotoxicity side effects caused by the large doses of this antibiotic required to achieve therapeutic levels

in the lungs. There would be a lot of interest in a medication delivery method that improved the therapeutic index of aminoglycosides by raising the concentration of the drug at the infection site and/or decreasing nephro- and ototoxicity. Amikacin dry powder inhaler, intratracheal gentamycin delivery, high dose nebulization of amikacin, and DPI formulation for cystic fibrosis patient solid lipid nanoparticles (SLNs) of amikacin were designed, optimised, and characterised in vitro are just a few of the delivery systems that have been investigated so far to reduce these toxicities [8]. The administration of local drugs through the lungs is a potential alternate route. In addition to achieving increased medication concentration at the primary site of infection, direct drug administration to lung infection conditions may also finding in decreased systemic toxicity. When added to appropriate vehicles, such as aerosols or dry powders, particles in the size range of 100–500 nm can successfully deposit into various respiratory tract regions. It is anticipated that in the near future, inhalable therapeutic agents loaded into nanoparticles may be used to treat pulmonary diseases. The pulmonary distribution of colloidal carriers has been the subject of various investigations published recently. Amiloride hydrochloride is a good example of a medication that is delivered through the lungs using colloidal carriers [9].

Materials and Methods

Materials

While Guggul lipid was obtained from Sami Labs Limited, Bangalore, Karnataka, India, the free sample of Diclofenac Sodium was provided by Asoj Soft Caps, Baroda, India. All of the additional compounds were of analytical grade and purchased from Sigma-Aldrich, including glyceryl monostearate (1-stearoyl-rac-glycerol), stearic acid (octadecanoic acid), and Poloxamer 188 (polyethylene-polypropylene glycol) (New Delhi, India). The commercial version of Voltaren Emulgel (Novartis) contained 1.16% weight-per-weight diclofenac diethylammonium, which is comparable to 1% weight-per-weight diclofenac sodium.

Methods

Nanoparticle Formulation

The nanoparticles were made according to the composition using the melt-emulsion sonication and low-temperature solidification procedures. Lipid and drug were dissolved in ethanol (10 mL) and heated to the melting point of the lipid. The melted oil phase was then given a 70°C mixture of Poloxamer 188 and double-distilled water. The final emulsion was mechanically agitated at 10,000 rpm for 10 minutes before being sonicated with a probe sonicator for 15 minutes at 100 W amplitude to create a nanoemulsion. This nanoemulsion was then quickly dipped into ice water (0°) to solidify the nanoparticles. The dispersion was then passed through a membrane filter to remove any particles larger than 0.45 m.

Size Distribution and Charge Characteristics

Size and form were determined using TEM together with phosphotungstic acid negative staining. A drop of the sample was placed on a copper grid, a 1% w/v solution of phosphotungstic acid was added, and the mixture was dried. The samples were examined using Philips CM-10. Using the Zetasizer NanoZS, which has a 4 mW He-Ne laser (633 nm), photon correlation spectroscopy was used to calculate the polydispersity indices and zeta potentials. The formulations were suspended in phosphate buffer (pH 7.4) before analysis.

In Vitro Drug Release through Synthetic Membrane

A synthetic membrane made of cellulose acetate with a molecular weight limit of 12 KDa was used to estimate drug release. Prior to the experiment, the membrane was placed in a Franz diffusion cell with a nominal surface area of 3.14 cm2 and adjusted to equilibrium in buffer (pH 5.5) at °C. After applying 1 g of the formulation to the donor side, the acceptor compartment was filled with buffer (pH 5.5). At certain intervals, aliquots were obtained, and an assay was used to determine the amount of medication in each one. A new buffer was used to replace the volume.

Gel Preparation [10]

Carbopol 934 was used to create gel from each and every SLN formulation (carboxyvinyl polymer). Using a mechanical stirrer, a suitable amount of carbopol 934 was dissolved in water to form 1% w/w dispersion. The dispersion was then neutralised with 0.5% v/v triethanolamine. To allow for any trapped air to be removed, the gel was left on for the entire night. The drug concentration was then maintained at 1% w/w before SLNs were introduced.

Preparation of SLNs of Amikacin

To produce particles with the ideal particle size and drug loading effectiveness, the SLNs of amikacin were synthesised as previously reported. In a nutshell, deionized water containing 1% w/w Tween 80 was used to dissolve 50 mg of amikacin or 99mTc-amikacin in PBS. This solution was then homogenised at 11000 rpm to reach a maximum volume of 50 mL. A 3:1 combination of ethanol and acetone was heated to 70°C while being stirred to dissolve 150 mg of cholesterol in the lipid phase. The hot oily phase was then mixed with the aqueous phase at 25°C while being homogenised at 11000 rpm. To produce nanoparticles, the produced emulsion was placed in a bath sonicator and sonicated before being cooled to room temperature.

Animal Studies

For the in vivo tests, male Wistar rats weighing 180–200 g from the animal house of Isfahan University of Medical Sciences' School of Pharmacy and Pharmaceutical Sciences were employed. The animals were kept in colony cages with free access to regular chow pellets and water, in uniform housing under environmentally controlled conditions (22 2°C, 12 h of light-dark cycle, 55-65% humidity), and they were kept in the lab for 4-6 days during the acclimatisation period and during the course of the study.

Drug Dosing Methods

We examined eight groups of male Wistar rats, weighing between 180 and 200 g. The first group (group A) received radio-labeled amikacin SLNs via the pulmonary route, the second group (group B) received free radio-labeled amikacin pulmonary, the third group (group C) received radio-labeled amikacin i.v., and the fourth group (group D) received radio-labeled amikacin i.v. Three of the nine rats in each of these groups were euthanized at time points Blood samples were obtained from the tail vein at the desired time periods (5, 15, 60, 120, 180, 240, and 360 minutes) from three rats in each of these four groups that were killed after 6 hours. The tail vein was used to place the i.v. catheter. Administration of medication and gathering blood samples. The control groups, Groups E and F, were given i.v. and pulmonary administration of blank SLNs. corresponding routes a free, non-radioactive amikacin solution was administered intravenously to Groups G and H, two additional control groups. And respiratory pathways to rule out any potential interactions with the drug testing

procedure. The rats in each subgroup were killed using CO_2 gas in the desired time point to detect drug concentration; before that, gamma scintigraphy was done to provide direct image of radio-labeled drug in whole animal bodies. Each control group contained three animals, and one of them was killed in each desired time point to remove organs for examination of drug concentration.

Gamma Scintigraphy Analysis

By visualising the particles that had been accumulated in the lung using -scintigraphy, medicinal compounds were discovered. The amount and location of the medication that was deposited in the lung after injection could be directly determined by imaging. For each study, a single-headed camera with a high resolution low energy gamma collimator was employed.

Results

The stability of the chosen SLN formulations (GMN-3, SAN-3, and GLN-3) was examined over a 180-day period. SAN-3 underwent the most significant changes in particle size (74.5 nm), PDI (0.09), entrapment efficiency (12.93%), and drug release (7.54%), while GMN-3 underwent only minor changes in particle size, PDI, entrapment efficiency, and in vitro drug release. Compared to GMN and SAN, GLN-3's zeta potential exhibited a greater backward trend. While GLN-3's zeta potential increased, that of GMN-3 and SAN-3 decreased. It was discovered that GLN-3 had the most stable formulation, with almost any changes to the physical properties. CG and CEG versus the penetration profile of the drug from gel formulations across the whole thickness of human skin. In contrast to CG and CEG, which showed drug penetration lasting only up to 14 hours, SLN formulations demonstrated drug permeation lasting up to 24 hours. Additionally, GLNG-3 (141.32 g/cm2) showed the highest penetration levels. The GLNG formulations followed by GMNG and SANG formulations had the maximum drug penetration to the receptor fluid. According to the permeation of drugs through human skin, steady state drug flux, lag time, permeability coefficient, diffusion parameter, and enhancement ratio were computed. The maximum flux (6.363 g/cm2/h) and enhancement ratio (39.43) with regard to CG were displayed by GLNG-3. After 6-7 days, SLN gels started to swell slightly. No component of the SLN gel produced any erythema or other skin responses. Every SLN gel formulation has an irritation index under 0.5.

After being administered for 0.5 and 6 hours, respectively, gamma scintigraphy images of the animals receiving 99mTc-amikacin SLNs by intravenous and pulmonary routes. These numbers demonstrate that following pulmonary delivery as opposed to intravenously, amikacin SLNs stayed in the lungs for a longer period of time.

Additionally, when comparing the pulmonary and intravenous methods, the medication concentration in the lungs lasted longer than it did in other organs. The SLNs were disseminated throughout the body after intravenous treatment, but after pulmonary injection, even six hours later, the medication was more concentrated in the lungs. The drug concentration in the lungs at various times was compared using a two-way ANOVA test. Data analysis in Group A receiving 99mTc-amikacin via intravenous SLNs revealed no significant difference between drug concentration in the lung at various time points (P > 0.001), but in Group B receiving intravenous free 99mTc-amikacin, differences in lung concentration at various time points were significant and decreased over time (P 0.05). Data analysis revealed no statistically significant variations in the kidneys between the analysed time points in the group receiving pulmonary free 99mTc-amikacin (P > 0.05). As an outcomes of the pulmonary distribution of free 99mTc-

amikacin, the drug concentration in the kidneys was stable and did not alter over time. However, at various time intervals, pulmonary delivery of 99mTcamikacin SLNs revealed a steady rise in drug concentration in the kidneys.

Discussion

Solid lipid nanoparticles play a key role in medication delivery. For controlled drug delivery through transdermal application in the current study, we created SLN formulations of GMS, SA, and Guggul lipid. The predominant lipid component in each SLN formulation was GMS, SA, or guggul lipid. Guggul lipid is a planar molecule, in contrast to GMS and SA, which both only have one hydrocarbon chain. The impact of their structure can be seen in the physical characteristics of the SLN and lipids stated above. Guggul lipid SLN were smaller than SLN synthesised with either GMS or SA, according to the particle size distribution. Lipid molecules may stack together to create a more compact nanoparticle, which could be the cause. Increased lipid content produced more evenly sized SLN, regardless of the type of lipid, as evidenced by the fact that polydispersity indices fell with increasing lipid content in each formulation group. Surface charge and surface area combine to form zeta potential. When compared to bigger size SLNs, smaller size SLNs typically yield higher surface area, as is the case with GLN formulations. Guggul lipid-containing SLN, however, was less negatively charged than SLN that included either SA or GMS. Because SA is 75% ionised at skin pH and GMS produces SA residues that give the negative charge, this is the case. The amount of lipid phase has an impact on encapsulation effectiveness. With more fat, encapsulation efficiency improved in each category. The most effective encapsulation was demonstrated by guggul lipid SLN. The electrostatic attraction between the negatively charged diclofenac molecule and the negatively charged lipid components in GMN and SAN formulations may be the cause of this observation. The highest drug release during the drug release trial was shown by SAN-1, followed by GMN-1. The formulation including guggul lipid demonstrated regulated drug release for 24 hours, and even then, GLN-3 was still able to hold onto nearly 26.46% of the medication. However, GLN formulations maintain a significant amount of drug despite being in the smaller size range, which further enforces the possibility of better packing of drug. Usually, smaller particles release more drug content due to their large surface area and low diffusional distance to be travelled by the drug molecule.

After pulmonary administration, analyse the biodistribution of amikacin SLNs. Other research was done to target the site of infection using medications to lessen adverse effects in conditions like cystic fibrosis. In the current investigation, amikacin was chosen as the antibiotic of preference for treating lung infection in cystic fibrosis patients. High aminoglycoside doses are required for this condition, which has side effects, particularly nephrotoxicity. This demonstrates the significance of utilising a proper drug delivery mechanism to locally transport the medicine to the site of action and reduce drug toxicity. Amikacin SLNs were created with the correct size to control drug release and the ability to reach the deep alveolar regions of the lungs in order to do this. In order to compare the biodistribution of the drug via pulmonary and intravenous routes, as well as to look into the possibility that amikacin SLNs might reduce kidney concentrations of the drug while potentially increasing lung concentrations, the optimised amikacin SLNs and free drug were given to animals. Use of gamma scintigraphy to monitor the accumulation and elimination of liposomes containing amikacin in healthy male volunteers. While in our investigation, amikacin was prelabeled by 99Tc for in vivo studies, allowing us to follow the free and loaded drug at each time, the lipid

Citation: Toe L (2022) Solid Lipid Nanoparticles of Guggul Lipid after Pulmonary Delivery. J Biochem Cell Biol, 5: 163.

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employed in the formation of liposomes was labelled by the other group. Comparatively to the uncoupled drug loaded in nanoparticles and plain drug solution, lactose-nanoparticle coupling considerably improved the drug's lung uptake, leading to a higher percentage of doses retrieved from the lungs. When animals were intravenously injected with azithromycin liposomes or free azithromycin solution, the liposomes produced slower clearance, an extended half-life, and a 7.4-fold increase in lung AUC relative to the drug solution. Dexamethasone SLNs achieved a 17.8-fold greater area under the curve of dexamethasone than its solution, according to biodistribution studies and lung-targeting effectiveness in mice following intravenous injection.

Conclusion

Three different lipids were assessed for SLN formulation in the current study because SLN is a significant medication delivery method. Based on the findings, it can be said that these SLNs demonstrated ideal physical properties and a permeation profile, as well as promising stability and high skin compatibility. The formulation with the highest concentration of Guggul lipid among all of the formulations was discovered to be GLNG-3, making it the most promising.

Acknowledgement

Conflict of Interest

The author has no known conflict of interest associated with this paper.

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