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Application of solid lipid nanoparticles preparation in infection caused by antibiotic-resistant bacteria

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ABSTRACT

Solid lipid nanoparticles (SLN) is a potential alternative method for drug delivery due to its good stability, low toxicity, can be modified and controlled in drug release, as well as can be produced on a large scale. The SLNs are composed of solid lipids stabilized by emulsifiers. The lipids used for SLN are physiological lipids that easily tolerated by the body. These characteristics make SLN as a potential delivery system that can increase the efficiency of an antibiotics preparation. The development of bacterial resistance to antibacterial has become serious health problems in infectious diseases. Solid lipid nanoparticles is a compelling choice as a drug delivery system that can reduce the problem of the bacterial resistance. This review discussed the role of SLN as a drug carrier system which includes the characteristics and structure of SLN, its pharmacokinetic properties, bacterial resistance mechanism, and SLN mechanism in reducing bacterial resistance.

ABSTRAK

Nanopartikel solid lipid (*solid lipid nanoparticle/*SLN) merupakan metode alternatif penghantaran obat yang potensial karena stabilitasnya yang baik, toksisitasnya rendah, dapat dimodifikasi dan dikendalikan pelepasan obatnya, serta dapat diproduksi dalam skala besar. Nanopartikel solid lipid tersusun dari lipid padat yang distabilkan oleh pengemulsi. Lipid yang digunakan untuk SLN merupakan lipid fisiologis yang ditoleransi oleh tubuh. Karakteristik tersebut menjadikan SLN sebagai sistem penghantaran potensial untuk meningkatkan efisiensi sediaan antibiotik. Perkembangan bakteri yang resisten terhadap antibiotik telah menjadi masalah kesehatan yang serius dalam penyakit infeksi. Nanopartikel solid lipid menjadi pilihan sebagai sistem penghantaran obat yang dapat mengurangi masalah resistensi antibiotik. Dalam ulasan ini, dibahas peran SLN sebagai sistem penghantar obat yang mencakup karakteristik dan struktur SLN, sifat farmakokinetik, mekanisme resistensi bakteri, dan mekanisme SLN dalam menurunankan kemampuan resisten mikroba.

INTRODUCTION

Nanoparticles are used in medical treatment as drug carrier in various diseases. Lipid-based nanoparticles use in drug delivery systems were discovered early on by professor RH Müller from Germany and professor M. Gascon from Italy since the early 19th century.[1] Nanoparticles are produced with nano-sized structures by pharmaceutical nanotechnology, to deliver drugs in particles that can be easily absorbed and provide a pharmacological response with minimal side effects.[2]

The advantages of lipid-based nanoparticles are ease of production on a large scale, non-toxicity, controlled drug release, and increased drug solubility.[3] The use of lipid-based solid nanoparticles as an antibiotic delivery agent is quite promising in overcoming drug resistance problems. Bacterial resistance to antibiotics can occur

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when bacteria develop the ability to evade the effects of drugs that can inhibit growth or kill bacteria. The high number of antibiotic resistance has prompted the development of various strategies to overcome multidrug-resistant (MDR) bacteria. One of them is by utilizing an optimization approach to the pharmacokinetic profile of drugs through nanoparticles. This approach can be applied to overcome the problem of bacterial resistance.[4]

The use of nanoparticles in improving the effectiveness of various therapeutic drugs in complex diseases, such as cancer and autoimmune diseases, has provided new solutions in the development of drug delivery systems for the treatment of infections caused by MDR.[5] This review is focused on seeing how the application of SLN can be a promising strategy to overcome or reduce the occurrence of antimicrobial resistance (AMR). The main topics of discussion include the characteristics and structure of SLN, pharmacokinetic properties, AMR mechanism, and SLN mechanism in reducing microbial resistance ability.

DISCUSSION

Solid Lipid Nanoparticle (SLN)

Solid lipid nanoparticles are spherical colloidal drug delivery systems with diameters ranging from 40-1000 nm.[6] The main components of SLN are a biodegradable physiological lipid such as glycerides, sterols, fatty acids, and waxes with a ratio 0.1% (w/w) to 30% (w/w). The lipid is used as a nanoparticle matrix which is supported by lipid properties that can solidify at room temperature. Another important component of SLN is surfactants with a concentration of 0.5% (w/w) to 5% (w/w) which is used to stabilize the dispersion of lipid in liquid medium.[7] Additionally, the material used for SLN preparation has a generally recognized as safe (GRAS) status approved by FDA.[8]

Types of SLN

In general, the structure of SLN is presented in FIGURE 1. Based on drug incorporation position, SLN is classified into three models 1) SLN type 1 or homogenous matrix model, characterized by uniformly dispersed drug in the lipid matrix; 2) SLN type 2 or drug-enriched shell model, characterized by the drug concentrated in the still liquid outer part, and 3) SLN type 3, drug-enriched core model, characterized by the drug that concentrated in the core part of SLN.
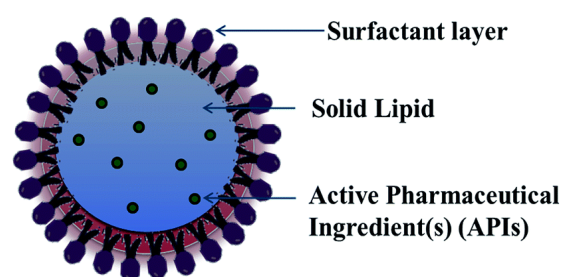

FIGURE 1. The structure of SLN.[9]

The position of drug incorporation can significantly affect drug release from nanoparticles. Homogenous matrix model and drug enriched core model are suitable for controlled release, while drug-enriched shell model is suitable for burst release. These models and their release system can be prepared by various methods.[10]

Preparation of SLN

Solid lipid nanoparticles and drug incorporation can be prepared by hot homogenization and cold homogenization methods which are the most common methods in SLN production.[12] In the hot homogenization method, the lipid must be melted at 5–10°C above its melting point to solubilize the drug. The drug-containing melt is then dispersed into a hot surfactant solution to obtain pre-emulsion. To produce nanoemulsions, pre-emulsion is homogenized at high pressure (500-1500 bar) and upon cooling at room temperature, nanoemulsions are solidified to form solid lipid nanoparticles (FIGURE 2).[13]

FIGURE 2. Hot homogenization method

In high temperature from hot homogenization process may cause degradation of temperature-sensitive drugs. Therefore, cold homogenization is more suitable than hot homogenization to incorporate these drugs into nano-carriers. This method performs rapid cooling using liquid nitrogen or dry ice to obtain solid solution. The solution is then converted into microparticle and dispersed into cold surfactant solution. In the next step, high pressure is applied to microparticle at room temperature to produce solid lipid nanoparticle.[14]

Pharmacokinetic Properties of SLNs

Absorption

Solid lipid nanoparticles possess physical and chemical properties that improve drug stability in gastrointestinal (GI) tract. The small size of SLNs can increase surface area and provide bioadhesion at the intestinal wall. The ability to perform bioadhesion can prolong the residence time of the drug and maintain close contact with epithelial membrane, resulting in better absorption. In addition, triglycerides, the main component of SLNs, will undergo hydrolysis by lipase to produce mono- and diacylglycerol. Mono- and diacylglycerol then induce bile salt secretion to form a structure called micelle that enhances drug absorption.[15] Furthermore, SLNs also induce chylomicron formation which is secreted into lymphatic system. Drugs are then transported through lymphatic system to avoid first-pass metabolism.[16]

Biodistribution

Encapsulation with SLNs can affect drugs absorption and transport pathway. The difference between long-chain triglycerides and medium-chain triglycerides also has an impact on intestinal uptake and lymphatic transportation. The SLNs with long-chain triglycerides require the formation of chylomicron for transport via lymphatic circulation which can increase drug bioavailability, while SLNs with medium-chain do not induce chylomicron formation.[17] The uptake of SLNs into lymphatic system can occur via two mechanisms: M-cell-mediated uptake and paracellular/transcellular route.[18] Basically, SLN properties such as particle size and shape can affect their bioavailability. Small particles tend to adhere to cell-surface while large particles (>200 nm) are filtered out by reticular meshwork.[19]

The capability of SLNs to enter blood circulation through lymphatic system

leads to differences in the distribution of the drug incorporated into SLN with conventional drugs. Luo et al.[20] reported that the oral administration of puerarin in SLN (Pue-SLN) showed a higher concentration compared to convention puerarin (the C_{max} for Pue-SLNs were 2.06 times of conventional puerarin). Conventional puerarin reached C_{max} in rats around 110 min while the time to reach C_{max} for Pue-SLNs was about 40 min. For the tissue concentration-time profiles, the Tmax value of Pue-SLNs was 1 h in the liver, spleen, kidney, and lung, 6 h in the heart, and 2.5 h in the brain. Increased concentration of puerarin in the lung and heart indicates a protective effect against cardiocerebrovascular disease. In a separate study by Wang et al.[19] using another substance (genoposide), it was reported to have similar results. The concentration of genoposide loaded into SLN was increased in various tissues, especially in the target organs such as liver, spleen, lung, and brain. The ability of SLN to penetrate the blood-brain barrier (BBB) is probably due to the interaction of SLN with ApoE, Apo C-II, albumin, and immunoglobulin G which belong to brain-specific targets. In addition, genoposide-SLN with its small size (116.5 nm) can pass through the reticuloendothelial system causing prolonged circulation time.

Elimination

Incorporation of drug can lead to alteration in the drug excretion pattern. Excretion of drug usually becomes slower after the oral administration of SLN drug resulting in a decrease of clearance rate. These alterations can be caused by two factors 1) properties of SLN that allow particles to perform bioadhesion thereby prolonging the residence time of the drug in the GI tract; and 2) the tendency of SLNs to enter lymphatic system thus avoiding first-pass metabolism which in turn increases bioavailability and reduces the clearance of drugs.[20,21]

Antibiotic Resistance Mechanism

Antibiotic resistance can develop both naturally and acquired. Natural resistance occurs intrinsically, where it is always expressed on bacteria without prior exposure to antibiotics, or induced, where bacteria expressing resistance after antibiotic exposure. In intrinsic resistance, the mechanism is mainly caused by reduced permeability of bacterial cell membrane, especially on Gram-negative bacteria which has lipopolysaccharide on the cell membrane, and efflux pump activity. Acquired resistance is caused by horizontal genetic material transfer from one bacterium to another, by either transformation, transposition, or conjugation. DNA mutation on bacterial chromosomes can also cause resistance. Resistance gene transmitted mainly by plasmid.

There are 4 main mechanisms in resistance i.e. 1) limiting drug uptake; 2) drug target modification; 3) drug inactivation; and 4) active drug efflux. Intrinsic resistance can occur by limiting uptake, drug inactivation, and drug efflux. Meanwhile, acquired resistance can be done by drug target modification and drug efflux. There are different mechanisms between Gram-negative and Gram-positive bacteria because of their different membrane structure. Gram-negative bacteria can reach resistance by all those mechanisms stated above, but on Gram-positive bacteria there is no resistance caused by limiting uptake and some mechanisms of drug efflux.[22]

Limiting uptake

Characteristics of bacteria affect antibiotic uptake. On Gram-negative bacteria which have lipopolysaccharide, the cell membrane acts as a barrier against certain molecule, so that Gram-negative bacteria are resistant against drug with large molecule. Mycobacterium has an outer membrane with high lipid content which allow hydrophobic drug

to enter its cell. Gram-positive bacteria do not have an outer membrane so there is no resistance caused by limiting drug uptake. Bacteria with no cell wall like Mycoplasma are resistant to antibiotics targeting cell walls like glycopeptide and β-lactam.[22]

Drug target modification

Bacteria can experience changes that occur on antibiotic targets. These changes can be point mutation on genes which coding target site, enzymatic changes on binding site, and replacement/bypass on target.[23] All those mechanisms reduced the affinity between antibiotics and their target. For example, mutation on *rpsL* gene which is located on *M. tuberculosis* chromosome that codes ribosomal protein 12s caused MTB resistance to streptomycin. On MRSA and *S. pneumoniae* resistant to penicillin there is a mutation on PBP which reduce β-lactam antibiotic's affinity to the target.

Inactivation of antibiotic by bacterial enzyme

Bacteria can inactivate or degrade antibiotics by producing enzymes that cause hydrolysis, redox reaction, or modification of chemical groups. One of the most known enzymes is β-lactamase, which inactivates antibiotics by cleaving the β-lactam ring. Extended-spectrum β-lactamase (ESBL) has total resistance to β-lactam antibiotics. There are more than 180 types of ESBL that have been identified, and they are found in *E. coli*, *Proteus mirabilis*, and *K. pneumoniae*.[24]

Efflux pump

Bacteria can form a system which pumps toxic substance out of their cells to extracellular environment. Efflux pump system was first found on the beginning of 1980s, where *E. coli* can expel tetracycline out of its cytoplasm with efflux pump. Efflux pump is present on both Gram-positive and Gram-negative bacteria. Efflux pump can either be specific to a substrate or nonspecific to some substrate (broad substrate specificity). Broad substrate specificity can be found on MDR bacteria. Efflux pump protein is coded by genes on mobile genetic element (MGE) or chromosome. Resistance mechanism by efflux pump occurs to various types of antibiotics like protein synthesis inhibitor, fluoroquinolone, β-lactam, carbapenem, and polymyxin.[22]

Biofilm-based AMR

Biofilm is a bacterial defense mechanism against the environment by forming a layer of extracellular polymeric dense matrix composed of various bacteria species which adheres to a surface. Biofilm-forming bacteria have 1000-fold resistance compared to planktonic bacteria. Antibiotic resistance caused by biofilm formation has 2 main mechanisms i.e. 1) bacteria are protected from antibiotic penetration by biofilm layer; 2) physiological changes in biofilm-forming bacteria, like slowed growth until dormant phase. *S. aureus* including MRSA are bacteria that is most likely to form biofilm.[25] Biofilm formation can reduce the susceptibility of sensitive bacteria that are part of biofilm.[26]

SLN/NLC Mechanism to Overcome AMR

The use of nanocarrier as a drug vehicle can overcome problems related to biological barriers like AMR mechanism.[27] Solid lipid nanoparticles allow higher surface contact which increases the permeability of cell membrane to drugs and efflux pump. Solid lipid nanoparticles can deliver poorly water-soluble drug and have higher efficiency in encapsulating water-soluble antibiotic. Some studies had been conducted *in vitro* with SLN as an antibiotic carrier. Bazzaz *et al.* reported the use of rifampin carried on SLN against biofilm-forming *S. epidermidis*. It was inferred that the rifampin loaded SLN was able to reduce the biofilm mass as concentration increased.[28] The nanoparticle may interact with the biofilm matrix and help remove bacterial

biofilms. Severino *et al.* showed that SLN-loaded polymyxin can be used effectively in resistant strains of *P. aeruginosa.*[27] Vancomycin loaded in SLN can be held for up to 54 h and increase clearance of MRSA in murine models.[29] Solid lipid nanoparticles improve antimicrobial activity by increasing drug permeation through bacterial cell wall and other barriers. They can reduce drug expulsion caused by efflux pump activity and protect antibiotics from enzymes that can modify antibiotics. However, there is no publication that explains about the protection of SLN against bacterial enzymatic activity. Furthermore, SLNs increase cell uptake of antibiotic because of its nano size.

Nanostructured lipid carrier (NLC) is the improved version of SLN, which has advantages like larger capacity and stability in carrying more amount of drug because its solid lipid component is replaced by oil. The disadvantage of SLN like drug expulsion caused by SLN polymorphism can be minimalized by NLC structure. Nanostructure lipid carrier has been used as an antibiotic vehicle. Vairo *et al.* studied NLC loaded with sodium colistimethate and amikacin *in vitro* and *in vivo*. The encapsulation process with NLC did not reduce the efficacy of both drugs and they were effective against extensively resistant *A. baumanii.*[30] The size of NLCs and their charges increase the penetrability so that the drug can penetrate the biofilm. Muraca *et al.* reported that ciprofloxacin loaded NLCs are potent for *P. aeruginosa* biofilm infections.[26]

CONCLUSION

Solid lipid nanoparticles have been used as an antibiotic carrier and proven to be effective to be used on resistant bacterial strains *in vitro*. The use of NLC which is the enhanced form of SLN has a higher effectivity rate since it has larger capacities to carry more amounts of drug. Nanocarrier is quite promising for overcoming antibiotic resistance problem, but the effectiveness of antibiotic formulation with SLN/NLC carrier needs to be evaluated by clinical trial on patients.

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