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Reviews

Liposome-encapsulated aminoglycosides in pre-clinical and clinical studies

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Liposome-encapsulated amikacin has recently entered clinical trials. The rationale for liposome encapsulation of aminoglycosides is the possibility to increase the therapeutic index of this class of antibiotics by increasing aminoglycoside concentrations at the site of infection and/or by reducing the toxicity of these drugs. Three approaches can be distinguished: the use of liposomes as a depot formulation for local drug administration; targeting of (relatively) short circulating conventional liposomes to the cells of the mononuclear phagocyte system (MPS) for treating intracellular bacterial infections; and targeting of long-circulating liposomes to infectious foci localized outside the MPS. This review discusses the pre-clinical and clinical data in connection with recent developments in liposome technology.

Introduction

Aminoglycosides

After the introduction of streptomycin in 1944, aminoglycosides developed into an important class of antibiotics. Their broad antimicrobial activity, post-antibiotic effect, synergy with β-lactam antibiotics, rapid, concentration-dependent bactericidal activity and low cost contributed to their success, as well as a low frequency of resistance to them.[1–4] However, they require parenteral administration. Moreover, dose-related adverse effects on kidneys and audio-vestibular apparatus make it necessary for the plasma concentrations to be maintained within a narrow range.[5–8] Therefore, aminoglycosides are currently used for the treatment of severe (nosocomial) Gram-negative and Gram-positive infections, especially in immunocompromised patients, and for the treatment of mycobacterial infections.[9–12]

A drug delivery system that helps to increase the therapeutic index of the aminoglycosides by increasing the concentration of the drug at the site of infection and/or reducing the nephro- and ototoxicity would attract considerable interest, and liposomal encapsulation of aminoglycosides may provide this.

Liposomes

Liposomes are spherical vesicles, with particle sizes ranging from 30 nm to several micrometres, consisting of one or more lipid bilayers surrounding aqueous spaces.[13,14] Hydrophilic drugs, such as aminoglycosides, can be encapsulated in the internal aqueous compartment, whereas hydrophobic drugs may bind to or are incorporated in the lipid bilayer.[13,15] The bilayers are usually composed of natural or synthetic phospholipids and cholesterol, but the incorporation of other lipids or their derivatives, as well as proteins, is also possible.[13–15] The physicochemical characteristics of the liposome, like particle size, surface charge, sensitivity to pH changes and bilayer rigidity, can be manipulated.[14] Manipulation of these characteristics can have marked effects on the *in vivo* behaviour of liposomes and therefore have a major impact on therapeutic success. Liposomes have also been studied as model membranes regarding the interaction of aminoglycosides with phospholipids in relation to aminoglycoside toxicity.[16–19] The

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present review will focus exclusively on liposomes as a drug delivery system for aminoglycosides.

In vitro data

Extracellular bacteria

The earliest publications on liposome-encapsulated aminoglycosides appeared some 20 years ago. Variable results were reported on the antibacterial activity of liposomal antibiotics against extracellular bacteria. It was generally shown that the concentrations of the liposome-encapsulated aminoglycoside necessary to obtain growth inhibition and killing needed to be substantially higher compared with the free drug.[20–22] Encapsulation of the antibiotic reduces its antibacterial activity because the bacteria are separated from it by the liposomal bilayer. Variability of the *in vitro* data is probably the consequence of the variations in the liposome lipid compositions used, resulting in the encapsulated agents having various release profiles.

In contrast to this general observation, Beaulac *et al.*[23] and Sachetelli *et al.*[24] reported that a liposome formulation composed of dipalimitoylphosphatidylcholine and dimyristoylphosphatidylglycerol encapsulating tobramycin showed a considerable antibacterial effect against a range of Gram-positive and Gram-negative bacteria at concentrations below the MIC of the free antibiotic *in vitro*. They argued that the enhanced antibacterial effect may be due to a fusion mechanism of this liposome formulation with bacteria.[24]

Intracellular bacteria

In vitro studies using intracellularly infected phagocytic cells demonstrated that the phagocytosis of aminoglycoside-loaded liposomes yielded therapeutic intracellular drug concentrations,[25] and consequently enhanced killing of intracellular microorganisms such as *Staphylococcus aureus*,[26,27] *Escherichia coli*,[28] *Brucella abortus*,[29–31] *Brucella canis*[30] and *Mycobacterium avium* complex (MAC).[32–35] A recent report addressed the possibility of further improving liposomal drug efficacy towards infected cells. Liposomes encapsulating gentamicin composed of pH-sensitive bilayers based on dioleoylphosphatidylethanolamine showed an improved antibacterial effect against intracellular *Salmonella typhimurium* and *Listeria monocytogenes* in murine macrophage-like J774A cells when compared with non-pH-sensitive liposome formulations.[36] It is believed that the pH sensitivity of the liposomes promotes drug release in the acidic environment of the lysosomes after phagocytosis by the infected cells.

Local application

Local application of large, multilamellar aminoglycoside-containing liposomes exploits the possibility of using liposomes as a reservoir from which the encapsulated drug can be released slowly, resulting in therapeutically active drug concentrations that are present at the site of infection for prolonged periods of time. Research in this area has focused on intravitreal or subconjunctival injection or topical application of liposomes for treatment of bacterial endophthalmitis or keratitis.[37–43] All studies reported prolonged presence of therapeutic aminoglycoside concentrations compared with administration of the free drug, offering the opportunity of reducing the number of injections necessary for successful treatment. In addition, systemic drug levels remained low. Research has been carried out mainly in rabbits but a single study reported excellent therapeutic results in eye infections affecting AIDS patients.[44]

Similar results to those obtained in the ophthalmic studies were reported after the prophylactic local application of aminoglycoside-loaded liposomes in models of soft tissue infection, burn wounds, prosthetic vascular grafts or surgical wound infections,[45–50] and after intrabronchial/intratracheal administration of liposomal aminoglycosides in rodents.[51–54] Following intrabronchial administration, liposome-encapsulated tobramycin was shown to eradicate mucoid *Pseudomonas aeruginosa* in a model of chronic pulmonary infection.[53] Interestingly, treatment results were dependent on the lipid composition of the liposomal formulation. Free tobramycin as well as tobramycin encapsulated in liposomes with rigid lipid bilayers showed no bactericidal effect, whereas tobramycin in liposomes composed of fluid lipid bilayers was able to eliminate the bacteria. These data are in agreement with data from *in vitro* experiments that have shown that fluid liposomes tend to release encapsulated aminoglycosides faster compared with their rigid counterparts.[54]

Intravenous administration

Conventional liposomes

Circulation kinetics and tissue distribution. Extensive research on liposome behaviour after iv administration has shown that many liposome types rapidly accumulate in the cells of the mononuclear phagocyte system (MPS), particularly in the liver and spleen.[55–57] It is believed that the relatively rapid clearance of the liposomes is the result of opsonization in the bloodstream facilitating MPS recognition and uptake.[58,59] Such liposomes are generally termed 'conventional' liposomes. The rate at which conventional liposomes are taken up by the MPS can be manipulated by controlling the liposome dose, but also by variation of liposomal characteristics such as charge, size and lipid composition. Generally, large, charged liposomes composed of fluid lipid bilayers tend to accumulate in the MPS more rapidly than small, neutral, rigid liposomes.[60] With the objective of reducing the MPS uptake of conventional liposomes, it has been shown that by increasing the liposome

dose, the proportion of liposomes that remains in the circulation can be increased because of saturation of MPS uptake.[61] However, saturation of the MPS should be avoided as it will impair the body's ability to clear micro-organisms from the circulation, which is an important defence mechanism in patients with severe infections.[62,63]

The pharmacokinetics of intravenously administered conventional liposome-encapsulated aminoglycosides generally show that plasma half-lives are prolonged compared with the free drug.[64–68] The blood levels reported in some representative studies of (liposomal) aminoglycosides are shown in Figure 1. Free and liposome-encapsulated drug were administered at equivalent doses. It is important to realize that when injected in the free form the aminoglycoside is completely active therapeutically, while after injection of the liposome-encapsulated form only the released portion is expected to show antimicrobial activity. The tissue distribution of aminoglycosides is greatly changed by liposomal encapsulation, as is illustrated in Figure 2. Free and liposome-encapsulated drug were again administered at equivalent doses. Renal concentrations of aminoglycosides are approximately similar after administration of either the free or the liposome-encapsulated forms, whereas much higher concentrations were observed in the liver and spleen after the injection of the liposome-encapsulated aminoglycosides. The absolute uptake of the liver exceeds that of the spleen when their respective weights are taken into consideration. Swenson *et al.*[66] reported measurable gentamicin levels in the liver and spleen up to 2 and 15 weeks, respectively, after injection of a single liposomal gentamicin dose of 20 mg/kg. Concentrations in other organs achieved with these conventional liposomes are generally insignificant, although a few reports indicated increased concentrations in the lung.[65,68] Interestingly, Ladigina & Vladimirsky[65] showed that in the lungs of mice infected with *Mycobacterium tuberculosis*, a six-fold increase was seen in the amount of drug localizing in the infected lungs. However, absolute drug concentrations remained low.

It has been suggested that after liposome uptake and processing by the MPS cells, the drug may be released into the blood, prolonging drug blood levels. Bermudez *et al.*[69] showed that substantial urinary excretion of amikacin continued for up to 7 days after injection of 50 mg/kg liposomal amikacin, whereas mice that received an equivalent dose of the free drug excreted most of the administered dose within the first day and had an undetectable level in the urine by day 4. Similar results were obtained by Swenson *et al.*,[66] showing cumulative gentamicin urinary excretion continuing up to 10 days after injection of liposomal gentamicin 20 mg/kg. Even at that time point, only 80% of the injected dose was excreted cumulatively.

Safety. Considering the prolonged presence of aminoglycosides in the body, it is unfortunate that studies on nephro- or ototoxicity of 'conventional' liposomal formulations of aminoglycosides are lacking. There are, however, reports comparing the acute toxicity (characterized by convulsions or death as a result of neuromuscular blockade) of free versus liposome-encapsulated aminoglycosides in mice.

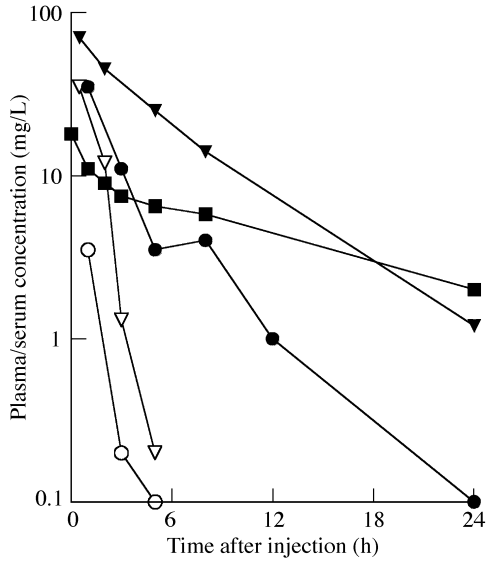

Figure 1. Circulation kinetics of conventional liposome encapsulated aminoglycosides (closed symbols) and free aminoglycosides (open symbols). Aminoglycoside concentrations at indicated time-points after injection of a single dose of gentamicin 20 mg/kg in rats (triangles),[66] amikacin 40 mg/kg in mice (circles)[68] or gentamicin 5.1 mg/kg in AIDS patients (squares).[80]

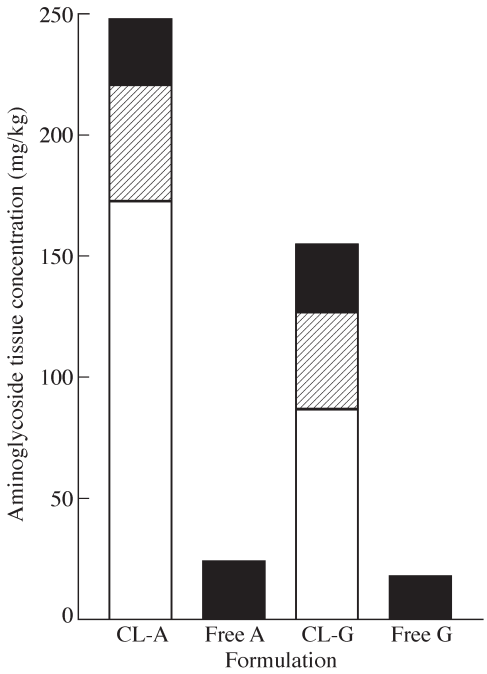

Figure 2. Tissue distribution of conventional liposome (CL)-encapsulated aminoglycosides and free aminoglycosides. Concentrations in tissues (□, spleen; ▨, liver; ■, kidney) at 24 h after injection of a single dose of gentamicin 20 mg/kg (G) in rats[66] and amikacin 40 mg/kg (A) in mice.[68]

Without exception all studies showed a substantial reduction in acute toxicity for the liposome-encapsulated drug.[67,69–71]

Therapeutic efficacy. Generally, because of their hydrophilic nature, aminoglycosides are not the drug of choice for treating intracellular infections inside phagocytic cells. However, conventional liposomes readily accumulate in the MPS.[72–74] Therefore, aminoglycoside-loaded conventional liposomes were initially studied using *in vivo* models of intracellular infections inside the MPS cells. An overview of treatment results achieved with conventional liposome formulations is presented in Table 1.

Promising results are reported regarding a bactericidal effect in the liver and spleen in intracellular infections caused by *Mycobacterium* spp., *Salmonella* spp. and *Brucella* spp.[30,67–71,75–89] A pH-sensitive liposome formulation further increased therapeutic efficacy in liver and spleen in a murine intracellular *S. typhimurium* infection.[88] In some studies a reduced bacterial load in lungs, blood and/or kidneys was also reported, but the antibacterial effects in these organs were always less pronounced and were only achieved at higher dosages. These results illustrate both the strengths and weaknesses of conventional liposomes as carrier systems for antibiotics. On the one hand, liposome-encapsulated aminoglycosides are very efficiently transported into the MPS cells in liver and spleen and consequently high intracellular concentrations can be achieved, resulting in good therapeutic efficacy as shown by prolonged survival and the opportunity to increase the dosing interval. On the other hand, owing to the relatively fast and efficient uptake of the liposomes by the MPS cells, relatively low levels of active drug are seen in organs outside the liver and spleen, and thus only moderate therapeutic effects are observed in these organs.

A limited number of reports describe the therapeutic efficacy of conventional liposomes encapsulating aminoglycosides directed against foci of infection outside the cells of the MPS. The prolonged presence of drug in the body after administration of conventional liposome-

Table 1. Clinical and preclinical therapeutic efficacy of aminoglycosides in conventional liposomes

Infection	Drug used	Result	Comments
Intracellular *B. canis*,[30] *B. abortus*[30,86] and *B. melitensis* infection of liver and spleen[87]	gentamicin,[86,87] streptomycin[30]	Compared with free drug: reduction of number of bacteria in spleen,[30,87] liver[87] and other organs,[30] prolonged survival[86] and high drug levels in liver and spleen.[87]	Empty cationic liposomes did also prolong survival.[86]
Intracellular *S. typhimurium*,[66] *S. dublin*[71] and *S. enteritidis* infection of liver and spleen[67,85]	gentamicin,[66,71] streptomycin[67,85]	Compared with free drug: prolonged survival,[66,67,71,85] reduced acute toxicity,[67,71] and high drug levels in liver and spleen.[85]	No reduction of number of bacteria in lung compared with free drug.[85]
K. pneumoniae sepsis, pneumonia, and thigh infection,[66,89] *E. coli* sepsis[66]	gentamicin[66,89]	Compared with free drug: enhanced therapeutic efficacy in *K. pneumoniae* pneumonia and thigh infection in neutropenic animals,[89] prolonged survival when administered prophylactically,[66] prolonged dosing interval allowed.[89]	Similar efficacy of free and liposomal drug when administered immediately after inoculation.[66]
Intracellular *M. avium–intracellulare* complex,[68,69,75–84] *M. tuberculosis* infection of lung, liver and spleen[70]	gentamicin,[69,76,79–81] amikacin,[68,69,75,79,82, 83] streptomycin,[70,77,78] kanamycin[84]	Compared with free drug: reduction of number of bacteria in liver, spleen,[68–70,75–79] blood,[80] lung[84] and kidneys,[75,84] prolonged survival,[70,82,83] reduced acute toxicity,[69,70] prolonged dosing interval and allowed[82,83] reduction in pulmonary lesions.[84]	No reduction of number of bacteria in lung,[68,70,75–79,82,83] or lymph nodes,[75] compared with free drug. Transient renal insufficiency in one patient,[80] no reduction of number of bacteria in any of the bone marrow core biopsy specimens.[81]

References 80 and 81 concern clinical studies.

encapsulated aminoglycosides has been the rationale behind studying their prophylactic activity against extracellular bacterial infections. Swenson *et al.*[66] showed that the dose of liposome-encapsulated gentamicin needed for protection against a lethal ip infection caused by *K. pneumoniae* or *E. coli* was substantially lower than for the free drug, when administered from 7 up to 2 days before bacterial inoculation. This result is not surprising, since the free drug is almost completely excreted within 24 h after injection. In a single dose study in a murine model of *K. pneumoniae* infection, a single dose of liposome-encapsulated gentamicin 20 mg/kg was more effective than an 80 mg/kg dose of free drug.[89] The prolonged residence time of gentamicin in the body by liposome-encapsulation is probably responsible for the enhanced efficacy.

Long-circulating liposomes (LCLs)

Circulation kinetics and tissue distribution. To enable the liposomes to reach infectious sites outside the major MPS-organs, such as the liver and spleen, it is necessary to decrease the rate of uptake of the liposomes by the phagocytic cells. One way to achieve this is by preparing small, neutral vesicles with a rigid bilayer. Using this approach, NeXstar Pharmaceuticals (currently Gilead Sciences Inc.) have developed MiKasome, a small (*c.* 50 nm) unilamellar liposome formulation containing amikacin. This formulation is currently in clinical trials. Another approach to prolonging the circulation time of liposomes is the incorporation of poly(ethylene glycol) (PEG) coupled to phosphatidylethanolamine in the liposome bilayers. It is believed that the hydrophilic PEG provides a layer of steric hindrance around the liposome reducing liposome opsonization and thereby rapid recognition and uptake by the MPS cells. These liposomes are therefore termed 'sterically stabilized liposomes' (SSLs). The low MPS uptake of the SSLs is to a high degree irrespective of liposome lipid composition, which is an important advantage when tuning the liposome lipid composition for optimal targeting, retention and release.[90–97] Using this approach in our laboratory, we have developed a long-circulating SSL formulation containing gentamicin.[98] Such flexibility in tailoring the liposome characteristics does not apply, for example, to MiKasome, as the lipid composition of MiKasome is restricted to a rigid membrane structure to retain its long half-life.

Studies with aminoglycosides encapsulated in both types of LCL show that drug plasma half-lifes are markedly prolonged. Blood levels obtained for MiKasome and SSL-gentamicin are shown in Figure 3. Studies in rats receiving MiKasome 50 mg/kg demonstrated that the AUC in plasma is increased approximately 130-fold compared with the AUC of an equivalent dose of free amikacin.[99] Similar findings were also seen in rabbits, dogs, rhesus monkeys and humans.[100,101] In man, the mean plasma half-life of MiKasome was 114 h. After 1 week of daily dosing with

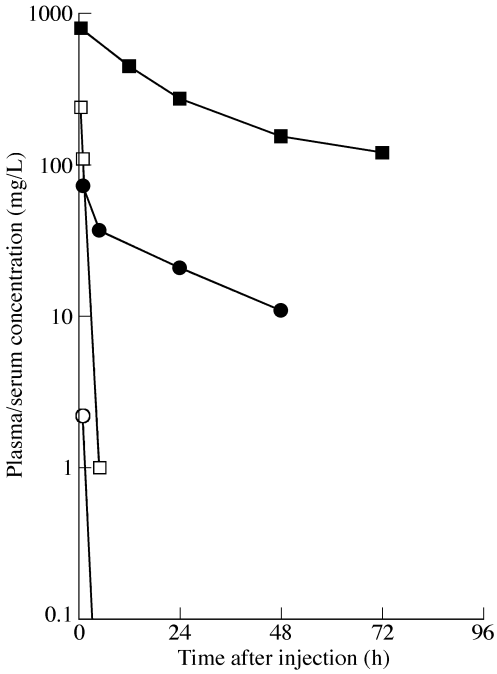

Figure 3. Circulation kinetics of LCL-encapsulated aminoglycosides (closed symbols) and free aminoglycosides (open symbols). Aminoglycoside concentrations at indicated time-points after injection of a single dose gentamicin 5 mg/kg in rats (circles)[98] or amikacin 50 mg/kg in rats (squares).[99]

2.5 or 5 mg/kg/day mean plasma concentrations were 120 and 215 mg/L, respectively. One week later, plasma concentrations still amounted to 10–20 mg/L. Yet, the concentrations of free amikacin released from the liposome never exceeded 4 mg/L. Our experimental studies with SSL-gentamicin showed a similar picture in rats, with 70- to 130-fold increase in AUC compared with the free drug.[98]

The tissue distribution of aminoglycosides is greatly changed after administration in the liposome-encapsulated form of both types of LCL to rats, as is illustrated in Figure 4. Equivalent doses of free and liposome-encapsulated drug were administered. Relatively high tissue concentrations are seen in the liver and spleen compared with free drug. In addition, higher drug concentrations are observed in other organs such as bone marrow, lungs, intestine, lymph nodes, skin and heart. MiKasome has been recovered from microvacuolated macrophages in most tissues after injection, which indicates that phagocytic cells could serve as a depot of amikacin. The urinary recovery of unchanged amikacin after injection in the MiKasome formulation is dramatically reduced compared with that in case of the free drug. Whereas practically all amikacin is excreted within 24 h after injection of the free drug, MiKasome showed less than 40% recovery in urine by day 10.[100]

In addition to the reduced affinity of LCL for the MPS and increased localization in other organs, it was demonstrated in our laboratory by Bakker-Woudenberg *et al.*[102] that in a rat model of a unilateral pneumonia caused by

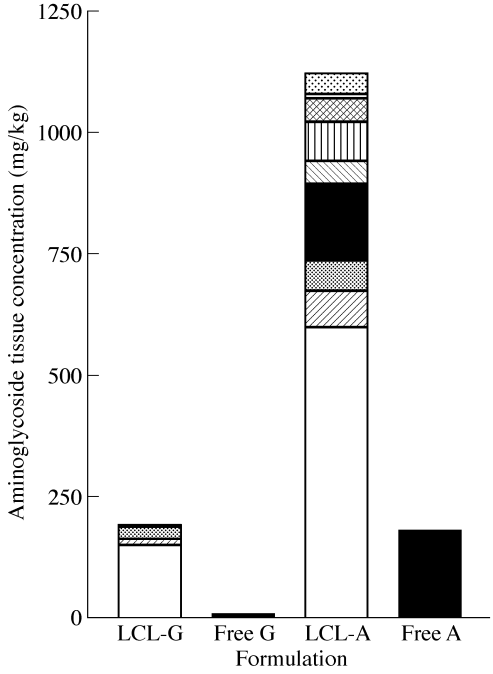

Figure 4. Tissue distribution of LCL-encapsulated aminoglycosides and free aminoglycosides. Concentrations in tissues at 24 h after injection of a single dose of gentamicin 5 mg/kg (G) in rats (data are based on liposome distribution, note the increased liposome levels in the infected lung tissue; organs shown are the only organs investigated)[98] or amikacin 50 mg/kg (A) in rats.[99] Key: ⊠, heart; ▨, skin; ◫, duodenum; ▧, bone marrow; ■, kidney; ▩, lung; ▨, liver; □, spleen.

K. pneumoniae, the localization of SSL in the infected left lung was approximately four-fold higher than the localization in the contralateral non-infected right lung. In the same model, 10-fold lower levels of localization in the infected lung were observed when liposomes with a relatively short circulation time were used.[103] Recent studies indicate that a prolonged liposomal circulation time is essential for substantial localization in the target site. An increase in AUC of the liposome formulation, achieved by tuning the lipid composition, was reflected by a proportional increase in localization at the infectious focus.[97] Similar findings of selective liposome localization at the target site in models of inflammation such as adjuvant arthritis, osteomyelitis, intra-abdominal abscesses, colitis, allergic encephalomyelitis, focal thigh infection and contact hypersensitivity have been reported.[104–113] The selectivity of the localization of LCL at the site of infection or inflammation is mediated by the locally increased capillary permeability as a result of the inflammatory response.[114–116] The nature of the inflammatory stimulus seems not important since instillation of 0.1 M hydrochloric acid or lipopolysaccharide into the lung also induced increased capillary permeability and localization of the liposomes.[116] A contribution of infiltrating inflammatory cells to selective target site localization of liposomes has been suggested by some authors.[105,115] Studies in the animal model using unilateral *K. pneumoniae* pneumonia indicate that the contribution of infiltrating inflammatory cells is not required for substantial target site localization of liposomes, as the degree of localization was similar in leucopenic rats as well as in immunocompetent rats.[116] This is an important observation as these results would indicate that targeted liposomal drug delivery could also be beneficial to immunocompromised patients, who suffer from severe infections and have a higher risk of failure of their treatment.

Safety. Much work has been done on the safety of MiKasome. Parameters tested in a 1 month study with daily or every third day injection of MiKasome in Beagle dogs were based on clinical chemistry, haematology, urine analysis and coagulation together with body weights, clinical observations and vital signs. Gross necropsy and histopathologic examination of tissues was performed at the end of the study period.[100] Daily doses of 20 mg/kg or every third day doses of 60 mg/kg were not associated with the occurrence of adverse effects despite mean steady state plasma concentrations above 750 mg/L and pre-dose levels >600 mg/L. Surprisingly, kidney concentrations above 1 mg/g did not lead to elevation of blood urea nitrogen or creatinine concentrations. The study shows that the ratio of cortical to medullary amikacin was substantially reduced by liposome encapsulation compared with the free drug. Therefore, it appears that liposome encapsulation results in a different kidney localization, preventing aminoglycoside-induced nephrotoxicity.[100]

A clinical study of safety in HIV-positive patients showed that after 1 week of daily dosing of 2.5 or 5 mg/kg, plasma levels were approximately 120 and 215 mg/L, respectively. Plasma amikacin levels of 10–20 mg/L persisted for 2 weeks after the last dose. However, no renal or audiovestibular toxicity was noted in any of the subjects participating in the study.[100]

Administration of gentamicin in rats showed acute toxicity after a single dose of 40 mg/kg, characterized by convulsions. A similar dose of SSL-gentamicin showed no acute toxicity.[117]

Therapeutic efficacy. Results of the treatment studies with aminoglycosides encapsulated in LCL are shown in Table 2.[98,117–122] The majority of studies report that the efficacy of LCL-encapsulated aminoglycosides is superior to that of the free aminoglycosides. Most studies relate to the use of MiKasome. The long half-life of LCL in the circulation allows for prolonged dosing intervals or even single dose treatments. A clinical trial in urinary tract infection patients shows that a single dose of MiKasome 40 mg/kg produced a high cure rate and the efficacy was comparable to seven daily infusions of 10 mg/kg.[118] In two rabbit models of endocarditis, it was shown that single daily doses of MiKasome improved survival and were as efficient in reducing bacterial numbers as twice daily doses of the free

Table 2. Clinical and preclinical therapeutic efficacy of aminoglycosides in LCLs

Infection	Drug used	Result	Comments
(Low-susceptible) *K. pneumoniae* pneumonia[98, 117]	gentamicin[98,117]	Compared with free drug: prolonged survival,[98,117] reduction of number of bacteria in lung[98,117] and blood.[117]	In this model, selective liposome localization in the infected tissue was demonstrated, which was superior to that of conventional liposomes.[102]
Complicated urinary tract infection[118]	amikacin[a,118]	Good bacterial and clinical cure rate. High dose single infusion as efficient as low dose daily infusions. No significant side-effects noted.[118]	Trial is ongoing with two fixed doses of 2 and 3 g amikacin in MiKasome formulation.[118]
S. aureus endocarditis,[119] *P. aeruginosa* endocarditis[120]	amikacin[a,119,120]	Compared with free drug: prolonged survival,[120] prolonged dosing interval allowed regarding vegetation density, relapse, reduction of renal and splenic abscesses.[119,120]	Treatments were combined with suboptimal doses of oxacillin. Both combinations preserved myocardial function.[119] Rate of vegetation sterilization was higher for free drug compared with liposome-encapsulated drug.[120]
K. pneumoniae sepsis[121]	amikacin[a,121]	Compared with free drug: prolonged survival, superior prophylactic activity, reduction of number of bacteria in liver and lungs.[121]	
M. avium complex infection in lung, liver and spleen[122]	streptomycin[122]	Conventional and long-circulating liposomes were equipotent in reduction of number of bacteria in spleen, liver and lungs.[122]	Liposomal circulation times not investigated.[122]

Reference 118 concerns a clinical study.
[a]The liposomal form of amikacin used in these studies was MiKasome.

drug, which is probably related to the prolonged residence time in the body of the liposomal formulation.[119,120] In contrast, the rate of vegetation sterilization was higher in the animals treated with the free drug, probably as a result of the short-lasting, but high peak-levels of the free drug in the circulation. In the endocarditis models, treatments were combined with suboptimal doses of oxacillin to take advantage of the documented synergy between aminoglycosides and β-lactams. The studies do not show whether differences in strength of the synergic interaction exist between free amikacin or MiKasome. A recent study reported that liposomal-co-encapsulation of gentamicin and ceftazidime resulted in a synergic interaction of both drugs against a (resistant) *K. pneumoniae* pneumonia in rats, in contrast to combination of the free drugs.[123] This study shows that liposomal formulation does not inhibit and may even promote synergic drug interactions.

In immunocompromised mice, the relatively high tissue concentrations of MiKasome are probably responsible for the enhanced prophylactic activity of the liposomal drug in prolonging survival and reduction in bacterial numbers (both outside and within the liver and spleen).[121] The studies related to SSL-gentamicin demonstrated in a *K. pneumoniae* pneumonia model in rats that the therapeutic efficacy was clearly superior to the free drug in a single dose schedule.[98] Evaluation of its efficacy in a multidose schedule in leucopenic rats showed that addition of a single dose of SSL-gentamicin to free gentamicin treatment showed complete survival, using a seven-fold lower cumulative amount of gentamicin compared with treatment with free gentamicin alone. In leucopenic rats infected with *K. pneumoniae* having a low susceptibility to gentamicin, free gentamicin at the maximum tolerated dose did not result in survival. Addition of SSL-gentamicin was needed

for therapeutic success. Complete survival was obtained by adding an SSL-gentamicin formulation with a fluid lipid bilayer, whereas adding a rigid SSL-gentamicin formulation showed only 50% survival. The increased gentamicin release from the fluid liposomes presumably improved rat survival, thus showing the importance of liposome lipid composition for therapeutic efficacy.[117]

Only one single study failed to show a superior effect of LCL-encapsulated aminoglycoside compared with conventional liposomal drug in the treatment of MAC infection.[122] Unfortunately, the preparations used in this study were not characterized with respect to their circulation time as well as their tissue distribution, so the underlying cause of the results cannot be traced.

Concluding remarks

Liposome-encapsulated aminoglycosides offer possibilities for increasing the therapeutic index of this class of antibiotics. Local application of liposomes may provide a reservoir that prolongs therapeutic drug concentrations at the site of infection. Readily accessible infected tissues such as in the eye, wounds and lungs could benefit from this local administration. In order to optimize therapeutic efficacy it is important to balance drug release from and retention in the liposome. Specific liposome compositions may enhance bacterial killing by interacting with the infectious organism.

Conventional liposomes are mostly taken up by the MPS after iv administration, the targeted delivery of drugs to MPS cells in the liver and spleen seems to be the most relevant application of this liposome type. Treatment of intracellular infections in the MPS cells may benefit from the high amounts of aminoglycosides that can be delivered intracellularly. By making liposomes pH-sensitive, the therapeutic availability of the liposome-encapsulated drug that is phagocytosed may even be increased. Research is needed on the nephro- and ototoxicity of conventional liposomal aminoglycosides, with respect to their prolonged presence in the body. This research should also include the potential danger of promoting microbial resistance as a result of the prolonged exposure of the resident microbial flora to the drug.

In case the infectious focus is located outside the MPS, conventional liposomes are of limited value. Therefore, research has been aimed at decreasing the MPS uptake of liposomes and consequently increasing their circulation time. LCLs were the result of these efforts. Intravenously administered LCLs potentially offer drug targeting to sites of infection not restricted to the MPS. A number of reports have demonstrated enhanced therapeutic efficacy of LCL-encapsulated aminoglycosides compared with free drugs or conventional liposomes. Unfortunately however, most studies with liposome-encapsulated aminoglycosides have, up to now, been performed in animal models with an intact host defence and infected with bacteria susceptible to the antibiotic. Treatment failure in clinical practice, however, particularly occurs in patients with impaired host defences or in patients infected with bacteria of low susceptibility. A single study addressed both issues in determining the efficacy of SSL-gentamicin.[117] These issues should be incorporated more in animal models to demonstrate the value of liposomes in clinically relevant settings. So far, MiKasome has shown an excellent safety profile. Yet, similar to the conventional liposome formulations, the effects that the prolonged tissue drug concentrations have on development of resistance need to be addressed. The results that have been reviewed indicate promising prospects for liposome-encapsulated aminoglycosides and warrant further clinical investigations into the use of these formulations for the treatment of severe infections.

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