Targeted drug delivery to enhance efficacy and shorten treatment duration in disseminated *Mycobacterium avium* infection in mice

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Objectives: Improvement of the efficacy of drug treatment in mycobacterial infection by the development and application of targeted drug delivery.

Methods: In disseminated *Mycobacterium avium* infection in mice, the relative efficacy of the antimycobacterial agents that are currently used in combination therapy was investigated. Next, the effect of the addition of targeted delivery of amikacin to the infected tissues in the initial phase of treatment was studied. Amikacin was chosen because of its unique rapid and high mycobacterial killing capacity. As drug delivery tool, long-circulating sterically stabilized liposomes were used.

Results: Treatment with clarithromycin alone daily (6 days a week) slowly killed most of the mycobacteria in the lung, liver, spleen, inguinal and mesenterial lymph nodes. However, after 24 weeks of treatment, persistence of substantial numbers of mycobacteria in the infected organs was observed. The addition of ethambutol to the clarithromycin regimen did not significantly enhance the efficacy of treatment, neither did rifampicin as a third agent. In contrast, the addition of liposomal amikacin in the initial phase of therapy resulted in rapid and complete elimination of the mycobacteria in all infected organs within 12 weeks of treatment without relapse of infection. As a result, total treatment duration could be significantly reduced to 12 weeks.

Conclusions: In *M. avium* infection in mice, the approach of targeted drug delivery was successful. The rapid decrease in the mycobacterial load followed by complete killing, including the persistent mycobacteria considered responsible for relapse of infection, allows a significant reduction of the total treatment duration.

Keywords: liposome, carrier, mycobacterial infection, drug targeting

Introduction

Mycobacteria are responsible for infections that cause immense morbidity and mortality. Infections caused by *Mycobacterium tuberculosis* and *Mycobacterium leprae* are endemic in most developing countries,¹,² and the major factor contributing to the resurgence of tuberculosis (TB) is the AIDS pandemic.³ Whereas *M. tuberculosis* commonly infects AIDS patients in developing countries, in western AIDS patients the pathogen most involved is *Mycobacterium avium* complex (MAC).⁴,⁵ Disseminated infections caused by MAC are hardly seen in patients with a normal cellular immunity, but are frequently encountered in patients who are severely immunocompromised.

The treatment of mycobacterial infections is complicated. Mycobacteria are intracellular pathogens that reside inside macrophages, and show low metabolism, necessitating prolonged courses of treatment (6 months or longer) with combinations of agents to minimize the emergence of antimicrobial resistance. Despite long-term treatment of MAC infection for over 24 months with a three-drug or four-drug regimen,
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significant relapse rates occur.6 Owing to the long duration and complexity, the treatment of TB, particularly in developing countries is often sub-optimal and non-compliance of patients to prescribed regimens is high. As a result, a significant mortality and relapse rate, as well as a high risk of development of resistance is observed. There is clear evidence that the incidence of drug-resistant mycobacterial infections is increasing.7

A higher efficacy of treatment and a significant reduction of treatment duration are urgently needed. In our opinion, a major step forward would be the introduction of targeted drug delivery effecting rapid and complete elimination of the mycobacteria, resulting in a higher cure rate, prevention of relapse, reduced treatment duration and hence improved patient compliance, as well as reduced risk of emergence of resistance.

The value of drug delivery systems in the treatment of mycobacterial infections has been studied by a number of investigators.8–12 In most studies a drug delivery system was developed to achieve sustained drug release, which would allow the reduction of the dosing frequency, and hence would improve patient compliance. The concept of drug delivery in the present study is targeted delivery of antimycobacterial drug to achieve increased drug concentrations at the site of infection and inside the infected cells (‘site-specific drug delivery’). At the same time, toxic side effects of drug would be minimized (‘site-avoidance drug delivery’). In this way, the delivery-based therapy is aiming for an increased rate of mycobacterial killing including the dormant mycobacteria in their intracellular location considered responsible for relapse of infection. This would allow reduction of treatment duration, and as a result improved patient compliance. In addition, with the rapid decrease in mycobacterial load in the infected organs, a reduced risk of development of resistance may be achieved.

The drug delivery systems under investigation in the treatment of mycobacterial infections are primarily lipid- or polymer-based nanoparticulate systems. With respect to the lipid systems, the role of liposomes as a drug delivery tool has been studied in experimental models of MAC infection and TB infection by a number of investigators who encapsulated a variety of antimycobacterial agents.9–13 In these studies, promising results with liposomal agents were reported regarding a mycobacterial effect in the liver and spleen. However, the antimycobacterial activity in the lung and lymph nodes was limited. The results can be explained by the fact that in these studies ‘classical’ or ‘conventional’ liposomes were applied. After intravenous administration, these liposomes are easily recognized by the cells of the mononuclear phagocyte system (MPS), particularly in the liver and spleen. As a consequence, the targeted delivery of drugs to intracellular infections localized in MPS tissues, primarily the liver and spleen, is the most relevant application of this liposome type. If the infectious focus is also located outside the MPS, conventional liposomes are of limited value.

The innovative approach of the present study is the application of stERICally stabilized liposomes (SSLs) as a drug delivery tool in mycobacterial infection. Unlike conventional liposomes, SSLs are able to avoid rapid hepatosplenic uptake. Owing to the poly(ethylene) glycol (PEG) coating of the liposomal surface, the adherence of opsonins to the liposomes is hindered. As a result, SSLs show relatively long blood circulation, and their use creates possibilities for targeting infected tissues outside the liver and spleen. In disseminated infections caused by MAC, exhibiting a high load of mycobacteria especially in the lymph nodes, bone marrow, spleen, liver and lung, the application of SSLs, as carriers of antimicrobial agents, might be of great value in enhancing the therapeutic efficacy.

In previous studies, we have shown the successful use of SSLs for improving treatment of bacterial lung infections.14,15 We demonstrated that the SSLs, after long-term circulation in blood, extravasate in infected tissues and eventually end up in deep-seated tissue macrophages. In this way, SSLs may act as a site-specific drug delivery system also in mycobacterial infections. In the present study, the SSL drug delivery concept was investigated in a mouse model of disseminated MAC infection. Human disseminated MAC infections are difficult to treat and an improved therapy strategy is needed. The MAC strain used was strain 101 (serovar 1), a clinical isolate that is frequently used in experimental infections. Amikacin was used for the delivery-based therapy as in our previous studies amikacin showed a unique rapid and high mycobacterial killing capacity at low concentrations in vitro and in this respect was far superior to other antimycobacterial agents.16 In addition, it has been shown in animal models of MAC that aminoglycosides including amikacin reduce the incidence of resistance in macrolide-containing regimens.17

In the first part of the study, we investigated the relative efficacy of antimycobacterial agents that are currently used in combination therapy by comparing the efficacy of the macrolide clarithromycin alone or in combination with ethambutol, as well as the potential of rifampicin as a third agent. Although the macrolides are recognized to be the most active of the drugs available for MAC treatment, important questions remain with respect to the relative contribution in terms of antimycobacterial activity of each of the components in the multidrug macrolide-containing regimen.18 In the second part of the study, the effect of targeted drug delivery using amikacin encapsulated in SSLs added in the initial phase of clarithromycin/ethambutol treatment was investigated.

Materials and methods

Animals

Female specific-pathogen-free C57BL/6 mice (IFAA Credo, l’Arbresle, France) 10–12 weeks old and weighing 20–25 g were used in all experiments. All animals received water and food ad libitum. The experimental protocols adhered to the rules laid down in the Dutch Animal Experimentation Act (1977) and the published Guidelines on the Protection of Experimental Animals by the Council of the EC (1986). The present protocols were approved by the Institutional Animal Care and Use Committee of the Erasmus University Medical Center Rotterdam, The Netherlands.

Antimycobacterial agents

Clarithromycin was obtained from Abbott (Saint Remy, France), ethambutol and amikacin were purchased from Sigma Chemical Co. (St Louis, MO, USA) and rifampicin was obtained from Hoechst Marion Roussel (Hoeyevelaken, The Netherlands). Dilutions of antimycobacterial agents were prepared according to the manufacturer’s instructions.
Bacteria

The *M. avium* strain used in this study was MAC strain 101 (serovar 1), originally isolated from the blood of an AIDS patient with disseminated MAC infection and was kindly supplied by L. S. Young, Kuzell Institute for Arthritis and Infectious Diseases, San Francisco, CA, USA. MAC organisms were cultured on Middlebrook 7H10 agar medium (Difco laboratories, Detroit, MI, USA) supplemented with oleic acid–albumin–dextrose–catalase enrichment (OADC; Baltimore Biological laboratories, Baltimore, MD) for 14 days at 37°C. MAC suspensions were prepared in Middlebrook 7H9 broth (Difco) supplemented with OADC and stored at ~80°C. Drug susceptibility of the MAC strain in terms of MIC was performed using a broth-based method (macrodiffusion) as described previously.18 The MICs of clarithromycin, ethambutol, rifampicin and amikacin for the MAC strain were 4, 16, 4 and 1 mg/L, respectively.

Infection model

MAC infection was produced by intravenous inoculation with 0.2 mL of a suspension containing $2 \times 10^8$ cfu MAC (end-log phase) via the tail vein. Intravenous inoculation resulted in disseminated multi-organ infection. The course of infection was assessed by quantification of the number of viable bacteria in the lungs, spleen, liver, inguinal lymph nodes, mesenterial lymph nodes and blood at indicated time points. Animals were sacrificed by CO2 inhalation. A blood sample was taken via retro-orbital bleeding in heparinized tubes on ice. The infected organs were dissected and homogenized (Polytron, Kinematica, Luzern, Switzerland) in 2 mL of 7H9 broth supplemented with OADC for 30 s at 30 000 rpm. Tissue homogenate suspensions and blood were cultured as described later in this section.

Liposome-encapsulated amikacin

Liposomes composed of partially hydrogenated egg phosphatidylcholine (PHEPC, iodination value 40) (Asahi Chemical Industry Co. Ltd, Ibaraki, Japan), cholesterol (Chol) (Sigma Chemical Co.) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[polyethylene glycol-2000] (PEG-DSPE) (Avanti Polar Lipids, Alabaster, AL, USA), in a molar ratio of 1.85:1:0.15, respectively, were prepared as described previously 19 with some modifications. Lipids (100 μmol of total lipid per batch) were dissolved in a round-bottomed flask in a chloroform/methanol (1:1, v/v) solution of PHEPC and Chol. After evaporation of the solvent under constant rotation and reduced pressure in a rotary evaporator, the lipid mixture was dried under nitrogen for 10 min, dissolved in 2-methyl-2-propanol (Sigma Chemical Co.), frozen and freeze-dried overnight. The resulting lipid film was hydrated for 2 h in 2.5 mL aqueous 200 mg/mL amikacin (Sigma Chemical Co.), subsequently 7.5 mL HEPES/NaCl buffer, pH 7.4 [10 mM HEPES (Sigma Chemical Co.) and 135 mM NaCl (Merck, Darmstadt, Germany)] was added. Liposomes were obtained by sonication of the hydrated lipid dispersion for 8 min in an MSE Soniprep 150 (Sanyo Gallenkamp PLC, Leicester, UK), or by multiple extrusion of the hydrated lipid dispersion in three steps through two stacked polycarbonate membranes (Nuclepore, Pleasanton, CA, USA) with pore sizes of 1000, 600 and 400 nm, respectively. Non-encapsulated amikacin was removed by ultracentrifugation in two changes of HEPES/NaCl buffer at 265 000 g for 2 h at 4°C. Particle size distribution was measured using dynamic light scattering (4700 system; Malvern Instruments Ltd, Malvern, UK). The polydispersity of the liposome population was always below 0.3, indicating very small variations in particle size. The amount of encapsulated amikacin was determined after disintegration of the liposomes by 0.1% v/v (final concentration) Triton X-100 (Janssen Chimica, Geel, Belgium). Amikacin was measured using a diagnostic susceptibility test agar (Oxoid, Basingstoke, UK) diffusion test with *Staphylococcus aureus* Oxford strain (ATCC 9144) as the indicator organism and standards ranging from 8 to 0.5 mg/L amikacin, as described previously.19 Phosphate concentration was determined spectrophotometrically according to Bartlett as described. The sonicated liposomes obtained had a particle size of 97 nm and contained 47 μg of amikacin/μmol of total lipid. The extruded liposomes obtained had a particle size of 393 nm and contained 61 μg of amikacin/μmol of total lipid.

Antimycobacterial treatment

Treatment was started at 24 h after inoculation of mycobacteria. Clarithromycin (Biclar®, Abbott) at 100 mg/kg as single drug or combinations of clarithromycin with ethambutol (Myambutol®, Riemser, Greifswald-Insel Riems, Germany) at 75 mg/kg and rifampicin (Rifadin®, Hoechst Marion Rousell) at 40 mg/kg were administered once-daily for 6 days a week. Clarithromycin and ethambutol were administered subcutaneously in the neck, whereas rifampicin was administered intraperitoneally. Mice were treated for a period of 8 weeks, 12 weeks or 24 weeks.

Amikacin at 12 mg/kg in the free form or in the liposome-encapsulated form (LE-amikacin) and placebo liposomes (LE-placebo) were administered intravenously in the tail vein twice-weekly for only the first 3 weeks of the treatment period. Mice treated with LE-amikacin-1 received 97 nm amikacin liposomes only, and mice treated with LE-amikacin-2 received a mixture of 97 nm amikacin liposomes and 393 nm amikacin liposomes in a ratio of 3:1. Total lipid doses of LE-amikacin-1, LE-amikacin-2 and LE-placebo were always below 250 μmol total lipid/kg.

Parameters for therapeutic activity

The therapeutic effect of antimycobacterial treatment was assessed by quantification of decrease in viable MAC numbers recovered from the infected organs and blood, which were cultured at indicated time points during and after the treatment (n = 4 per time point). To prevent carryover of antimycobacterial agent (if still present in the tissues) to the subculture plates, tissue suspensions were centrifuged at 2400 g for 10 min, and pellets were re-suspended in 7H9 broth supplemented with OADC. Samples were serially diluted and plated onto 7H10 agar supplemented with OADC. After dilution, the remaining homogenates were subjected to the pour plate method. Plates were incubated for 14 days at 37°C, and cfu numbers were counted. Change in susceptibility of the MAC organisms recovered from the tissues towards clarithromycin or ethambutol was monitored by sub-culturing on plates containing 8 or 16 mg/L clarithromycin and plates containing 32 or 64 mg/L ethambutol.

Statistical evaluation

Differences in quantitative cultures of mice (comparison of two treatment groups) were assessed. Since we only performed statistics between two treatment groups, related to the value of the addition of one agent to the regimen, the Student’s t-test was used. Because in none of the conventionally treated mice was elimination of mycobacteria from the infected organs achieved, and the addition of liposomal amikacin to the conventional regimen resulted in complete
mycobacterial elimination in all infected organs investigated, statistical analysis was not performed.

Results

Mouse model of MAC infection

Intravenous inoculation of MAC into mice resulted in disseminated infection in lungs, spleen, liver, inguinal lymph nodes and mesenterial lymph nodes. The infection was characterized by persistence of MAC in these organs for at least 24 weeks (Figure 1). Only a small variation between individual animals was seen. Blood cultures of infected mice were positive only on day 1 and were always negative from day 1 at the time points indicated.

Efficacy of conventional therapy for 24 weeks

As shown in Figure 2, treatment for a period of 24 weeks with clarithromycin alone resulted in a gradual decrease in MAC numbers in the infected organs over time. After 24 weeks of treatment, MAC numbers were substantially decreased, and mycobacterial killing up to \( \geq 99\% \) in the infected organs was obtained. However, complete elimination of MAC was not achieved. Change in susceptibility of the MAC organisms recovered from the tissues towards clarithromycin was never observed.

Addition of ethambutol to the clarithromycin regimen resulted in a further decrease in MAC numbers in infected organs, however differences were not significant compared with treatment with clarithromycin alone. Change in susceptibility of the MAC organisms towards clarithromycin or ethambutol was never observed.

When rifampicin was added to the clarithromycin/ethambutol regimen again MAC numbers in infected organs further decreased, however they were not significantly lower compared with those of the clarithromycin/ethambutol-treated mice. After 24 weeks of treatment with all schedules, dormant populations of MAC in all organs remained.

Efficacy of targeted therapy with liposome-encapsulated amikacin added to conventional therapy for 24 weeks

As shown in Figure 3, addition of LE-amikacin-1, which are 97 nm amikacin liposomes twice-weekly during the first 3 weeks of conventional therapy with clarithromycin/ethambutol for 24 weeks resulted in a rapid decrease of MAC numbers followed by complete elimination of MAC from all organs except the spleen. Within 12 weeks mesenterial lymph nodes were sterile and within 18 weeks lungs, liver and inguinal lymph nodes were

Figure 1. Disseminated \( M. \text{avium} \) infection in mice. At indicated time points after intravenous inoculation of \( 2 \times 10^8 \) \( M. \text{avium} \) organisms, mice were sacrificed and quantitative cultures of infected organs and blood were performed \((n = 4\) per time point). Results are expressed as means ± SD (error bars). Any sample with a value below the limit of detection (LOD) was assigned as the highest possible value below LOD (log cfu = 0.2). d, day; w, week.
sterile, whereas MAC cultures from the spleen detected of 1.30 ± 0.49 and 0.45 ± 0.36 log cfu at week 18 and week 24, respectively. The administration of the same amount of free non-encapsulated amikacin or placebo liposomes at the same dosage schedule as the amikacin liposomes had no effect, as demonstrated in mice dissected only at week 4 and week 12 after inoculation.

Figure 4 shows that compared with the addition of LE-amikacin-1, which are 97 nm amikacin liposomes only, after addition of LE-amikacin-2, which is a combination of 97 nm amikacin liposomes and 393 nm amikacin liposomes at the same liposomal lipid dose and schedule to the conventional therapy, the MAC-infected organs were sterilized more rapidly. In addition, with LE-amikacin-2, MAC was completely eliminated from the spleen within 18 weeks.

Efficacy of targeted therapy with liposome-encapsulated amikacin added to conventional therapy at reduced duration

The data in Figure 5 show that as a result of the rapid decrease in MAC load from infected organs following targeted therapy with LE-amikacin-2 total treatment duration could be reduced from 24 weeks to 12 weeks, without the occurrence of relapse of MAC infection. Whereas conventional therapy alone for a duration of 24 weeks only resulted in substantial decrease of MAC numbers, mycobacteria were rapidly and also completely eliminated from all infected organs with conventional therapy for only 12 weeks duration when LE-amikacin-2 was added during the first 3 weeks of treatment.

Discussion

For treatment of disseminated MAC infections mostly macrolide-containing multidrug treatment schedules are applied. The regimens mostly include clarithromycin or azithromycin, rifampicin or rifabutin and ethambutol. The addition of an initial aminoglycoside such as streptomycin in a four-drug regimen did not result in clinical improvement or significant differences in the sputum relapse rates.

Combinations of agents are used primarily in order to reduce the incidence of macrolide resistance during monotherapy. However, uncertainty persists regarding the optimal drugs that should accompany the macrolide. For the combination of clarithromycin and ethambutol, it
has been shown in a mouse model that the incidence of resistance is reduced,20 however, the addition of ethambutol appeared to have no significant effect on the reduction of the mycobacterial numbers.21 Also a clinical study showed that the addition of ethambutol to a clarithromycin regimen resulted in a reduction of development of resistance but did not enhance clearance rates of MAC from the blood.22 The role of the rifamycins, including rifampicin, in the clarithromycin/ethambutol regimen as a third agent is also not fully clarified.

The present study shows that the clarithromycin treatment regimen removed at least 99% of the mycobacteria, however, persistence of substantial numbers of mycobacteria in the infected organs after 24 weeks of treatment was observed. The susceptibility of the mycobacteria recovered from the infected organs towards clarithromycin was unchanged. The addition of ethambutol to the clarithromycin regimen substantially, but not significantly, further decreased mycobacterial numbers compared with treatment with clarithromycin alone. These findings are in agreement with similar studies in mice by Furney et al.21 In the present study, clarithromycin- or ethambutol-resistant mycobacteria were never found, which is in contrast to the studies of Düzgünes et al. demonstrating in a MAC mouse model that ethambutol has a role in reducing the incidence of clarithromycin-resistant mutants.20 In the present study, the addition of rifampicin to the clarithromycin/ethambutol regimen did not significantly enhance the mycobactericidal effect of treatment. Actually, the role of rifampicin in the multidrug regimen can be questioned also in view of the drug interaction between rifampicin and clarithromycin resulting in reduced bioavailability of clarithromycin as demonstrated in patients following addition of rifampicin to the regimen.23,24

In the second part of the study, we investigated the therapeutic value of liposomal targeted delivery of amikacin in mice with M. avium infection. Conventional therapy with clarithromycin/ethambutol alone, chequered bars; untreated controls, black bars. Treatment was started at 24 h after M. avium inoculation. Clarithromycin/ethambutol at 100 and 75 mg/kg, respectively, were administered subcutaneously once-daily for 6 days a week. LE-amikacin-1 or amikacin at 12 mg/kg or LE-placebo were administered intravenously twice-weekly for only the first 3 weeks of the treatment period. At indicated time points, mice were sacrificed and quantitative cultures of infected organs and blood were performed (n = 4 per time point). Mice treated with amikacin or LE-placebo were dissected only at week 4 and week 12. Results are expressed as means ± SD (error bars). Any sample with a value below the LOD was assigned as the highest possible value below LOD (log cfu = 0.2). d, day; w, week.
compartment. The physico-chemical characteristics of the liposomes such as lipid composition (bilayer rigidity), surface charge and particle size can be manipulated. The in vivo behaviour of the liposomes is dependent on these characteristics and is the major determinant of the therapeutic effect of the encapsulated agent. The so-called conventional type of liposomes are mostly studied and are limited in their usefulness because of their rapid and significant hepatosplenic uptake from the bloodstream. These liposomes have also been applied in mouse models of MAC infection by a number of investigators who encapsulated a variety of antimycobacterial agents. Besides first-line antimycobacterial agents like rifampicin, rifabutin and clofazimine, also aminoglycosides like amikacin, gentamicin, streptomycin, kanamycin and capreomycin were applied in the liposome-encapsulated form. In general, in these studies promising results are reported regarding an increased bactericidal effect of the liposomal agents in the liver and spleen, compared with the agents in the free form. Unfortunately, in most studies it was not investigated whether prevention of relapse of infection was achieved after termination of treatment. By some investigators, a reduced mycobacterial load in the lungs, lymph nodes and blood was also reported, however, this was less pronounced compared with liver and spleen. These results illustrate the limitations of the conventional liposomes in the treatment of infections beyond the liver and spleen. Also in MAC infection in man, it was demonstrated that low frequent administration of liposomal gentamicin in MAC bacteraemia in AIDS patients resulted in substantial decrease in mycobacterial numbers in blood. In contrast, another study using the same liposomal gentamicin formulation could not detect reduced numbers of mycobacteria in bone marrow specimens of AIDS patients.

To enable the liposomes to reach infectious sites outside the major MPS organs, research has been aimed at decreasing the MPS uptake of liposomes and consequently increasing their circulation times. One example of long-circulating liposomes is MiKasome, a small, neutral, unilamellar liposome formulation containing amikacin. In experimental disseminated MAC infection in mice even with MiKasome, eradication of the mycobacteria from tissues outside the MPS was never achieved. Despite the use of high dosage, a substantial mycobacterial load in the infected organs was still detectable.

Another approach to prolong circulation time of liposomes is the incorporation of PEG coupled to DSPE in the liposome bilayers. The hydrophilic PEG provides steric hindrance around...
the liposome reducing liposome opsonization and thereby reducing rapid recognition and uptake by MPS cells. These liposomes are therefore termed as SSLs. An important characteristic of these SSLs is that their prolonged blood circulation time is, to a high degree, independent of liposome characteristics such as liposome particle size, charge and lipid composition (rigidity) of the bilayer, as well as liposomal lipid dose.44,45 This provides the opportunity to manipulate liposomal characteristics needed for optimal targeting, retention and release of encapsulated drug without compromising blood circulation time. This in contrast to MiKasome in which the lipid composition, being a rigid membrane structure, is needed to retain its long blood circulation half-life.46

In previous studies, in our rat model of left-sided Klebsiella pneumoniae pneumonia, we have demonstrated that the localization of SSLs in the infected left lung was significantly increased compared with the contralateral non-infected right lung.15,46,47 Critical liposome-related factors that contributed to liposome target localization in the infected lung tissue appeared to be PEG density, particle size, bilayer fluidity, negative surface charge and circulation kinetics.48,49 With respect to host factors, the localization of SSLs at the site of infection appeared to be mediated by the locally increased capillary permeability as a result of the inflammatory response.50 Liposomes after labeling were visualized principally in the infected tissues, and liposomal uptake by macrophages was demonstrated. The degree of target localization was observed to be similar in leucopenic rats as well as in immunocompetent rats, indicating that targeted drug delivery using SSLs could also be beneficial to the immunocompromised host. We demonstrated that the treatment of the experimental K. pneumoniae lung infection was significantly improved when gentamicin, ceftazidime or ciprofloxacin were administered in the SSL-encapsulated form.19,51 Also in leucopenic rats with lung infection caused by low gentamicin-susceptible K. pneumoniae, the superior therapeutic efficacy of liposomal gentamicin over free gentamicin was shown.52 Moreover, liposome-co-encapsulated gentamicin and ceftazidime resulted in a synergistic interaction of both drugs in contrast to the combination of free drugs,53 demonstrating that liposomal formulation may promote synergistic drug interactions.54

Following the successful use of SSLs for targeting infected tissues in the present study, we applied this drug delivery
approach in the initial phase of treatment of experimental disseminated MAC infection. Antibiotic release at the site of infection would result in prolonged high antibiotic concentrations, and hence effective mycobacterial killing including the dormant mycobacteria. We chose amikacin for liposomal encapsulation as we have shown that this antibiotic has strong mycobacterial killing potency \textit{in vitro} which was far superior to the other antimycobacterial agents tested.\textsuperscript{16} The poor intracellular penetrating capability and potential toxic side effects of amikacin, being possible important drawbacks for therapeutic activity, were overcome by liposomal encapsulation of the agent.

The results obtained in the present study support the feasibility of our concept of targeted drug delivery to improve treatment in mycobacterial infection. Liposomal amikacin added in the initial phase of treatment significantly improved the activity of the clarithromycin/ethambutol therapy in two ways. A rapid decrease in mycobacterial load in the infected organs was obtained. In addition, complete elimination of mycobacteria from all infected organs was achieved. As a consequence, total treatment duration could be reduced to 12 weeks without relapse of infection. This is a major step forward, as full eradication of mycobacteria was not observed after the multidrug treatment courses investigated for 24 weeks.

A study by Gangadharam \textit{et al.}\textsuperscript{54} showed that conventional and long-circulating liposomes containing streptomycin were equipotent in reduction of mycobacterial numbers in the spleen, liver and lungs. Unfortunately, the liposome preparations used in this study were not characterized with respect to their circulation time as well as their tissue distribution, which makes the explanation of the results obtained difficult.

Summarizing, our approach of targeted drug delivery in the treatment of experimental MAC infection meets the expectations. The rapid decrease in the mycobacterial load followed by effective killing, including the persistent mycobacteria considered responsible for relapse of infection, allows a significant reduction of the total treatment duration and may result in a lower risk of development of resistance. Aminoglycosides may be good candidates for the drug delivery-based therapy. The rationale for liposome encapsulation of aminoglycosides is the possibility to increase their therapeutic index by increasing concentrations at the site of infection and by reducing the toxic side effects of these agents.

The benefits of this new treatment concept in the management of TB are currently under investigation in our mouse model of pulmonary TB.

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