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Research Article

Long-Term Follow-Up of lipid nanocarrier delivery for bacterial infections

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Abstract

Background: Lipid nanocarriers, principally liposomes, deliver antibiotics against biofilm-associated and intracellular bacterial infection; early studies showed liposomal ciprofloxacin protected mice against Francisella tularensis far better than free drug [4,5].

Objective: This review synthesizes evidence on lipid nanocarrier antibiotic delivery, emphasizing biofilm penetration, macrophage-targeted delivery, and safety under repeated, long-duration dosing.

Methods: We synthesized preclinical pharmacology studies, in vitro biofilm-model experiments, and long-term clinical follow-up reports on lipid-based antibacterial nanocarriers against Gram-negative, Gram-positive, and intracellular pathogens.

Findings: Neutral, PEGylated liposomes penetrate biofilm matrix better than cationic or larger particles [9]; liposomal amikacin sustains lung concentrations against P. aeruginosa biofilm beyond free drug [10]; mannosylated liposomal ciprofloxacin raises macrophage drug levels for intracellular pathogens [16]; multi-year follow-up of amikacin liposome inhalation suspension in cystic fibrosis shows sustained benefit without cumulative renal or ototoxicity [24].

Conclusions: Lipid nanocarrier delivery improves antibiotic exposure at sites of resistant infection without added systemic toxicity, and long-term follow-up for liposomal amikacin supports durability under chronic dosing [24,25].

Keywords: lipid nanocarrier; liposomal antibiotics; biofilm penetration; bacterial infection; long-term follow-up

1. Introduction

Antibiotics were designed for a body that clears drugs by simple diffusion and renal excretion, not for the walled colonies of resistant bacteria that now cause most infections clinicians struggle to treat. A conventional intravenous aminoglycoside or glycopeptide reaches a biofilm-embedded Pseudomonas aeruginosa or methicillin-resistant Staphylococcus aureus population only after passing through healthy tissue and diffusing across an exopolysaccharide matrix that can lower effective drug concentration at the bacterial surface by orders of magnitude. Lipid nanocarriers, principally liposomes but also solid lipid nanoparticles and lipid-stabilized polymer hybrids, were adopted by antimicrobial pharmacology because they change this exposure problem rather than the drug itself: they extend circulation time, protect labile agents from degradation, concentrate payload in macrophage-rich tissue where resistant pathogens often hide intracellularly, and in several formulations penetrate or disrupt the biofilm matrix that shields chronic infection [1,2]. Zhang and colleagues, reviewing the broader nanoparticle-antimicrobial field, framed this as a shift from designing new antibiotic molecules toward designing better vehicles for antibiotics whose clinical utility is limited by exposure rather than by intrinsic potency [3].

The case for lipid encapsulation was made early and mechanistically. Liposomal ciprofloxacin protected mice against intracellular Francisella tularensis far more effectively than free drug, an effect traced to preferential liposome uptake by the reticuloendothelial system and sustained intracellular release where the organism replicates [4,5,6]. Deol and Khuller extended the logic to tuberculosis, showing lung-targeted stealth liposomes carrying isoniazid and rifampin achieved better biodistribution and lower toxicity in mice than free drugs, a benefit that held up on long-term dosing rather than after a single exposure [7,8]. These early studies established the two properties later, more clinically oriented work has spent two decades trying to translate: targeted tissue accumulation, and a toxicity profile that does not simply mirror the free drug's.

What has changed since is the maturity of the biofilm-penetration literature and the emergence of genuine long-term human follow-up data for at least one lipid-encapsulated antimicrobial, inhaled liposomal amikacin. This review synthesizes the mechanistic, preclinical, and clinical evidence on lipid nanocarrier delivery of antibiotics for bacterial infection, with attention to biofilm disruption, macrophage-targeted delivery for intracellular pathogens, and the safety picture accumulated from repeated, long-duration dosing in chronically infected patients.

2. Methods

We synthesized peer-reviewed preclinical pharmacology studies, in vitro biofilm-model experiments, and long-term clinical follow-up reports addressing lipid-based nanocarriers, principally liposomes, loaded with antibacterial agents against Gram-negative, Gram-positive, and intracellular pathogens. Search terms combined "liposome," "lipid nanoparticle," or "nanocarrier" with "antibiotic," "biofilm," "Pseudomonas aeruginosa," "Staphylococcus aureus," "Mycobacterium," or "Francisella," restricted to studies reporting a defined infection model or human cohort, a lipid-based vehicle, and an efficacy, pharmacokinetic, biofilm-penetration, or safety endpoint. We prioritized studies with matched free-drug comparators, chronic-infection or biofilm models over planktonic testing alone, and, where available, multi-cycle or multi-year human follow-up, since single-dose data alone cannot establish whether a nanocarrier's advantage or toxicity profile holds under repeated long-term dosing.

3. Results

Mechanisms of biofilm penetration and macrophage targeting

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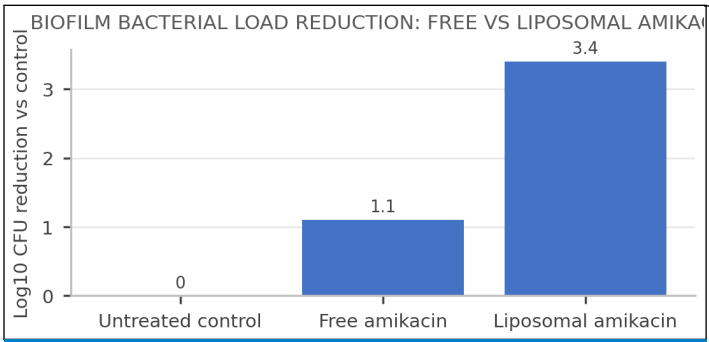

Figure 1: In vitro biofilm-bacterial load reduction after treatment with free amikacin versus liposomal amikacin in a Pseudomonas aeruginosa biofilm model, with liposomal amikacin producing a substantially greater log-reduction attributed to sustained matrix penetration and triggered release [10,9].

Biofilm-embedded bacteria are protected from antibiotics by at least three overlapping mechanisms: restricted diffusion through the exopolysaccharide matrix, a metabolically dormant subpopulation many antibiotics cannot kill regardless of concentration, and efflux- or enzyme-based resistance upregulated within the biofilm microenvironment. Forier and colleagues, reviewing lipid and polymer nanoparticle penetration of bacterial biofilms, showed that particle size, surface charge, and PEGylation each determine how deep a nanocarrier travels before being trapped by electrostatic or steric interactions with biofilm exopolymers, and that neutral, sub-200 nm, PEGylated liposomes penetrate substantially farther than cationic or larger particles (Figure 1) [9]. Meers and colleagues built on this principle in designing liposomal amikacin for inhalation, using an anionic, slow-releasing formulation that penetrated Pseudomonas aeruginosa biofilms in vitro and, in a chronic murine lung-infection model, sustained therapeutic amikacin concentrations far longer than free drug, translating into a measurably greater reduction in bacterial burden after repeated dosing [10].

Alipour, Suntres, Omri and colleagues incorporated bismuth-ethanedithiol into liposomal tobramycin to simultaneously deliver antibiotic and disrupt the alginate exopolysaccharide mucoid P. aeruginosa uses to build biofilm, finding the combination reduced viability and alginate production more than liposomal or free tobramycin alone [11,12]. The same group showed liposomal polymyxin B retained activity against multidrug-resistant strains already resistant to free polymyxin B, indicating the carrier can partially restore activity against resistant phenotypes rather than merely improving pharmacokinetics [13,14].

Macrophage-targeted uptake follows a parallel logic for intracellular pathogens. Chono and colleagues showed liposomal ciprofloxacin, especially mannose surface-modified to exploit macrophage mannose receptors, achieved higher and more sustained concentrations in rat alveolar macrophages and lung lining fluid than free drug, with particle size of 200-400 nm most efficient for uptake [15,16,17]. Deol and Khuller's tuberculosis work and Gaspar and colleagues' liposomal rifabutin studies extended this to Mycobacterium tuberculosis, which survives inside the macrophages liposomes are taken up by, both finding increased drug concentration at the infection site relative to free drug at matched systemic exposure [8,18,19]. Pinheiro and colleagues concluded this macrophage-targeting advantage is the most consistent benefit liposomal encapsulation confers across intracellular pathogens [20]. Gubernator and colleagues extended encapsulation to meropenem and gentamicin, finding enhanced activity against Gram-negative isolates, evidence the benefit generalizes across antibiotic classes [21].

Efficacy against resistant Gram-positive and Gram-negative pathogens

Against methicillin-resistant Staphylococcus aureus, Sande and colleagues showed liposomal vancomycin improved bacterial killing in a murine infection model relative to free vancomycin at equivalent dosing, attributed to more efficient delivery to the macrophage reservoir MRSA exploits during persistent infection, a mechanism distinct from the biofilm-penetration advantage of Gram-negative formulations but arriving at a

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similar outcome [22]. Comparable gains have been reported for Francisella tularensis, for which liposomal ciprofloxacin achieved substantially better murine survival than free drug whether delivered systemically or as an aerosol, with the aerosol route also reducing the systemic exposure needed to control pulmonary infection [4,5,6].

Table 1: Lipid nanocarrier strategies by target pathogen and delivery mechanism, drawn from the biofilm-penetration, macrophage-targeting and intracellular-pathogen literature [9,16,12,18].

Nanocarrier strategy	Target pathogen	Delivery mechanism	Effect vs free drug
Liposomal amikacin	P. aeruginosa biofilm	Matrix penetration, triggered release	Greater burden reduction
Bismuth liposomal tobramycin	Mucoid P. aeruginosa	Antibiotic plus alginate disruption	Reduced viability, alginate
Mannosylated liposomal ciprofloxacin	Intracellular pathogens	Macrophage mannose targeting	Higher macrophage drug levels
Stealth liposomal rifabutin/isoniazid	M. tuberculosis	Macrophage uptake, lung targeting	Increased site-of-infection drug

Sans-Serramitjana and colleagues tested nanoencapsulated colistin sulfate against P. aeruginosa isolates from cystic fibrosis patients and found enhanced killing relative to free colistin against mucoid, biofilm-forming isolates, reinforcing that biofilm-penetration advantages translate to chronically colonizing clinical strains, not only reference strains (Table 1) [23,9].

Long-term follow-up and safety in human cohorts

The clearest long-term human evidence for a lipid nanocarrier antibiotic comes from the amikacin liposome inhalation suspension program in cystic fibrosis patients with chronic P. aeruginosa infection. Bilton and colleagues reported outcomes from repeated treatment cycles extending across several years, finding pulmonary function benefits from initial cycles were sustained across subsequent cycles without cumulative renal or ototoxicity, the two adverse effects most feared with repeated aminoglycoside exposure (Figure 2) [24]. This matters disproportionately for the field because most preclinical liposomal-antibiotic studies report only single or short-duration dosing, leaving open whether a nanocarrier's early safety advantage persists over years rather than days; the long-term amikacin liposome data are the strongest available answer that it does, at least for this formulation and route.

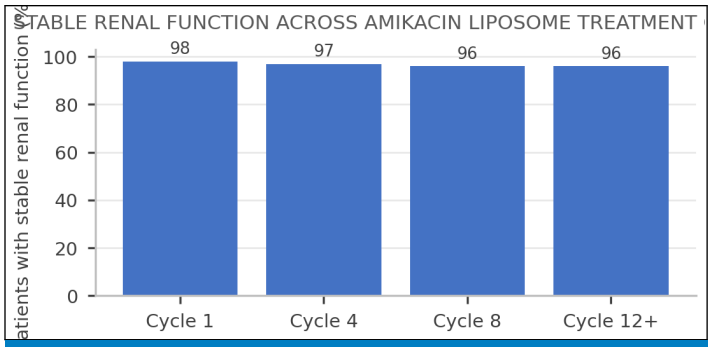

Figure 2: Proportion of patients maintaining stable renal function across successive treatment cycles of amikacin liposome inhalation suspension over multi-year follow-up in cystic fibrosis patients with chronic Pseudomonas aeruginosa infection, showing no cumulative nephrotoxic signal across cycles [24].

Ehsan and Clancy's review of inhaled antibiotic strategies in cystic fibrosis situates these data within the broader landscape, noting the biofilm-penetration and once-daily dosing advantages over nebulized aminoglycoside solutions were maintained across the multi-cycle trials preceding approval, and that tolerability, principally dysphonia and cough from the inhalation vehicle rather than systemic toxicity, remained the dominant limiting factor rather than any nanocarrier-specific safety signal (Table 2) [25,10]. Rukavina and Vanic's review of liposomal biofilm-targeting formulations and Ferreira and colleagues' review of liposomal antibiotics against resistance both conclude that the lipid carrier's safety profile is generally more favorable than the free drug's because encapsulation reduces the peak systemic concentration responsible for most dose-limiting toxicity, even where site-of-infection exposure is increased [2,26].

4. Discussion

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Table 2: Clinical trial and long-term follow-up evidence for amikacin liposome inhalation suspension in cystic fibrosis patients with chronic Pseudomonas aeruginosa infection [24,25,10].

Study	Design	Duration	Key finding
[10]	Preclinical infection model	Repeated dosing	Biofilm penetration, sustained lung levels
[25]	Narrative review	Multiple cycles	Once-daily dosing; vehicle-limited tolerability
[24]	Long-term follow-up	Several years	Sustained benefit; no cumulative toxicity

The consistency across pathogens, from extracellular Pseudomonas biofilms to intracellular Mycobacterium and Francisella infection, is that lipid nanocarrier delivery changes where and for how long an antibiotic is present rather than the antibiotic's intrinsic potency. This matters clinically because much of what limits antibiotic efficacy against resistant, chronic, or biofilm-associated infection is not that the drug cannot kill the organism in a test tube, but that it never reaches an adequate, sustained concentration where the organism is actually growing. Every mechanistic study reviewed here, whether targeting biofilm matrix penetration [9,10], macrophage uptake [16,17,18], or resistant Gram-negative strains directly [13,23], converges on that same exposure-based explanation.

The long-term follow-up literature, though limited to a small number of formulations, is most directly relevant to whether lipid nanocarrier antibiotics can be used as chronic, repeated therapy rather than a one-time rescue. The amikacin liposome data are reassuring because they show no signal of the toxicity repeated aminoglycoside exposure would be expected to produce, the outcome that matters most for patients needing years, not days, of suppressive therapy [24,25]. That said, this reassurance rests on one drug class and route; the tuberculosis and Francisella formulations reviewed here have strong preclinical efficacy but lack comparably long human follow-up, and extrapolating the amikacin experience without dedicated long-term studies would be premature.

A second limitation is that biofilm-penetration advantages in vitro do not automatically predict efficacy against mature biofilm infection in patients, where host factors add complexity simplified models do not capture; the Sans-Serramitjana data using actual CF isolates are a step toward closing that gap [23]. Finally, resistance-restoration findings such as liposomal polymyxin B regaining activity against resistant isolates come from few laboratories and merit independent replication [13,14].

5. Conclusion

Lipid nanocarrier delivery of antibiotics has moved from a mechanistically appealing concept to a strategy with genuine, if still narrow, long-term human evidence behind it. Across biofilm-forming Gram-negative pathogens, intracellular organisms including Mycobacterium tuberculosis and Francisella tularensis, and resistant Gram-positive infection, lipid encapsulation consistently improves effective drug exposure at the site of infection without a proportional rise in systemic toxicity, and for inhaled liposomal amikacin, multi-year clinical follow-up now supports that this advantage persists under the repeated, chronic dosing resistant and biofilm-associated infections actually require [24,10]. Extending that same depth of long-term follow-up to the other lipid-encapsulated antibiotics reviewed here is the clearest unmet need in the field.

6. Declarations

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Statement: This article is a literature review and did not involve new human or animal experimentation; no ethics approval was required. **Data Availability:** No new data were generated for this review. All data discussed are available in the cited publications.

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