Special Report

For reprint orders, please contact: reprints@futuremedicine.com

Future MICROBIOLOGY

Antibiotic-nanomedicines: facing the challenge of effective treatment of antibiotic-resistant respiratory tract infections

Jeffrey AS Ritsema[‡,1], Hessel van der Weide[‡,2], Yvonne M te Welscher[1], Wil HF Goessens[2], Cornelus F van Nostrum[1], Gert Storm[1], Irma AJM Bakker-Woudenberg[2] & John P Hays[*,2]

[1]Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands

[2]Department of Medical Microbiology & Infectious Diseases, Erasmus University Medical Center Rotterdam (Erasmus MC), Rotterdam, The Netherlands

*Author for correspondence: Tel.: +31 (0)10 703 2177; j.hays@erasmusmc.nl

[‡]Joint first authors

Respiratory tract infections are one of the most frequent infections worldwide, with an increasing number being associated with (multiple) antibiotic-resistant pathogens. Improved treatment requires the development of new therapeutic strategies, including the possible development of antibiotic-nanomedicines. Antibiotic-nanomedicines comprise antibiotic molecules coupled to nanocarriers via surface adsorption, surface attachment, entrapment or conjugation and can be administered via aerosolization. The efficacy and tolerability of this approach has been shown in clinical studies, with amikacin liposome inhalation suspension being the first inhalatory antibiotic-nanomedicine approved by the US FDA. In this special report, we summarize and discuss the potential value and the clinical status of antibiotic-nanomedicines for the treatment of (antibiotic-resistant) respiratory tract infections.

First draft submitted: 27 June 2018; Accepted for publication: 31 October 2018; Published online: 30 November 2018

Keywords: aerosols • antibiotic resistance • antibiotic nanomedicines • pulmonary administration • respiratory tract infections

Respiratory tract infections (RTIs) present a significant burden on global healthcare and have been estimated as the underlying cause of 6% of disability-adjusted life years in 2015 [1]. In primary care, many RTIs are often self-limiting viral infections and are usually not fatal unless a secondary bacterial infection occurs [2,3]. However, in secondary and tertiary care, bacterial RTIs predominate over viral infections, with bacterial infections being much more likely to lead to significant morbidity and/or mortality in affected patients [4]. The effective antimicrobial treatment of bacterial infections is a crucial component in reducing the disease burden of RTIs and may be a life-saving action in many cases [5]. However, pathogenic bacteria continue to demonstrate their ability to adapt to many different types of antimicrobial compounds. As a result, global antibiotic resistance continues to increase, while the pool of effective antimicrobial compounds is simultaneously drying up. There are several reasons why the current R&D portfolios of pharmaceutical companies are deficient in this area, largely due to the fact that antibiotic discovery is not as financially successful as the development of drugs for other disease areas. The consequence is that relatively few new candidate antibiotics have reached the market [6,7]. Global authorities such as the European Union (EU) and the United Nations (UN), as well as the World Health Organization (WHO), are aware of this problem and invest in trying to solve the worldwide endemic of antibiotic resistance by focusing on several key 'One Health' action areas [8–10]. These key areas include increased epidemiological reporting of antibiotic resistance, the implementation of guidelines for effective infection prevention policies, reducing the use of antibiotics as growth promoters in food animals, the development of novel rapid diagnostics and the development of new antibiotics which are active against extensively resistant microorganisms.

Future Medicine

Novel antimicrobial compounds

Most currently available antimicrobial compounds were initially discovered by screening microorganisms (usually fungi) for naturally produced antimicrobial compounds, for example, penicillins, macrolides, tetracyclines and aminoglycosides [7,11]. Additionally, synthetic chemistry approaches have been utilized to generate new types of antibiotics, for example the carbapenems. However, both of these approaches have failed to generate any new classes of antibiotics in recent years [12]. As of September 2018, only one novel class of antibiotics has entered clinical development, namely gepotidacin for the treatment of RTIs [13]. Further, two known, but undervalued, classes of antibiotics that have previously seen little therapeutic application are also being developed for the treatment of RTIs; namely antimicrobial peptides and pleuromutilins [14,15]. It is the discovery and development of these new/undervalued classes of antibiotics (rather than the continual adaptation of already existing classes of antibiotics) which is most likely to be successful in the worldwide fight against antibiotic resistant bacterial pathogens.

New treatment strategies

The treatment of RTIs in primary care occurs via the prescription of a course of oral antibiotics, whereas in secondary and tertiary care, more powerful antibiotics are usually prescribed and administered via the intravenous route [16,17]. However, although the parenteral (intravenous) application of antibiotics allows for high systemic bioavailability, this route of administration may not always achieve the necessary minimum inhibitory antibiotic concentration at the site of infection. Furthermore, the systemic application of antibiotics can cause unwanted toxic side-effects due to the antibiotic reaching tissues other than the infected tissue [18–21]. Another serious side-effect of systemic antibiotics is the enhanced selection of antibiotic-resistant bacteria residing in the endogenous microbiota, so-called collateral damage [22]. Such resistant subpopulations, selected from the endogenous microbiota, may cause invasive infections which prove very hard to treat in critically ill patients Therefore, in addition to the development of novel antibiotics, new treatment strategies are also being investigated including the administration of β-lactams combined with β-lactamase inhibitors, bacteriophage-based treatment and the synthesis of hybrid antibiotics [23–26]. Another promising approach is the use of antibiotic-nanomedicines, as outlined in this Special Report.

Role of aerosolized antibiotics

To effectively treat a patient suffering from an infection, it is important to deliver antibiotic molecules to the actual site of the infection such that the minimum inhibitory concentration (MIC) is achieved while causing minimal side-effects and collateral damage to the patient's own microbiota. For RTIs, the site of infection is the lung and over the last decade there has been an increasing interest in exploiting the pulmonary delivery of antibiotics [27]. The accurate delivery of antibiotics directly to the pulmonary site of infection would allow the antibiotics to reach higher local concentrations, thereby increasing their effective antimicrobial activity [27,28]. This local delivery approach also avoids 'first-pass' metabolism and may help reduce any toxic side-effects associated with systemic administration [29,30]. However, although some antibiotics have been non-invasively administered to patients via aerosols – in solid or liquid particles ranging in size from 0.01 to 100 microns in diameter – the majority of aerosolized antibiotics often show suboptimal therapeutic efficacy due to their short lung half-life and low therapeutic availability at the intrapulmonary site of infection [31]. This short half-life and limited therapeutic activity is primarily due to mucociliary-related pulmonary clearance mechanisms present in the host [32]. Exogenous particles and chemicals are typically trapped within the mucus layer in the lungs with cilia facilitating coordinated movement of these particles to the pharynx, where these are coughed up and swallowed. Deposited particles are also susceptible to alveolar macrophage clearance, as alveolar macrophages engulf, transport and thereby clear particles from the alveolar epithelium. The large surface area, epithelial permeability and high vascularization of the lung also facilitate the rapid absorption of antibiotics into the bloodstream (away from the lung) via passive diffusion or passage through tight junctions [32]. Additionally, many antibiotics possess hydrolytically susceptible chemical bonds (e.g., esters and amides), causing degradation (and subsequent loss of biological activity) via enzymes secreted by the lung.

Nanocarrier formulations

The protection of antibiotics from clearance, enzymatic/chemical degradation and rapid adsorption, as well as the reliable deposition and residence of aerosolized drug doses at predetermined locations in the lung, can prove challenging [33–35]. The incorporation of antibiotics in or on so-called 'nanocarriers' can potentially overcome

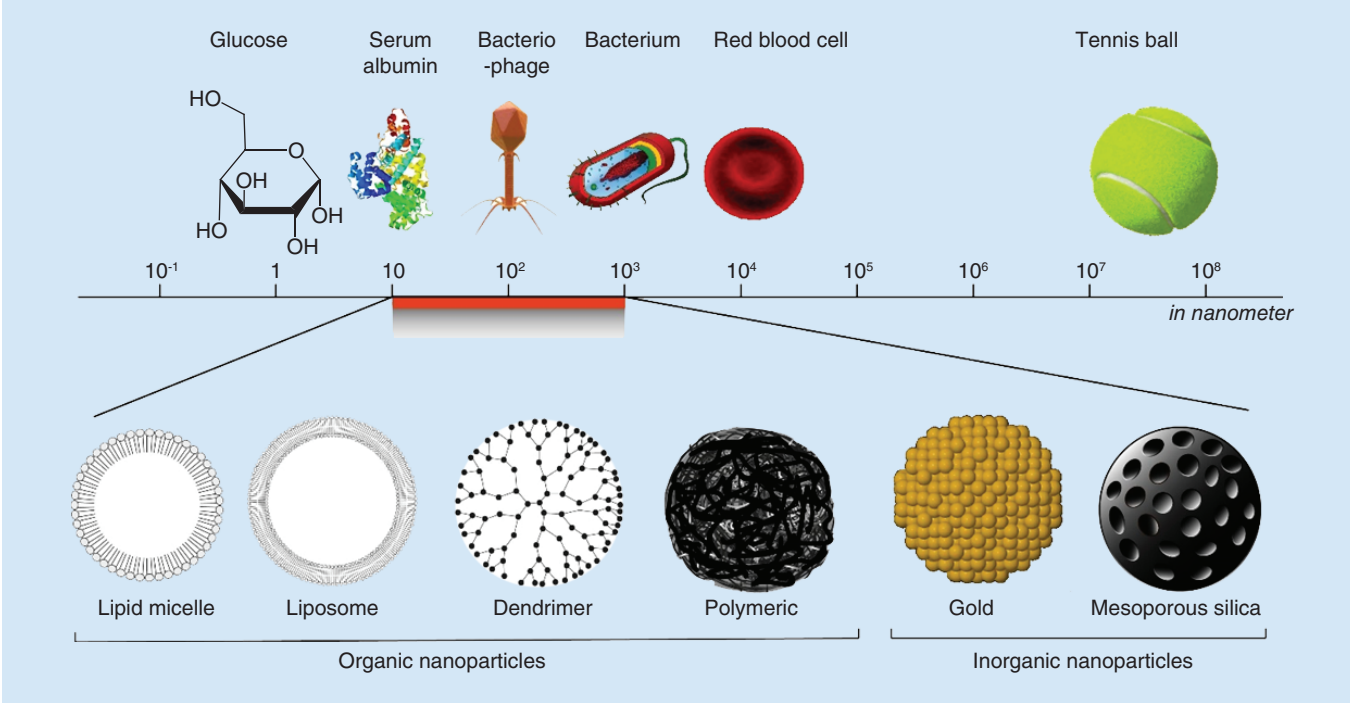

Figure 1. Pictorial representation of different types of nanoparticles and their size in comparison to various biological and physical objects. Nanocarriers can be classified into two essential groups: Organic nanoparticles (e.g. lipid micelles, liposomes, dendrimers, polymeric nanoparticles) and inorganic nanoparticles (e.g., gold and mesoporous silica nanoparticles as examples).

these hurdles, and in this respect, many different nanocarrier formulations have been developed for antibiotic encapsulation or coupling.

Nanocarriers are particles ranging in size from 10 to 1000 nm [36] and are used in a wide variety of medicines, where the active pharmaceutical ingredient is either adsorbed, covalently attached to the surface, entrapped or conjugated into the matrix of the nanocarrier (Figure 1) [37]. Nanocarriers may in general be classified based on the type of material from which the matrix is made, in other words, organic nanocarriers or inorganic nanocarriers.

Organic nanocarriers, especially 'liposomes', are the most widely studied nanoparticulate delivery systems [38]. Liposomes are self-assembling spherical nanostructures consisting of one or more lipid bilayers, formed via the intrinsic interfacial properties imparted by the constituent phospholipids. Other widely studied organic drug delivery systems include polymeric nanocarriers, which can be highly stable due to the high structural integrity afforded by the rigidity of their polymer matrix. Poly(lactic- *co*-glycolic acid), chitosan, dextran, alginates, polyvinyl alcohol (PVA) and polyethylene glycol (PEG) are examples of components of polymeric nanocarriers that are currently being extensively studied as drug delivery systems, owing to their minimal toxicity, biodegradability and biocompatibility [39]. In recent years, other nanocarrier-based drug delivery systems have also been described, including polymeric or lipid micelles, solid-lipid nanoparticles (NPs), dendrimers, polymersomes, nanogels, etc. [40].

Focusing on RTIs and nanomedicine delivery to the lung, a wide variety of nanocarriers could potentially be utilized [41]. The aerodynamic diameter, shape and surface properties of the aerosol are the primary factors, with the architecture of the respiratory tract and biological clearance mechanisms as key determinants of lung deposition pattern and retention of aerosols. For instance, when targeting the lower airways, aerosols with an aerodynamic diameter of 1–5 μm are believed to deposit most efficiently [42]. In practice, this means that micron-sized powder of agglomerated particles or liquid dispersions are currently mainly used for the pulmonary delivery of nanomedicines [43,44].

Advantages of pulmonary administration of antibiotic-nanomedicines

For RTI, aerogenic administration of antibiotic-nanomedicines possess several advantages over free inhaled antibiotics [32,45–48].

Increased target localization & efficacy at lower drug dose

To improve lung bioavailability, Pandey *et al.* administered poly(DL-lactide-*co*-glycolide) (PLGA) NPs containing rifampicin, isoniazid and pyrazinamide via the pulmonary route to *Mycobacterium tuberculosis*-infected guinea pigs [49]. The inhaled nanomedicines (as an aerosol) exhibited increased lung retention at therapeutic levels and improved dosage schedule (i.e., reduced dosing frequency as an aerosol) compared with the free drug given via oral or intravenous route. After a single nebulization of drug-loaded PLGA NPs, all 3 antibiotics were detected at therapeutic drug levels up to 11 days in lung homogenates, while oral or aerosol-administered free antibiotic at the same dose were not detectable after 24 h. Complete killing of *M. tuberculosis* in the lungs of infected guinea pigs was realized after nebulization of 5 doses of drug-loaded PLGA NPs at 10-day intervals, whereas 46 similar daily doses of orally administered drugs were required in order to provide similar therapeutic efficacy [49].

Protection against enzymatic/chemical degradation or unwanted interactions with other molecules

Nanocarriers can physically protect sensitive molecules from rapid degradation and reduce unwanted interactions with nonrelevant host biomolecules. For example, nano-sized drug delivery systems can be coated with (poly)ethylene glycol resulting in decreased uptake and degradation by cells of the mononuclear phagocyte system – a strategy to increase the blood circulation time after intravenous administration [50–52].

Nacucchio *et al.* showed that encapsulation of the beta-lactam antibiotic piperacillin by PC:Chol liposomes protected the drug from hydrolysis by Staphylococcal beta-lactamase. This resulted in enhanced antibacterial activity of liposomal piperacillin against Staphylococcal growth in biofilms in the presence of exogenous beta-lactamase [53]. Meers *et al.* showed that encapsulation of amikacin in DPPC:Chol liposomes is beneficial in the treatment of chronic *Pseudomonas aeruginosa* biofilm infections via improvement of biofilm access and/or reducing undesirable interactions with biofilm matrix components. Measurement of amikacin release and efficacy in the rat lung, as measured by fluorescence polarization immunoassay and viable bacterial count, showed that inhaled liposomal amikacin was released in a slow, sustained mode in normal rat lungs and was superior in antimicrobial activity compared with inhaled free amikacin in infected lungs in a 14 day *P. aeruginosa* infection model. Further, the use of a filter assay and epifluorescence/confocal scanning laser microscopy showed that fluorescently labeled DPPC:Chol liposomes could significantly penetrate the *P. aeruginosa* biofilm [54]. Magabe *et al.* compared the antibacterial activity of liposomal gentamicin versus free gentamicin against gentamicin-resistant strains of *P. aeruginosa* [55]. Gentamicin encapsulated in DMPC:Chol, DPPC:Chol or DSPC:Chol liposomes exhibited a higher antimicrobial activity compared with free gentamicin. This effect was attributed to either enhanced diffusion of the liposome-encapsulated antibiotic across the bacterial cell envelope and/or to protection of the antibiotic from enzymatic degradation as a result of liposomal encapsulation.

Protection from pulmonary clearance mechanisms

The inclusion of mucoadhesives (e.g., cationic groups) via surface modification of nanocarriers has been suggested to improve the pulmonary delivery of drugs via an increased lung retention time. In the case of chitosan-modified PLGA nanospheres (approx. 650 nm) loaded with elcatonin (an antiparathyroid agent), the elimination rate constant was approximately a third compared with that of unmodified chitosan nanospheres, resulting in enhanced and prolonged pharmacological action compared with unmodified chitosan nanospheres [56].

Other studies have suggested that the retention of particles that adhere to airway mucus is limited due to mucus clearance mechanisms and that nanocarriers that do not adhere, or rapidly penetrate the mucus, allow uniform and long-lasting drug delivery to the airways following inhalation. Schneider *et al.* demonstrated in *in vitro* experiments that particles as large as 200 nm are able to rapidly penetrate the respiratory mucus of patients with cystic fibrosis (CF) if the particles are densely coated with (poly)ethylene glycol. On the other hand, mucoadhesive particles were unable to rapidly penetrate respiratory mucus regardless of the particle size [57]. When tested *in vivo*, the mucoadhesive particles were more rapidly eliminated from the lumen of the lung of mice, while the penetrating nanocarriers were uniformly distributed throughout the mucus layer and exhibited improved retention time. This resulted in improved therapeutic efficacy compared with both carrier-free drug or a drug delivered via a mucoadhesive nanocarrier system.

Enhanced internalization by target cells

In the context of the treatment of intracellular infections, one major challenge is the difficulty of antibiotic access to the protective environment within cells. For example, *Mycobacteria* spp. latently reside in the phagocytic

intracellular compartments of macrophages. Kisich *et al.* investigated the effects of moxifloxacin encapsulated in poly(butyl cyanoacrylate) (PBCA) NPs against *M. tuberculosis* residing in macrophages [58]. Drug-loaded PBCA NPs showed increased antibacterial activity via tenfold reduction of the MIC. In macrophages exposed to moxifloxacin PBCA NPs, the intracellular accumulation of moxifloxacin was increased threefold compared with exposure to free drug. Also, the intracellular retention time of moxifloxacin increased from 4 to 24 h.

Controlled antibiotic release

The use of different types of antibiotic nanomedicines can enable the controlled release of antibiotics into the lung. For example, antibiotic nanomedicines may allow the triggered release of an antibiotic at low pH conditions, such as is found in the inflamed lung environment, or may be positively charged to improve their affinity for negatively charged bacterial surfaces and biofilms at the site of infection [59,60]. Additionally, antibiotics with time-dependent activity may benefit from the use of sustained-release nanomedicines [61], resulting in optimal antimicrobial activity over time, while minimizing the chance of unwanted side-effects due to uncontrolled and massive release of antibiotic within a short period of time.

Finally, the inhalation of insoluble nondegradable or slowly degradable particles may lead to serious inflammatory responses and oxidative stress, resulting in irritation, cellular injury, edema, phagocytosis impairment and breakdown by host defense mechanisms [34,35]. However, the toxicity of NPs is mainly determined by the materials that they are composed of and their surface characteristics. Therefore, extensive *in vitro* and *in vivo* testing is performed as part of the development process of NP-based drug-carrier systems. This means that the materials and inhalation strategies established for a particular antibiotic nanomedicine formulation are carefully selected (e.g., the use of biodegradable or biocompatible materials), in order to minimize the possibility of adverse reactions when inhaled by patients.

Clinical status

The use of nanocarriers and the potential value of direct delivery of aerosolized nanocarrier-bound antibiotics to the lung has been shown in patients with inhaled liposomal formulations of ciprofloxacin (Lipoquin™) and a mixed formulation of non-encapsulated and liposomal ciprofloxacin (Pulmaquin™). These formulations have been evaluated in Phase III clinical trials in CF patients and non-CF patients with RTIs.

Initially, two Phase IIA clinical trials of liposomal ciprofloxacin formulations demonstrated that 2- and 4-week once-daily administration of Lipoquin in CF patients and non-CF bronchiectasis patients was safe and capable of reducing the *P. aeruginosa* bacterial load in sputum [62]. Although the results obtained using Lipoquin were encouraging, extra experiments using Pulmaquin were performed in order to determine if additional clinical benefit might be gained using a rapid antibiotic release (peak concentration) strategy when compared with using free antibiotic. In Phase I studies, Pulmaquin showed significantly higher maximum plasma concentrations of ciprofloxacin when compared with Lipoquin due to the presence of non-encapsulated antibiotic in the Pulmaquin formulation. The ciprofloxacin concentrations in plasma over time were more than two-fold lower following administration of Pulmaquin or Lipoquin compared with plasma levels of approved doses of oral or intravenous ciprofloxacin. This suggested that after administration of liposomal ciprofloxacin the potential risk of systemic side-effects, even upon repeated dosing with such ciprofloxacin-NPs, was significantly reduced. In Phase IIb clinical trials, named ORBIT-1 and ORBIT-2 (directed against non-CF bronchiectasis patients suffering from *P. aeruginosa* infection), both Lipoquin and Pulmaquin were investigated for their ability to provide the optimum dose of ciprofloxacin with minimal side-effects. Pulmaquin showed superior pulmonary safety profile with rapid and persistent reduction of bacterial load in sputum

Based on these results, Pulmaquin was selected and evaluated by Aradigm Corporation in a Phase III clinical trial in non-CF bronchiectasis patients (ARD-3150-1201, ORBIT-3 (NCT01515007) and ORBIT-4 (NCT02104245), which was followed by a 28-day open label extension study [63]. The Aradigm Corporation announced that analyses of combined data from both studies demonstrated a statistically significant reduction in *P. aeruginosa* load in the lungs at the end of the first on-treatment period. There was also a statistically significant reduction (27%) in pulmonary exacerbation over a 48-week double-blind treatment period between the Pulmaquin group and the placebo group. The median time to first moderate or severe pulmonary exacerbations - those exacerbations that require interventions with antibiotics or hospitalization – were statistically improved in the Pulmaquin treated group (198 days) versus placebo group (302 days). Additionally, Pulmaquin was safe and well tolerated in both studies. Therefore, in the first quarter of 2018, Aradigm Corporation submitted a marketing authorization request to the

European Medicines Agency (EMA) for Linhaliq™ (formerly Pulmaquin) as a treatment for non-CF bronchiectasis patients with a chronic *P. aeruginosa* lung infection.

Another antibiotic-nanomedicine – liposomal amikacin – has been studied in Phase II clinical trials (NCT00558844, NCT00777296, NCT01315236) comparing inhaled liposomal Amikacin (Arikayce®/Arikace®/ALIS®) to placebo in once-daily and multi-drug regimens. In CF patients with *P. aeruginosa* infection, once-daily, Arikayce was shown to improve lung function and improve patient-reported respiratory clinical symptoms over a 28 day period of treatment [64]. Additionally, a statistically significant reduction in *P. aeruginosa* density in sputum (>1 log) was observed compared to baseline measurements. In patients with antibiotic-resistant non-tuberculous mycobacteria (NTM) infections, negative bacterial cultures were obtained by day 84 in 11/45 patients, whereas this was only achieved in 3/45 patients receiving standard treatment [65]. Insmed Inc. also completed a European and Canadian registrational Phase III studies of Arikayce in CF patients - the CLEAR-108 (NCT01315678) and CLEAR-110 (NCT01316276) projects [66]. Overall, once daily Arikayce was non-inferior to inhalation treatment with tobramycin solution when taken twice daily in patients with CF and chronic bronchopulmonary *P. aeruginosa* infections. Furthermore, inhaled Arikayce was generally safe and well tolerated. Insmed Inc. also investigated Amikacin Liposome Inhalation Suspension (ALIS) in a Phase III clinical trial (INS-312, NCT02628600), which was established to investigate the treatment of adult patients with refractory NTM infections caused by *Mycobacterium avium* complex (MAC) [67]. The study demonstrated that ALIS when added to guideline-based therapy, eliminated the infection in 29% of patients, compared with 9% of patients treated using guideline-based therapy. Based on these results, in the first quarter of 2018, Insmed Inc. announced FDA acceptance for a new drug application (NDA) for ALIS for the treatment of NTM lung infections caused by MAC. In the fourth quarter, the FDA granted accelerated approval for the amikacin liposome inhalation suspension (Arikayce/ALIS) for the treatment of lung disease caused by MAC in adult patients left with a few or no suitable treatment options [68].

The EMA (EU) and FDA (US) have also granted the orphan drug designation to fusogenic liposomes loaded with tobramycin (Tobramycin Fluidosomes™, Axentis Pharma), for use in CF patient-associated RTIs [69,70]. Although the results of clinical trials using Fluidosomes™ are not available, *in vitro* studies have described the antimicrobial activity of Fluidosomes™ versus free tobramycin using biofilm infection models of *P. aeruginosa*, *Stenotrophomonas maltophilia* and *Burkholderia cepacia*. These studies showed an increased antimicrobial effect (> 17-50x) of Fluidosomes™ compared with free tobramycin in all biofilm models tested [71,72]. Also *in vivo* studies using Fluidosomes™ have shown increased bactericidal activity against infections caused by antibiotic-susceptible or resistant *P. aeruginosa* strains [73,74].

Conclusion & future perspective

The worrying failure of conventional antibiotics in the treatment of infected patients is attributable to the worldwide emergence of antibiotic-resistant bacteria, and therefore the development and testing of new or undervalued antibiotics, or new antibiotic treatment modalities, is urgently required. Furthermore, toxic side-effects associated with prolonged oral and parenteral delivery of high dose antibiotics to patients, and a lack of a targeting mechanism to guide antibiotics to the focus of infection, may lead to treatment with sub-therapeutic levels of antibiotic and therefore inadequate killing of infectious pathogens. The current lack of novel effective antibiotics, implies that the development of efficient delivery modalities, such as the use of inhaled, customized antibiotic nanomedicines containing existing and/or novel antibiotics, could result in improved treatment of pulmonary infections. In this respect, the sheer variety and versatility of nanocarriers available provide many opportunities for the development of antibiotic nanomedicines, including the possibility of targeting the antibiotics to both extracellular and intracellular pathogens in infected tissues, or to pathogens embedded within protective niches of the lung, within sputum or within pulmonary biofilms. That said, only one antibiotic-nanomedicine has entered the market as of this time, mainly due to several hurdles limiting their implementation. Some of these hurdles include factors relating to manufacturing processes (scaling-up production, good manufacturing practice etc.), the unique environment of the lung (e.g., overcoming host defence mechanisms) and the different delivery needs in case of treatment of acute versus chronic infections (creation of rapid versus slow antibiotic release profiles, respectively). At a time when existing antibiotics are becoming ineffective and few new antibiotics are in the developmental pipeline, the potential advantages (i.e., reduction of patient morbidity and mortality) that could be gained by using antibiotic-nanomedicine therapies should be taken very seriously.

Executive summary

Respiratory tract infections & antibiotic treatment

- Increasing worldwide antibiotic resistance means that the successful treatment of Respiratory tract infections (RTIs) is often challenging.
- One major critical factor involved in promoting the spread of antibiotic resistance is sub-optimal antibiotic dosing and inferior pharmacokinetic profiles (associated with antibiotic administration via oral and parenteral routes).
- Antibiotic therapy may also be associated with host tissue toxicity and damage to the host's protective microbiota.

New strategies to provide effective treatment against bacterial RTIs

- Novel antimicrobial compounds could be utilized to treat antibiotic-resistant RTIs, but there is currently a lack of novel antibiotics available for clinical use.
- The effective administration of antibiotics to the site of infection in patients with RTIs could be facilitated by the local delivery of (existing and/or novel) antibiotics via antibiotics incorporated in nanocarriers (antibiotic nanomedicines).
- antibiotic nanomedicines could be optimized for targeted deposition of (mixtures of) antibiotics at the infection focus in RTIs, thereby increasing the efficacy of antibiotic therapy.

Increased efficacy associated with the use of antibiotic nanomedicines for bacterial RTIs

- antibiotic nanomedicines can increase the antibiotic concentration at the site of infection and therefore can help to reduce the emergence of antibiotic resistance.
- Toxic side-effects and collateral damage by antibiotics to the host's bacterial flora can also be minimized.
- Custom antibiotic nanomedicines for tailored antibiotic release kinetics can be developed.
- Protection of the antibiotic from host-related pulmonary clearance mechanisms, enzymatic/chemical degradation or detrimental interactions with host biomolecules.

The potential value of direct delivery of aerosolized antibiotic nanomedicines to the lung has been shown in patients

- Inhaled liposomal formulations of ciprofloxacin (Lipoquin™ and Pulmaquin®/Linhaliq™) and amikacin (Arikayce®/Arikace®/Alis®) have been evaluated for their efficacy and safety in Phase III clinical trials.
- The US FDA granted accelerated approval for the amikacin liposome inhalation suspension (Arikayce®) for the treatment of lung disease caused by *Mycobacterium avium* complex in adult patients left with a few or no treatment options.

Conclusion

- The development and testing of antibiotic nanomedicines for the targeted delivery of antibiotics has the potential to be a powerful tool for the treatment of (antibiotic-resistant) bacterial RTIs.
- antibiotic nanomedicines can address critical challenges associated with conventional antibacterial therapies and administration routes.
- A better mechanistic understanding of the complex delivery pathways of inhaled antibiotic nanomedicines is required in order to be able to further improve lung deposition and maximize therapeutic efficacy.

Financial & competing interests disclosures

Erasmus University Medical Center Rotterdam (Erasmus MC) and Utrecht University received funding from the European Union's Seventh Programme for Research, Technological Development and Demonstration under grant agreement No. 604434, PneumoNP (www.pneumonp.eu). In addition, Utrecht University was supported by the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 604237 NAREB (www.nareb.eu). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1 Geneva WHO. Global Health Estimates 2016: Burden of disease by Cause, Age, Sex, by Country and by Region, 2000–2016 (2016). www.who.int/healthinfo/global_burden_disease/GHE2016_Deaths_Global_2000_2016.xls?ua=1

2 Vos T, Barber RM, Bell B *et al.* Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 386(9995), 743 (2015).

3 Fahey T, Stocks N, Thomas T. Systematic review of the treatment of upper respiratory tract infection. *Arch. Dis. Childhood* 79(3), 225–230 (1998).

4 Mccullers JA. Insights into the interaction between influenza virus and pneumococcus. *Clin. Microbiol. Rev.* 19(3), 571–582 (2006).

5 Md PFW, Md KBK, Md JFM. When to consider the use of antibiotics in the treatment of 2009 H1N1 influenza-associated pneumonia. *New Engl. J. Med.* 361(24), e112 (2009).

6 Davies J, Davies D. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.* 74(3), 417–433 (2010).

•• **This review summarizes the development of antibiotic resistance in the past half-century and the current urgency for new antibiotic treatment.**

7 Lewis K. Antibiotics: Recover the lost art of drug discovery. *Nature* 485(7399), 439–440 (2012).

8 Council of the European Union. Council conclusions on the impact of antimicrobial resistance in the human health sector and in the veterinary sector – a 'One Health' perspective. C 211/2 (2012). www.consilium.europa.eu/uedocs/cms_data/docs/pressdata/en/lsa/131126.pdf

9 United Nations. Draft political declaration of the high-level meeting of the General Assembly on antimicrobial resistance 16–16108(E) (2016). www.un.org/pga/71/wp-content/uploads/sites/40/2016/09/DGACM_GAEAD_ESCAB-AMR-Draft-Political-Declaration-1616108E.pdf

10 Shallcross LJ, Davies SC. The World Health Assembly resolution on antimicrobial resistance. *J. Antimicrobial. Chemother.* 69(11), 2883–2885 (2014).

11 Aminov RI. A brief history of the antibiotic era: lessons learned and challenges for the future. *Front. Microbiol.* 1, 134 (2010).

12 Lewis K. Platforms for antibiotic discovery. *Nat. Rev. Drug Discov.* 12(5), 371–387 (2013).

• **Explores the diminishing returns from the traditional platforms for antibiotic discovery and current strategies for establishing new platforms.**

13 Pew Charitable Trusts. Antibiotics currently in global clinical development (2018). www.pewtrusts.org/es/research-and-analysis/data-visualizations/2014/antibiotics-currently-in-clinical-development

14 Gordon YJ, Romanowski EG, Mcdermott AM. A review of antimicrobial peptides and their therapeutic potential as anti-infective drugs. *Curr. Eye Res.* 30(7), 505–515 (2005).

15 Novak R, Shlaes DM. The pleuromutilin antibiotics: a new class for human use. *Curr. Opin. Investig. Drugs* 11(2), 182–191 (2010).

16 Macgregor RR, Graziani AL. Oral administration of antibiotics: a rational alternative to the parenteral route. *Clin. Infect. Dis.* 24(3), 457–467 (1997).

17 Mcmullan BJ, Andresen D, Blyth CC *et al.* Antibiotic duration and timing of the switch from intravenous to oral route for bacterial infections in children: systematic review and guidelines. *Lancet Infect. Dis.* 16(8), e139–e152 (2016).

18 Falagas ME, Kasiakou SK. Toxicity of polymyxins: a systematic review of the evidence from old and recent studies. *Crit. Care* 10(1), R27 (2006).

19 Kaewpoowat Q, Ostrosky-Zeichner L. Tigecycline: a critical safety review. *Expert Opin. Drug Saf.* 14(2), 335–342 (2015).

20 Prayle A, Watson A, Fortnum H, Smyth A. Side effects of aminoglycosides on the kidney, ear and balance in cystic fibrosis. *Thorax* 65(7), 654–658 (2010).

21 Grill MF, Maganti RK. Neurotoxic effects associated with antibiotic use: management considerations. *Br. J. Clin. Pharmacol.* 72(3), 381–393 (2011).

22 Paterson DL. 'Collateral damage' from cephalosporin or quinolone antibiotic therapy. *Clin. Infect. Dis.* 38(Suppl. 4), S341–S345 (2004).

23 Parkes AL, Yule IA. Hybrid antibiotics – clinical progress and novel designs. *Expert Opin. Drug Discov.* 11(7), 665–680 (2016).

24 Hanlon GW. Bacteriophages: an appraisal of their role in the treatment of bacterial infections. *Int. J. Antimicrob. Agents* 30(2), 118–128 (2007).

25 Drawz SM, Bonomo RA. Three decades of β-lactamase inhibitors. *Clin. Microbiol. Rev.* 23(1), 160–201 (2010).

26 Zhanel GG, Chung P, Adam H *et al.* Ceftolozane/tazobactam: a novel cephalosporin/β-lactamase inhibitor combination with activity against multidrug-resistant gram-negative bacilli. *Drugs* 74(1), 31–51 (2014).

27 Yang W, Peters JI, Williams RO. Inhaled NPs: a current review. *Int. J. Pharm.* 356(1), 239–247 (2008).

28 Patton JS, Byron PR. Inhaling medicines: delivering drugs to the body through the lungs. *Nat. Rev. Drug Discov.* 6(1), 67–74 (2007).

29 Sung JC, Pulliam BL, Edwards DA. NPs for drug delivery to the lungs. *Trends Biotechnol.* 25(12), 563–570 (2007).

30 Bailey MM, Berkland CJ. Nanoparticle formulations in pulmonary drug delivery. *Med. Res. Rev.* 29(1), 196–212 (2009).

31 Patton JS, Brain JD, Davies LA *et al.* The particle has landed – characterizing the fate of inhaled pharmaceuticals. *J. Aerosol Med. Pulmon. Drug Deliv.* 23(Suppl. 2), S71–S87 (2010).

•• **Provides a better understanding of the determinants of pulmonary drug deposition, dissolution, absorption and metabolism.**

32 El-Sherbiny I, Elbaz N, Yacoub Magdi H. Inhaled Nano- and microparticles for drug delivery. *Glob. Cardiol. Sci. Pract.* 2015, 2 (2015).

33 Byron PR. Drug delivery devices: issues in drug development. *Proc. Am. Thorac. Soc.* 1(4), 321–328 (2004).

34 Abdelaziz HM, Gaber M, Abd-Elwakil MM *et al.* Inhalable particulate drug delivery systems for lung cancer therapy: nanoparticles, microparticles, nanocomposites and nanoaggregates. *J. Control. Rel.* 269, 374–392 (2018).

35 Haque S, Whittaker MR, Mcintosh MP, Pouton CW, Kaminskas LM. Disposition and safety of inhaled biodegradable nanomedicines: Opportunities and challenges. *Nanomedicine* 12(6), 1703–1724 (2016).

36 Duncan R, Gaspar R. Nanomedicine(s) under the microscope. *Mol. Pharm.* 8(6), 2101–2141 (2011).

37 Shen S, Wu Y, Liu Y, Wu D. High drug-loading nanomedicines: progress, current status, and prospects. *Int. J. Nanomedicine* 12, 4085–4109 (2017).

38 Lammers T, Kiessling F, Hennink WE, Storm G. Drug targeting to tumors: principles, pitfalls and (pre-) clinical progress. *J. Control. Rel.* 161(2), 175–187 (2012).

39 Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. *J.Control. Rel.* 70(1), 1–20 (2001).

40 Bei D, Meng J, Youan BB. Engineering nanomedicines for improved melanoma therapy: progress and promises. *Nanomedicine (London, England)* 5(9), 1385–1399 (2010).

41 Mansour HM, Rhee Y-S, Wu X. Nanomedicine in pulmonary delivery. *Int. J. Nanomedicine* 4, 299–319 (2009).

42 Heyder J, Gebhart J, Rudolf G, Schiller CF, Stahlhofen W. Deposition of particles in the human respiratory tract in the size range 0.005–15 μm. *J. Aerosol Sci.* 17(5), 811–825 (1986).

43 Adam B, Jorrit W, Moritz B-B, Mingshi Y. Nanoembedded microparticles for stabilization and delivery of drug-loaded nanoparticles. *Curr. Pharm Des.* 21(40), 5829–5844 (2015).

44 Mangal S, Gao W, Li T, Zhou Q. Pulmonary delivery of nanoparticle chemotherapy for the treatment of lung cancers: challenges and opportunities. *Acta Pharm. Sin.* 38, 782 (2017).

45 Huh AJ, Kwon YJ. 'Nanoantibiotics': a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. *J. Control. Release* 156(2), 128–145 (2011).

•• **Summarizes emerging efforts in combating infectious diseases using nanomaterials.**

46 Costa-Gouveia J, Aínsa JA, Brodin P, Lucía A. How can nanoparticles contribute to antituberculosis therapy? *Drug Discov. Today* 22(3), 600–607 (2017).

47 Moreno-Sastre M, Pastor M, Salomon CJ, Esquisabel A, Pedraz JL. Pulmonary drug delivery: a review on nanocarriers for antibacterial chemotherapy. *J. Antimicrobial Chemother.* 70(11), 2945–2955 (2015).

48 Bobo D, Robinson KJ, Islam J, Thurecht KJ, Corrie SR. Nanoparticle-based medicines: a review of FDA-approved materials and clinical trials to date. *Pharm. Res.* 33(10), 2373–2387 (2016).

49 Pandey R, Sharma A, Zahoor A, Sharma S, Khuller GK, Prasad B. Poly (dl-lactide-co-glycolide) nanoparticle-based inhalable sustained drug delivery system for experimental tuberculosis. . *Antimicrob. Chemother.* 52(6), 981–986 (2003).

50 Shan X, Liu C, Yuan Y *et al.* In vitro macrophage uptake and in vivo biodistribution of long-circulation nanoparticles with poly(ethylene-glycol)-modified PLA (BAB type) triblock copolymer. *Colloids Surfaces. B Biointerfaces* 72(2), 303–311 (2009).

51 Allen TM, Hansen C. Pharmacokinetics of stealth versus conventional liposomes: effect of dose. *Biochim. Biophys. Acta* 1068(2), 133–141 (1991).

52 Bhattarai N, Bhattarai SR, Khil MS, Lee DR, Kim HY. Aqueous solution properties of amphiphilic triblock copolymer poly(p-dioxanone-co-l-lactide)-block-poly(ethylene glycol). *Eur. Polymer J.* 39(8), 1603–1608 (2003).

53 Nacucchio MC, Bellora MJ, Sordelli DO, D'aquino M. Enhanced liposome-mediated activity of piperacillin against staphylococci. *Antimicrobial Agents Chemother.* 27(1), 137–139 (1985).

54 Meers P, Neville M, Malinin V *et al.* Biofilm penetration, triggered release and *in vivo* activity of inhaled liposomal amikacin in chronic *Pseudomonas aeruginosa* lung infections. *J. Antimicrobial Chemother.* 61(4), 859–868 (2008).

55 Mugabe C, Azghani AO, Omri A. Liposome-mediated gentamicin delivery: development and activity against resistant strains of Pseudomonas aeruginosa isolated from cystic fibrosis patients. *J. Antimicrobial Chemother.* 55(2), 269–271 (2005).

56 Yamamoto H, Kuno Y, Sugimoto S, Takeuchi H, Kawashima Y. Surface-modified PLGA nanosphere with chitosan improved pulmonary delivery of calcitonin by mucoadhesion and opening of the intercellular tight junctions. *J. Control. Release* 102(2), 373–381 (2005).

57 Schneider CS, Xu Q, Boylan NJ *et al.* Nanoparticles that do not adhere to mucus provide uniform and long-lasting drug delivery to airways following inhalation. *Sci. Adv.* 3(4), (2017).

58 Kisich KO, Gelperina S, Higgins MP *et al.* Encapsulation of moxifloxacin within poly(butyl cyanoacrylate) nanoparticles enhances efficacy against intracellular Mycobacterium tuberculosis. *Int. J. Pharm.* 345(1–2), 154–162 (2007).

59 Paliwal SR, Paliwal R, Vyas SP. A review of mechanistic insight and application of pH-sensitive liposomes in drug delivery. *Drug Deliv.* 22(3), 231–242 (2015).

60 Sercombe L, Veerati T, Moheimani F, Wu SY, Sood AK, Hua S. Advances and challenges of liposome assisted drug delivery. *Front. Pharmacol.* 6, 286 (2015).

61 Levison ME, Levison JH. Pharmacokinetics and pharmacodynamics of antibacterial agents. *Infect. Dis. Clin. North Am.* 23(4), 791–815 (2009).

62 Cipolla D, Blanchard J, Gonda I. Development of liposomal ciprofloxacin to treat lung infections. *Pharmaceutics* 8(1), pii:E6 (2016).

- **A complete overview of Aradigm Corporations' liposomal ciprofloxacin products: from development to clinical studies.**

63 Aradigm. Aradigm Announces Top-Line Results from Two Phase 3 Studies Evaluating Pulmaquin for the Chronic Treatment of Non-Cystic Fibrosis Bronchiectasis Patients with Lung Infections with *Pseudomonas aeruginosa* (2016). https://aradigm.gcs-web.com/news-releases/news-release-details/aradigm-announces-top-line-results-two-phase-3-studies

64 Clancy JP, Dupont L, Konstan MW *et al.* Phase II studies of nebulised Arikace in CF patients with *Pseudomonas aeruginosa* infection. *Thorax* 68(9), 818–825 (2013).

65 Olivier KN, Griffith DE, Eagle G *et al.* Randomized trial of liposomal amikacin for inhalation in nontuberculous mycobacterial lung disease. *Am. J. Respir. Crit. Care Med.* 195(6), 814–823 (2017).

66 Konstan M, Pressler T, Clancy JP *et al.* Long-Term Study of Liposomal Amikacin for Inhalation in Patients With Cystic Fibrosis and Chronic Pseudomonas aeruginosa Infection, Presented at: *29th Annual North American Cystic Fibrosis Conference (NACFC)*, Denver, CO, 8–10 October 2015.

67 Insmed. Insmed Announces Additional Data from ALIS (Amikacin Liposome Inhalation Suspension) Phase 3 Clinical Program for Adult Patients with Treatment Refractory NTM Lung Disease Caused by MAC and Reports Progress with Commercial Preparations (2018).

68 FDA. FDA approves a new antibacterial drug to treat a serious lung disease using a novel pathway to spur innovation (2018). www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm622048.htm

69 Axentispharmaag. Axentis Pharma (2018). www.axentispharma.com/

70 FDA. Cumulative List of all Products that have received Orphan Designation (2009). www.fda.gov/downloads/forindustry/developingproductsforrarediseasesconditions/howtoapplyfororphanproductdesignation/ucm162066.xls

71 Beaulac C, Sachetelli S, Lagace J. *In vitro* bactericidal evaluation of a low phase transition temperature liposomal tobramycin formulation as a dry powder preparation against Gram negative and Gram positive bacteria. *J. Liposome Res.* 9(3), 301–312 (1999).

72 Beaulac C, Sachetelli S, Lagace J. *In vitro* bactericidal efficacy of sub-MIC concentrations of liposome-encapsulated antibiotic against gram-negative and gram-positive bacteria. *J. Antimicrobial Chemother.* 41(1), 35–41 (1998).

73 Beaulac C, Clement-Major S, Hawari J, Lagace J. Eradication of mucoid *Pseudomonas aeruginosa* with fluid liposome-encapsulated tobramycin in an animal model of chronic pulmonary infection. *Antimicrobial Agents Chemother.* 40(3), 665–669 (1996).

74 Omri A, Beaulac C, Bouhajib M, Montplaisir S, Sharkawi M, Lagace J. Pulmonary retention of free and liposome-encapsulated tobramycin after intratracheal administration in uninfected rats and rats infected with *Pseudomonas aeruginosa*. *Antimicrobial Agents Chemother.* 38(5), 1090–1095 (1994).