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Antimicrobial molecular nanocarrier–drug conjugates

Andrzej S. Skwarecki[a], Sławomir Milewski[b], Marta Schielmann[b], Maria J. Milewska[a,*]

[a]Department of Organic Chemistry, Faculty of Chemistry, Gdańsk University of Technology, Gdańsk, Poland
[b]Department of Pharmaceutical Technology and Biochemistry, Faculty of Chemistry, Gdańsk University of Technology, Gdańsk, Poland

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Abstract

Many antimicrobial drugs are poorly active against pathogenic microbes causing intracellular infections, such as *Mycobacterium tuberculosis* or *Plasmodium falciparum*. On the other hand, several known antimicrobial agents are not effective enough because of their limited cellular penetration. A common feature of both challenges is the inability of an active agent to cross the biological membrane(s). One of the possible approaches facing these challenges is conjugation of an active substance with a molecular organic nanocarrier. The conjugate thus formed should be able to penetrate the membrane(s) and, once internalized, the active component could reach its intracellular target, either after release from the conjugate or in an intact form. Several molecular nanocarriers have been proposed: oligopeptides, including cell penetrating peptides, carbon nanotubes, siderophores, dendrimers, terpenoids and molecular umbrellas. A comprehensive review of the current status of molecular organic nanocarrier–drug conjugates and the future perspectives of their application as novel antimicrobials is presented.

Key words: Peptides; Siderophores; Terpenoids; Carbon nanotubes; Dendrimers; Molecular umbrellas

For many years, antimicrobial drugs have been used to inhibit or kill bacteria and other microbes. Unfortunately, overconsumption and inappropriate use of these drugs have created major environmental pressures for microbial pathogens to evolve towards resistance. In consequence, resistance to antimicrobial drugs has become increasingly widespread and this has resulted in a significant threat to public health and a substantial challenge for antimicrobial chemotherapy.[1] There are numerous mechanisms of microbial resistance but the most challenging is that of the multi-drug type, resulting from disturbance of drug transport across the microbial membranes, including an impaired uptake and/or active efflux of antimicrobials. Moreover, some of the antimicrobial chemotherapeutics also exhibit strongly reduced activity against biofilm-forming micro-organisms. These pathogens are often able to synthesize and secrete a matrix consisting of an extracellular polymeric substance (EPS) which accumulates and eventually surrounds the population of microbial cells. The EPS matrix is a barrier to diffusion of intact antibiotic molecules and, in consequence, microbes in biofilms are up to 1000 times more resistant to antibiotics than the planktonic ones.[2]

Another challenge for contemporary chemotherapy is the treatment of intracellular microbial pathogens. Several human pathogenic micro-organisms, including *Staphylococcus aureus*, *Salmonella enterica* serovar Typhimurium, *Mycobacterium tuberculosis*, *Plasmodium falciparum* and *Cryptococcus neoformans*, have developed the ability to persist in mammalian cells, making the infection latent or recurrent. The intracellular location provides a particular shelter for microbial pathogens because they are protected not only from host defenses but also from antimicrobial therapy. Indeed, among the antibiotic families, some of them, such as, β-lactams and aminoglycosides, exhibit restricted cellular penetration owing to their high hydrophilicity and, some others, like fluoroquinolones and macrolides, display low intracellular retention. Therefore, intracellular active concentration of these agents is often subtherapeutic, resulting in the emergence of resistance that cannot be managed by high doses of antibiotics, generating many side effects and toxicity.[3]

It is hoped that discovery of novel antimicrobials or appropriate modification of the known antimicrobial agents could afford new potential drugs, active against microbes resistant to current antibiotics and/or against intracellular microbial pathogens. One

Abbreviations: AmB, amphotericin B; CNT, carbon nanotube; CPP, cell penetrating peptide; DFO, desferrioxamine B; MDR, multidrug resistance; MIC, minimal inhibitory concentration; MWCNT, multiwalled CNT; PAMAM, poly(amidoamine); PEG, polyethylene glycol; PPI, poly(propyleneimine); SWCNT, single-walled CNT.

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*Corresponding author at: Department of Organic Chemistry, Gdańsk University of Technology, Gdańsk, Poland.

E-mail address: marmilew@pg.gda.pl (M.J. Milewska).

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of the possibilities of such modification is conjugation of antimicrobials with molecular nanocarriers.

Nanocarrier-antimicrobial agent conjugates—basic aspects

A common feature of the two challenges mentioned above, i.e., microbial resistance due to the inefficient drug uptake and diminished activity of a drug against intracellular pathogens, is the inability of a biologically active substance to cross the biological membrane(s). One of the possible strategies to overcome this problem has been the use of biodegradable nanoparticles, such as, liposomes, polymeric nanosystems; e.g., polymeric nanoparticles, nanofibrils, polymer micelles and solid lipid nanoparticles (SLN), as carriers for antimicrobials to ensure their delivery to the target site. These nanoparticles can load antimicrobial agents through physical encapsulation or adsorption and deliver their cargoes into target cells through different pathways; e.g., contact release, adsorption and endocytosis. The enhanced cellular uptake and subsequent sustained release of nanoparticle adsorbed/entrapped antimicrobial agents could effectively improve their antibacterial effects, due to both the direct action by contact of nanoparticles with microbial cells and diffusion of the released antimicrobial agents to bacteria located sites. Details of this strategy and examples of its application have been reviewed by other authors.[2,4,5]

Another approach, known as the "Trojan horse strategy", is based on the idea of conjugation of an active substance with a molecular organic nanocarrier that should be able to penetrate the membrane(s) and, once the conjugate is internalized, the active component could reach its intracellular target, either after release from the conjugate or in an intact form. The rational design of molecular nanocarrier–drug conjugates, their biological activity and perspectives of clinical application are the subject of this review.

In some cases, drug and nanocarrier are joined by a direct covalent linkage or form complexes stabilized by noncovalent interactions, but usually in the nanocarrier–drug conjugates, the two components are connected by a stable or cleavable linker (Figure 1, *A*). The latter type is used if the drug must be released from the conjugate to reach its intracellular target, however, the conjugate should be stable before internalization. The intracellular cleavage can be achieved by taking advantage of the activity of hydrolases, the reductive properties of cytosolic glutathione or the acidic environment of endosomes formed during endocytosis. Structures of some specific linkers applied in nanocarrier–drug conjugates are shown in Figure 1, *B-F*. A disulfide bond present in 5-thiol(2-nitrobenzoyl) B and *o*-dithiobenzyl carbamate C is stable in human serum but is a subject of thiolate-disulfide interchange in reaction with glutathione in cellular cytosol. The (acyloxy)alkyl esters D and "trimethyl locks" E are hydrolyzed by intracellular esterases or phosphatases. The hydrazone bonding F is stable at pH close to 7.0 but readily decomposes in acidic conditions.

Molecular organic nanocarriers and their conjugates with antimicrobials

Several types of molecular organic nanocarriers used for the formation of conjugates with antimicrobials have been proposed so far. Two of them take advantage of specific transport systems operating in microorganisms. Siderophores are the components of microbial iron acquisition systems and oligopeptides are transported by oligopeptide permeases. Particular types of cell penetrating peptides, carbon nanotubes and terpenoid derivatives enter the cells by direct translocation. Dendrimers and large cell penetrating peptides (CPPs) are internalized by endocytosis. An extensive survey of reported conjugates of antimicrobial agents with these nanocarriers is presented in the following paragraphs.

Oligopeptides and cell penetrating peptides

Biological membranes are generally impermeable to peptides which are zwitterionic or bear a net charge species. However, some peptides of specific sequences, called the cell penetrating peptides, are able to penetrate the membranes by direct translocation or by endocytosis. On the other hand, microbial cells possessing the membrane proteins known as oligopeptide permeases may take up small oligopeptides built of 2-8 amino acid residues. Such accumulation occurs against the oligopeptide concentration gradient, as an active transport driven by metabolic energy, usually the proton motive force. Oligopeptide permeases exhibit a broad spectrum of substrate specificity and tolerate substantial structural diversity of oligopeptides transported. Therefore, peptides can be used as nanocarriers for biomolecules, including the low molecular weight drugs, especially those containing charged functionalities, which do not diffuse through the cell membrane. To allow internalization of such molecules and, thus, enhance their potential as antimicrobials, two peptide-based strategies were developed in the past. One of them, called the "warhead delivery", "illicit transport" or "smuggling" concept, was based on the idea of incorporation of an amino acid enzyme inhibitor into a small peptide (2-4 amino acid residues), an active transport of the inhibitor-containing oligopeptide by oligopeptide permeases and release of an active inhibitor upon the intracellular enzymatic cleavage.[6] Nature has already taken advantage of this idea and some antibiotic substances acting according to this mechanism are known, including: tetaine (bacilysin) **1**, phaseolotoxin **2** and nikkomycin Z **3** (Figure 2). Several antimicrobials were designed following the "warhead delivery" concept, synthesized and characterized. Structures of some of them are shown in Figure 2. Oligopeptides containing an inhibitor of alanine racemase, Ala(P) i.e., a phosphonate analog of L-alanine, exhibited good antibacterial activity. A dipeptide L-Ala–L-Ala(P) **4**, was known under a trivial name alafosfalin and L-Nva–L-Ala(P) demonstrated especially high antibacterial *in vitro* activity.[7,8] Other peptide "smugglings" were obtained as potential antifungals. Those included oligopeptides containing L-glutamine analogs belonging to the group of N^3-acyl derivatives of L-2,3-diaminopropanoic acid, inhibitors of glucosamine-6-phosphate synthase, a key enzyme in biosynthesis of chitin and mannoproteins, components of the fungal cell wall. The most effective of these inhibitors was N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP). FMDP-containing oligopeptides exhibited high antifungal activity *in vitro*,[9] paradoxically enhanced activity against multidrug-resistant *C. Candida albicans*,[10] and high chemotherapeutic activity in the