Fighting bacterial infections—Future treatment options

Jenny Fernebro

Swedish Research Council, Vetenskapsrådet, Klarabergsviadukten 82, Box 1035, 101 38 Stockholm, Sweden

Abstract

This review summarises ongoing research aimed at finding novel drugs as alternatives to traditional antibiotics. Anti-virulence approaches, phage therapy and therapeutic antibodies are strategies that may yield drugs with high specificity and narrow spectra. Several candidates are currently being evaluated in clinical trials, mostly for topical applications, but so far, none have been approved for market authorization. Candidates based on antimicrobial peptides (natural, semisynthetic and synthetic) are also being tested in clinical trials, mostly for the topical treatment of chronic infections. An alternative to the development of new antibiotics is to find potentiators of traditional antibiotics; in this respect, beta-lactamase inhibitors are already in clinical use. Novel variants are under investigation as well as efflux pump inhibitors.

1. Introduction

Because of the development of antibiotic resistance in virtually all clinically important pathogens, alternatives to conventional antibiotics are urgently needed. The majority of the antibiotics used today originate from Actinomycetales, mostly Streptomyces, and were isolated during the “golden age” of antibiotics discovery, the period from the 1940s to the 1970s. Natural products have proven to be highly efficient for the treatment of bacterial infections and, not surprisingly, the variety of drugs based on natural products is enormous. There are drugs with broad and narrow spectra for oral, topical or parenteral administration and with activities against almost all known pathogens. Most natural product-based candidates currently under development are new, improved versions of old drugs, exemplified by the recently FDA-approved glycopeptide telavancin (Guskey and Tsuji, 2010). The chemical modification of existing drugs has proven to be the most efficient way to develop novel drugs active against resistant strains. However, these new agents are doomed to suffer from resistance development as well. Therefore, the development of antibacterial drugs with completely new modes of action is much needed. In 1995, the complete genome of Haemophilus influenzae was published, marking the beginning of the genomic era. Hopes were high that novel targets would be identified, leading to new drug candidates. The results, however, have been poor. Employees at GlaxoSmithKline published a review in 2007 summarizing the efforts made by the company in target-based drug research (Payne et al., 2007). Seventy screenings of libraries containing between 260,000 and 530,000 molecules resulted in five candidates, none of which have subsequently passed clinical trials to become licensed. The study reveals the complexity in finding novel antibacterial drugs. Still, alternatives to conventional antibiotics are needed; research to find those alternatives is ongoing. This report attempts to review the efforts made within seven fields: antimicrobial peptides (AMPs), antivirulence strategies, phage therapy, therapeutic antibodies, vaccines, potentitors of currently used antibiotics and antibacterial biomaterials. Characteristics of antibacterial drugs that may result from these research fields can be found in Table 1.

2. Antimicrobial peptides

2.1. Background

Antimicrobial peptides (AMPs), which are present in all animals, are evolutionary conserved components of the innate immune defense (Zasloff, 2002). Eukaryotic AMPs are small (10–50 amino acids), cationic and contain both hydrophobic and hydrophilic parts (Hancock and Sahl, 2006). Traditionally, they have been described as “antimicrobial”, with most having the ability to disrupt bacterial membranes, killing the bacteria. The exact mechanism of action is
unknown. It has recently been discovered that some AMPs are not directly bactericidal, but rather exert their effects by immunomodulation (Bowdish et al., 2005). Generally, AMPs are broad-spectrum antibiotics active against not only bacteria but also certain viruses and fungi. At higher concentrations, many may exhibit toxicity to eukaryotic cells. Magainins, which are AMPs from frogs, are among the best-studied AMPs (Berkowitz et al., 1990). AMPs of prokaryotic origin are called “bacteriocins” and often have a narrower spectrum (Riley and Wertz, 2002). Nisin, a bacteriocin, is the commercially most important AMP. It has been used extensively for food preservation (Riley and Wertz, 2002). The polymyxins, bacterial lipopeptides, were introduced into the clinic in the 1960s, but owing to their toxicity, they were replaced by other antibiotics (Falagas and Kasiakou, 2005). They are now commonly used only in topical therapy and considered a last-resort treatment of severe infections caused by multidrug-resistant Gram-negatives such as Pseudomonas aeruginosa and Acinetobacter baumannii.

### 2.2. Clinical potential

The broad-spectrum activity and rapid mode of action of AMPs make them promising drug candidates. The level of induced resistance against AMPs is also anticipated to be low (Zasloff, 2002). Most AMPs in preclinical and clinical trials today have been developed for topical applications (Hancock and Sahl, 2006). Examples of indications are catheter site infections, cystic fibrosis, acne and wound healing. The development of AMPs into drugs, however, has encountered several difficulties. Not only may some AMPs be toxic but also the production cost for synthetic peptides is high and their in vivo stability, especially toward proteases, is an issue (Hancock and Sahl, 2006). A theoretical concern for the pharmacological use of AMPs closely related to human ones is that selection for bacterial resistance could generate organisms of higher virulence potential. The fact that AMP function does not depend on specific amino acid sequences, but rather on biochemical properties, has opened the opportunity to develop synthetic peptide mimics for therapeutic use.

### 2.3. Ongoing research

Two AMPs (omiganan and pexiganan) have shown efficacy in Phase III clinical trials, but neither of them has been approved for clinical use (Hancock and Sahl, 2006). Pexiganan, a synthetic analogue to the magainins, was developed for the topical treatment of diabetic foot ulcers, whereas omiganan was investigated for the prevention of catheter-related infections. Development programs for these two drug candidates are still running (www.dipexiumpharmaceuticals.com, www.migenix.com). Several synthetic peptide mimics have shown efficacy in animal models (Choi et al., 2009; Livne et al., 2009; Radzishevsky et al., 2007; Rotem et al., 2008; Sarig et al., 2010). Oligomers of acylated lysines (OAKs) (Livne et al., 2009; Radzishevsky et al., 2007; Rotem et al., 2008; Sarig et al., 2010) are all classes of broad-spectrum peptide mimetics that have been used successfully in vivo. Moreover, candidates from the groups of OAKs and arylamide foldamers have shown efficacy against staphylococcal infection in mice when delivered systemically (Choi et al., 2009; Livne et al., 2009; Sarig et al., 2010). Some synthetic peptide mimics are also in clinical trials. Promising results were recently reported from a Phase Ib trial of the intravenously delivered defensin-mimetic PMX-30063, active against Staphylococcus spp. Phase II trials for skin and soft tissue infections are underway (www.polymedix.com). LTX-109, a broad-spectrum synthetic peptidomimetic, is currently being evaluated for nasal decolonization of MRSA in a Phase I/IIa trial (www.lytixbiopharma.com). Peptides that are more closely related to endogenous ones are also being investigated. The human-derived peptide DPX-060 has successfully gone through Phase I/IIa clinical trials for topical use against atopic dermatitis (www.dermagen.se, Schmidtchen et al., 2009). Two other investigational drugs (drugs under study but not yet approved for clinical use), hLF1-11 and talactoferrin, both based on human lactoferrin, have been tested in clinical trials (Velden et al., 2009, www.agennix.com). These peptides appear to have both immunomodulatory and antibacterial properties. Talactoferrin has been shown to stimulate wound healing and was recently evaluated as oral therapy against severe sepsis in a Phase II trial with promising results (www.agennix.com, Engelmayer et al., 2008). Prophylactic oral administration of bovine lactoferrin has been reported to reduce the incidence of early onset sepsis in very-low birth weight premature infants (Manzoni et al., 2009). IMX942, another peptide–based candidate with immunomodulatory properties, has shown efficacy in animal studies and recently went through a Phase I clinical trial (delivered intravenously) (www.iminexpharma.com, Scott et al., 2007). A lantibiotic (bacterial AMP), NAI-107, is also under preclinical development (Jabes and Donadio, 2010). Finally, the first fungal AMP, plectasin, was identified a few years ago (Mygind et al., 2005). It is highly effective against Streptococcus pneumoniae, non-toxic to eukaryotic cells, stable, has the potential for systemic delivery and can be produced by recombinant expression (Mygind et al., 2005). Promising preclinical data have recently been published on plectasin and an optimized, more broad-spectrum version of it, NZZ114 (Andes et al., 2009; Ostergaard et al., 2009; Brinch et al., 2010). The mode of action of these peptides, targeting the bacterial cell wall precursor Lipid II, has also been elucidated (Schneider et al., 2010).

### 3. Antivirulence strategies

#### 3.1. Background

Most traditionally used antibiotics kill bacteria by interfering with essential cellular processes. An alternative to this approach
is to disarm the pathogens, making it easier for the host innate immune system to clear the infection. Virulence is defined as the ability of a pathogen to cause disease. Some of the major targets of antivirulence research are toxins, quorum-sensing, biofilm production, type III secretion and adhesion. Toxins are produced by numerous pathogenic bacteria and in many cases the immune defense can manage the infection well if the toxins are taken out of the equation. A large number of Gram-negative bacteria release their toxins by type III secretion, making the machinery used in this process another potential target. Type III secretion involves the formation of a needle-like structure that delivers toxins and other effector molecules directly into host cells (Galan and Wolf-Watz, 2006). Quorum-sensing – another potential target – can be described as the process by which bacteria “talk” to each other. Bacteria can sense each other by taking up small molecules secreted by other bacteria nearby; in this way, bacteria can act as a population instead of as individuals (Kauffmann et al., 2008). Quorum-sensing enables bacteria to form biofilms, an effective approach to becoming more resistant toward both antibiotics and host immune responses. Finally, host cell adhesion is a critical initial step in bacterial colonization and thus a promising target for antibacterial drugs.

3.2. Clinical potential

Targeting bacterial virulence is attractive since it is specific toward pathogenic bacteria and spares the commensal flora. Since bacterial viability is not directly targeted, it is also unlikely that inhibitors of virulence would show cross-resistance with existing antibiotics or even evoke novel modes of resistance. Antivirulence drugs could be used both systemically and locally. The indications would vary depending on the specific virulence mechanism targeted. One application could be prophylactic use under certain circumstances, such as travelling, a bioterrorism threat or during an epidemic. Developing drugs based on antivirulence has several issues that need to be addressed. The evaluation of new drugs relies on robust in vitro assays for pharmacological studies. Since many antivirulence drugs do not have a phenotypic effect that can be assayed in vitro, novel assays must be set up that mimic the in vivo setting. Others, such as inhibitors of toxins, may be easier to monitor. The narrow specificity of virulence inhibitors requires rapid, precise diagnostic methods, as well as novel assays for susceptibility testing.

3.3. Ongoing research

The development of anti-toxin antibodies is the antivirulence strategy that is closest to clinical application (described below in the section about therapeutic antibodies). Several other alternatives, however, are under investigation (Table 2). Because of the bioterrorism threat, inhibitors of anthrax toxins have been identified (Laine et al., 2010; Min et al., 2004; Tonello et al., 2002; Karginov et al., 2005; Moayeri et al., 2006; Mourez et al., 2001; Shoop et al., 2005; Xiong et al., 2006; Panchal et al., 2004; Turk et al., 2004), with several of these having shown potency in animal models (Karginov et al., 2005; Moayeri et al., 2006; Mourez et al., 2001; Shoop et al., 2005; Xiong et al., 2006). At least one is under commercial preclinical development (Xiong et al., 2006).

Inhibition of toxins produced by Clostridium botulinum (Boldt et al., 2006; Burnett et al., 2006; Eubanks et al., 2007; Li et al., 2010; Pang et al., 2010; Roxas-Duncan et al., 2009; Schmidt and Stafford, 2002; Silhár et al., 2010), P. aeruginosa (Arndol et al., 2008), Shiga toxin-producing Escherichia coli (Armstrong et al., 1991; Kitov et al., 2008; Nishikawa et al., 2002; Paton et al., 2000; Watanabe-Takahashi et al., 2010; Kitov et al., 2000), Staphylococcus aureus (Ragle et al., 2010) and Vibrio cholerae (Hung et al., 2005) has also been reported. The mode of action of these inhibitors includes direct binding to the toxin, binding to the toxin receptor and manipulation of gene expression. One of these toxin inhibitors, Synsorb-Pk, was tested in clinical trials, but failed to show efficacy (to my knowledge the only non-antibody virulence inhibitor tested in clinical trials) (Trachtman et al., 2003). An elegant example of toxin inhibition is the use of a cholesterol biosynthesis inhibitor to block staphylococcal virulence (Liu et al., 2008). Similarities between crystal structures in human and bacterial biosynthetic pathways led the researchers to screen squelane synthase inhibitors for activity toward staphylococci, resulting in the identification of an inhibitor with potency in vivo. Several studies have identified type III secretion inhibitors active against important Gram-negatives such as Yersinia, E. coli, Chlamydia, Shigella, Pseudomonas and Salmonella (Aiello et al., 2010; Felise et al., 2008; Gauthier et al., 2005; Hudson et al., 2007; Kauppi et al., 2003; Larzabal et al., 2010; Muschiol et al., 2006; Negrea et al., 2007; Pan et al., 2009; Veenendaal et al., 2009; Wolf et al., 2006). Many of these inhibitors are effective toward more than one bacterial species and some are currently being investigated for commercial development (www.microbiotix.com, www.creativeantibiotics.com). In the search for inhibitors of adhesion, a group of compounds designated pilicides has been identified. These compounds inhibit E. coli pilus synthesis, thereby making the bacteria less adhesive (Pinkner et al., 2006; Svensson et al., 2001). Recently, modifications of a pilicide were shown to render it effective toward curli production as well, enhancing its anti-adhesive properties (Cegelski et al., 2009). Many companies and researchers are also investigating methods to prevent biofilm formation. RNAIII-inhibiting peptide (RIP) targets quorum sensing in S. aureus and has been shown to prevent biofilm formation in vivo (Balaban et al., 1998; Giacometti et al., 2003). Furanone-based compounds (Hentzer et al., 2003) and acyl-homoserine lactones (Geske et al., 2005) have been shown to prevent Pseudomonas biofilm formation via quorum sensing inhibition. Recently, novel inhibitors for quorum sensing in E. coli and V. cholerae were also identified (Gutierrez et al., 2009). Other targets for antivirulence drugs are Gram-positive sortases (Chenna et al., 2010; Kudryavtsev et al., 2009; Maresso et al., 2007; Oh et al., 2004; Suree et al., 2009), the heptose biosynthesis in Gram-negatives (De Leon et al., 2006; Desroy et al., 2009; Moreau et al., 2008), FimH in E. coli (Larsson et al., 2005; Wellens et al., 2008) and iron acquisition systems (Bain et al., 2008; Ferreras et al., 2005; Kaneko et al., 2007). Some of these are under commercial development (Desroy et al., 2009; Moreau et al., 2008). Furthermore, an inhibitor of QseC signaling, LED209, was recently discovered (Rasko et al., 2008). QseC is a sensor histidine kinase required for regulation of virulence factors in many important Gram-negatives. LED209 was shown to attenuate virulence in a mouse model of Salmonella infection.

4. Bacteriophages and lysins

4.1. Background

Bacteriophages (phages) are bacteria-specific viruses present in nearly all environmental niches. Most known phages are “lytic” phages, which ultimately lyse and kill the host bacterial cells to release their progeny (O’flaherty et al., 2009). Other phages are “lysogenic” and do not kill the host, being incorporated into the bacterial genome as prophages and only attack and lyse the host organism on rare events (O’flaherty et al., 2009). Phage therapy, investigated worldwide during the first half of the 20th century, has a long and controversial history. It was abandoned by the Western world after the introduction of antibiotics, but continued to be used in the former Soviet Union. The Elava Institute in Georgia, which has treated thousands of people for almost a
### 4.2. Clinical potential

Because phages are the natural predators of bacteria, using them as antibiotics may seem quite straightforward. Properties that make phages good drug candidates are that they are cheap to produce and very specific (Hanlon, 2007). However, the risk of resistance development is high and there may be clinical problems with neutralization of phages by the host immune response (Merril et al., 2003). Theoretically, the safety profile of phages should be excellent because of their specific action; however, little formal clinical data are available. Another major concern is the lack of data regarding efficacy and pharmacokinetics. Phages have very narrow specificity and thus phage therapy will require good diagnostics to identify precisely the infecting agent. The indications for phage therapy currently being evaluated include topical use on wound infections caused by, for example, *P. aeruginosa* or *S. aureus*, as well as other chronic infections, such as *P. aeruginosa* infection in cystic fibrosis patients. Another potential area of use is oral treatment for enteric infections. It may also be possible to use phages in a prophylactic fashion as decolonizers, eradicating the nasopharyngeal carriage of, for example, *S. aureus* in high-risk groups. An alternative to using whole phage particles is to use lysins. So far, lysins have only been found that are potent against Gram-positives. Promising in vivo data have been reported on lysins active against *Bacillus anthracis* (Schuch et al., 2002; Yoong et al., 2006), Enterococcus spp (Yoong et al., 2004), *S. aureus* (Daniel et al., 2010; Rashel et al., 2007), *Streptococcus agalactiae* (Chhibber et al., 2008; Vinodkumar et al., 2005; Kumari et al., 2010), *P. aeruginosa* (Debarbieux et al., 2010; Heo et al., 2009; McVay et al., 2007; Wang et al., 2006a; Watanebe et al., 2007; Vinodkumar et al., 2008), *Salmonella enterica* (Capparelli et al., 2010), *S. aureus* (Capparelli et al., 2007; Gupta and Prasad, 2011; Hoshiba et al., 2008; Kato et al., 2010; Matsuzaki et al., 2003; Wills et al., 2005; Zimecki et al., 2009, 2010) and *Vibrio vulnificus* (Cerveny et al., 2007). An alternative to the use of whole phage particles is to use lysins. So far, lysins have only been found that are potent against Gram-positives. Promising in vivo data have been reported on lysins active against *Bacillus anthracis* (Schuch et al., 2002; Yoong et al., 2006), Enterococcus spp (Yoong et al., 2004), *S. aureus* (Daniel et al., 2010; Rashel et al., 2007), *Streptococcus agalactiae* (Cheng and Fischetti, 2007; Cheng et al., 2005), *S. pneumoniae* (Entenza et al., 2005; Grandirard et al., 2008; Jado et al., 2003; Loeffler et al., 2003, 2001; Mccullers et al., 2007; Witzenrath et al., 2009) and *Streptococcus pyogenes* (Nelson et al., 2001). Bioengineered phages are also a possibility. An example.

### Table 2

Examples of inhibitors of virulence that have shown efficacy in animal models.

<table>
<thead>
<tr>
<th>Target</th>
<th>Inhibitor</th>
<th>Mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesion</td>
<td>Pilicides/curlicides (Cegelski et al., 2009)</td>
<td>Inhibits <em>E. coli</em> pilus and/or curli assembly</td>
</tr>
<tr>
<td>Biofilm production/Quorum-sensing</td>
<td>RNAIII-inhibiting peptide (Balaban et al., 1998)</td>
<td>Inhibits <em>S. aureus</em> quorum sensing</td>
</tr>
<tr>
<td>Toxins</td>
<td>Hydroxamate lethal factor inhibitor (Shoop et al., 2005)</td>
<td>Inhibits <em>B. anthracis</em> lethal factor protease activity</td>
</tr>
<tr>
<td>Toxins</td>
<td>Cispatin (Moayeri et al., 2006)</td>
<td>Inhibits <em>B. anthracis</em> protective antigen</td>
</tr>
<tr>
<td>Toxins</td>
<td>Polyvalent inhibitors of anthrax toxin (Moureze et al., 2001)</td>
<td>Inhibit <em>B. anthracis</em> toxin assembly</td>
</tr>
<tr>
<td>Type III secretion</td>
<td>INP0007 and INP0403 (Hudson et al., 2007)</td>
<td>Block type III secretion in <em>Y. pestis</em> and <em>Salmonella enterica</em> serовар Typhimurium</td>
</tr>
<tr>
<td>Virulence gene regulation</td>
<td>LED209 (Rasko et al., 2008)</td>
<td>Inhibits <em>QeC</em> signaling in several Gram-negative pathogens</td>
</tr>
<tr>
<td></td>
<td>Virstatin (Hung et al., 2005)</td>
<td>Inhibits <em>V. cholera</em> production of cholera toxin and the toxin coregulated pilus</td>
</tr>
</tbody>
</table>

...
of this is currently in clinical trials, evaluated for the decolonization of *S. aureus* (Fairhead, 2009, www.phicotherapeutics.co.uk). Finally, veterinary applications of phages are under investigation, especially in poultry (Johnson et al., 2008).

5. Therapeutic antibodies

5.1. Background

Therapeutic antibodies are already on the market, the majority of them for the treatment of cancer (Reichert et al., 2005). None of these antibodies have been approved for the treatment of bacterial infections, but several are undergoing preclinical and clinical trials. The concept of passive immunization is not a new one: before the antibiotic era, patients were regularly given antisera prepared from inoculated horses. Anti-bacterial antibodies can be divided into two categories: those that bind directly to the pathogen and those that aim to neutralize toxins or other virulence factors (Bebbington and Yarranton, 2008). Antibodies that bind directly to the bacteria usually work by opsonizing the bacteria for phagocytosis. Those that bind to virulence factors disarm the bacteria, thereby giving the host a chance to clear the infection immunologically.

5.2. Clinical potential

Therapeutic antibodies have the advantage of being very specific, which means that they do not affect the commensal flora. Intended for systemic use only, they can be produced toward any pathogen. Indications for antibodies currently in clinical trials include the prevention of *S. aureus* infection in high-risk groups, as well as prophylaxis and treatment of anthrax. Antibodies are widely used for cancer treatment and thus many questions regarding safety and pharmacokinetics may already have been addressed. However, as with antivirulence strategies and phage therapy, the narrow specificity increases the requirements for rapid diagnosis. Therapeutic antibodies are also relatively costly to produce and are usually intended for small markets, strongly suggesting that the final product may be expensive.

5.3. Ongoing research

Several antibody-based antibacterial drugs are currently being evaluated in clinical trials (Table 3). However, a number of promising candidates – *Veronate*, *Altastaph* and *Aurograb* – directed toward *S. aureus* recently failed Phase II/Phase III studies because of lack of efficacy (Bebbington and Yarranton, 2008). Many other staphylococci-specific antibodies have been identified (Brown et al., 2009; Hall et al., 2003; Ragle and Bubeck Wardenburg, 2009; Tilahun et al., 2010; Walsh et al., 2004; Park et al., 2007) and one of these, *Pagibaximab*, is currently being evaluated for prevention of infection in low-birth-weight infants in clinical trials (www.biosynexus.com, Weisman et al., 2009). Several studies also report antibodies that target *B. anthracis* toxins (Zhou et al., 2008; Albrecht et al., 2007; Mohamed et al., 2005; Peterson et al., 2006; Staats et al., 2007; Vitale et al., 2006; Cui et al., 2005; Maynard et al., 2002; Wild et al., 2003; Chen et al., 2009; Herrmann et al., 2006; Hull et al., 2005; Zhao et al., 2003). *Anthim*, *Raxibacumab* and *Valortim* are all anthrax-specific antibodies currently undergoing clinical trials (www.elusys.com, www.pharmathene.com, Subramanian et al., 2005). Another common target for therapeutic antibodies is *P. aeruginosa*. *KBPA101*, a monoclonal directed toward LPS O polysaccharide of *P. aeruginosa* serotype O11, is currently undergoing clinical trials (Horn et al., 2010; Lazar et al., 2009). *KB001*, also a *Pseudomonas*-specific antibody inhibiting type III secretion, is being evaluated in cystic fibrosis patients in clinical trials (Baer et al., 2009, www.kalobios.com). In addition, antibodies targeting *Pseudomonas* quorum sensing (Kauffman et al., 2006), alginate (Pier et al., 2004, www.aridispharma.com) and flagellin (Neville et al., 2005) have been reported. Polyclonal *Pseudomonas*-specific antibodies produced in hens and transported to the egg yolk are in clinical use for the treatment of cystic fibrosis patients in Sweden (Nilsson et al., 2008). These antibodies are not approved by the European Medicines Agency (EMA) or the FDA, but Phase III clinical trials are underway (www.immunsystem.se). Antibodies directed to shiga toxin-producing *E. coli* are also in development and two (ShigamAbs and *Urtoxazumab*) are currently undergoing clinical trials (Bitzan et al., 2009; Kimura et al., 2002; Lopez et al., 2010; Mukherjee et al., 2002). Further, the promising results of a Phase II clinical trial evaluating monoclonal antibodies directed toward *Clostridium difficile* were recently published (Babcock et al., 2006; Lowy et al., 2010). Most of the antibodies described thus far are directed toward toxins and are very specific to the bacterial species producing those toxins. A more broad-spectrum antibody has also been reported targeting the poly-N-acetylgalcosamine (PNAG) of bacterial polysaccharide. This antibody, currently under preclinical development, has shown in vivo protection toward both *S. aureus* and *E. coli* (Cerca et al., 2007; Pier et al., 2004, www.alopexx.com).

6. Vaccines

6.1. Background

The oldest vaccines are based on attenuated or killed whole cells (e.g. BCG against tuberculosis). New techniques have made it possible to produce vaccines based on modified toxins and protein-conjugated polysaccharides. Suitable antigens have traditionally been found by the immunization of animals and the identification of immunoreactive proteins. The breakthrough of genomics has provided novel methods for selecting antigens. Bioinformatics has made it possible to identify specific groups of proteins, such as surface-exposed proteins. This way of finding novel antigens has been called “reverse vaccinology” (Rappuoli, 2000). An alternative method, the ANTIGENome technology, has also been described (Meinke et al., 2005). Peptide libraries covering the whole genome of a pathogen are screened for immunogenicity by the addition of serum from humans previously exposed to the pathogen.

6.2. Clinical potential

A functional vaccine is probably the most cost-effective antibacterial drug possible. It would be hard to question the usefulness of the vaccine strategy—after all, it has been used to eradicate smallpox. However, vaccines are used prophylactically, often offered to general patient populations, i.e. the safety issue is extremely important.

6.3. Ongoing research

Research into vaccines is so extensive that it is hard to grasp the full picture. This section will focus on vaccine candidates currently undergoing clinical trials or in advanced preclinical development. Efforts to develop a *P. aeruginosa* vaccine have been ongoing for a long time, but though several have seemed promising in early clinical trials, none have been approved (Doring and Pier, 2008). However, IC43 has recently completed Phase II clinical trials (NCT00876252). This candidate vaccine is a recombinant fusion protein of OprF and OprI, two *P. aeruginosa* outer membrane proteins (www.intercell.com). Meanwhile, at least three staphylococcal vaccines are currently being evaluated in clinical trials. V710 is, based on the IdsB protein, involved in iron acquisition and identified using the ANTIGENome technology (Etz et al., 2002).
The other two candidates, GSK2392105A and SA3Ag, are multivalent vaccines containing three and four antigens, respectively (NCT01160172, NCT01018641). A previous attempt to develop a staphylococcal vaccine focused on polysaccharides, but this vaccine (StaphVAX) failed to show efficacy in Phase III trials (Schaller and Lee, 2008). The polysaccharide strategy has otherwise been very successful, exemplified by the novel conjugate vaccines that have been developed toward S. pneumoniae and Neisseria meningitidis. These vaccines show good efficacy but do not cover all clinically important strains. Therefore, efforts are made to develop vaccines with better coverage. At least one pneumococcal non-polysaccharide vaccine, IC47, is currently being evaluated in clinical trials. It is a multivalent vaccine containing three broadly conserved protein antigens (Gieffing et al., 2008, www.intercell.com). Another interesting candidate, killed whole cell vaccine (WCV), has shown potency in animal models and clinical trials are in preparation (Malley, 2010; Lu et al., 2010). For meningococci, serogroup B has been especially difficult to target because its polysaccharide is identical to a human one. A couple of vaccines based on outer membrane vesicles (OMVs) have been used locally, but no universal one has been approved. Several are now in clinical trials (Granoff, 2010), including variants based on multiple bioengineered strains (Van Den Dobbelsteen et al., 2007; Zollinger et al., 2010). Two promising protein-based vaccines are also undergoing clinical trials (Gorrione and Van Alphen, 2009), one containing two variants of a lipoprotein (rLP2086) (Fletcher et al., 2004) and one containing five antigens engineered into three recombinant proteins (Giuliani et al., 2006; Pizza et al., 2000). Efficient vaccines are also lacking against Group A streptococci (GAS) and Group B streptococci (GBS). StreptAvax, a 26-valent GAS vaccine candidate based on the M protein antigen, was recently evaluated in a Phase I clinical trial (Kotloff et al., 2004). Other GAS vaccines are in preclinical development, including PepVac StreptLinCor, which is based on the conserved parts of the M protein (Brandt et al., 2000; Guilherme et al., 2009), as well as two protein-based candidates with novel antigens identified via reverse vaccinology (Rodriguez-Ortega et al., 2006) and the ANTIGEnome technology (Fritzler et al., 2010). For GBS, the most common strategy has been to use polysaccharide conjugates and several monovalent or divalent candidates have been tested in clinical trials (Edwards, 2008). One 4-valent candidate is currently undergoing clinical trials (NCT01150123). Protein-based candidates, however, are under development (Maione et al., 2005; Doro et al., 2009). When it comes to vaccines toward gastrointestinal pathogens, several candidates are under evaluation for prevention of enterotoxicogenic E. coli (ETEC) disease (NCT01060748, NCT00993681). One C. difficile candidate, based on toxoids, is being evaluated in clinical trials (Kotloff et al., 2001). Other E. coli variants in addition to ETEC have been targeted in vaccine research. Several promising candidates have failed in late stage clinical trials, and are no longer in clinical evaluation. Two recent publications, however, identify novel antigens for future vaccine development (Alteri et al., 2009; Moriel et al., 2010). Finally, even though there is a licensed vaccine for protection against tuberculosis, efforts are being made to develop one with better efficacy. Several candidates are being evaluated in clinical trials. HybriD-I is based on a fusion protein of Ag85B and ESAT-6 (Weinrich Olsen et al., 2001) and Mtb72F/AS02A contains two antigens selected for their ability to boost pre-existing immunity induced by BCG or TB infection (Von Eschen et al., 2009). AERAS-402/Crucell Ad35 (Abel et al., 2010) and MVA85A/AERAS 485 (Mchane et al., 2004) are two additional candidates, both based on virus vectors.

7. Potentiators of currently used antibiotics

7.1. Background

An alternative to the development of novel antibiotics is to find potentiators of the already existing ones. These potentiators could function either by reversing resistance mechanisms in naturally sensitive pathogens or by sensitizing naturally resistant strains. The most common resistance mechanism toward clinically important beta-lactams is the production of beta-lactamases or alternative penicillin-binding proteins (PBPs). Treatment with a beta-lactam in combination with a beta-lactamase inhibitor is already used clinically, and three inhibitors have been registered: clavulanic acid, tazobactam and sulbactam (Drazw and Bonomo, 2010). Another resistance mechanism common among Gram-negatives is the overexpression of efflux pumps. This is also a possible target for potentiators, although no therapeutic efflux pump inhibitors are currently available on the market.

7.2. Clinical potential

The good news about potentiators is that they work, i.e. we already have them in the clinic. The bad news is that resistance to beta-lactamase inhibitors has been reported (Buynak, 2006). Most beta-lactamase inhibitors under preclinical investigation have been developed for use against Gram-negatives such as P. aeruginosa and E. coli. These bacteria, and S. aureus, are typical targets also for the novel efflux pump inhibitors.

7.3. Ongoing research

Several novel beta-lactamase inhibitors are currently being investigated. NXL104 is active against class A and C beta-lactamases, with shown potency in several Gram-negatives, including E. coli and K. pneumoniae (Stachyra et al., 2009). It is being evaluated in Phase II clinical trials in combination with ceftazidime. ME1071 (CP3242), currently undergoing clinical trials in Japan (www.meiji.com, Ishii et al., 2010), has shown efficacy in Gram-negatives such as P. aeruginosa, A. baumannii, E. coli and K. pneumoniae. BI-489 is a penem-based inhibitor with potency in combination with piperacillin in experimental infections with piperacillin-resistant E. coli and E. cloacae (Petersen et al., 2009). Several other agents with beta-lactamase inhibitory properties have been reported, including the 6-alkylidenepenem sulfofones (Pattanaik et al., 2009), the oxapenem analogues (Simpson et al., 2003), LK-157 (Paukner et al., 2009).
et al., 2009) and BAL30376 (a combination of three inhibitors) (www.basilea.com). The other major area of research within this field is the development of efflux pump inhibitors. Examples of compounds reported for Gram-negatives include pyrrolidinomethyl derivatives active in P. aeruginosa (Yoshida et al., 2007; Lomovskaya et al., 2001), aryl-piperazines for use in E. coli, Acinetobacter, Klebsiella and Enterobacter (Schaumberger et al., 2006; Pannek et al., 2006; Kern et al., 2006), quinolines for Enterobacter (Chevalier et al., 2001) and more recently quinazolines active in Enterobacter, Klebsiella and P. aeruginosa (Chevalier et al., 2010). Inhibitors with activity in Gram-negatives have also been identified, especially for S. aureus (Sangwan et al., 2008; Vidaillac et al., 2007; German et al., 2008).

As yet, no efflux pump inhibitor has been approved for clinical use. One candidate, MP-601,205, with intended use in combination with fluoroquinolone antibiotics, was tested in clinical trials but these trials were not pursued because of tolerability issues (Lomovskaya et al., 2007). Efflux pump inhibitors, however, are still included in the developmental programs of Mpx, the company that identified MP-601,205 (www.mpxpharma.com). Other creative examples of potentiating currently used antibiotics include the use of bioengineered phages (Lu and Collins, 2009) as well as activating a drug toward a pathogen that is naturally resistant to it, as exemplified by the combined use of meropenem and clavulinate to treat successfully tuberculosis in mice (Hugonnet et al., 2009). Another approach was reported last year when it was shown that targeting a regulator of stress response (AmpRS) in P. aeruginosa led to increased tobramycin sensitivity (Lee et al., 2009). A similar strategy, targeting the bacterial SOS response, is under preclinical development (www.achaogen.com).

8. Antibacterial biomaterials

8.1. Background

Bacterial infections at the sites of implanted medical devices are conditions of immense clinical importance. Both permanent implants and short-term biomedical devices such as catheters and endotracheal tubes may be colonized. Urinary tract infections related to catheters are the most prevalent form of nosocomial infections (Klevens et al., 2007). Bacterial biofilm formation on the medical device is the main reason for the high prevalence of infections. For devices used in urology, especially urinary catheters and ureteral stents, growth of bacteria may not only lead to infection but also to a phenomenon known as encrustation. Basically, urease produced by the infecting agent hydrolyzes urea present in the urine, resulting in an elevation of pH, ultimately leading to precipitation of salts and deposition of crystals on the surface (Morris and Stickler, 1998). Encrustation may lead to blockage of the catheter and severe complications. The bacteria most commonly associated with infections on medical devices are staphylococcal species, except for the ones on urinary devices, where Gram-negatives (e.g., E. coli, P. aeruginosa, Enterobacter aerogenes, Acinetobacter acuinas, Klebsiella spp and Proteus spp) are most common (Leonie et al., 2003). Proteus mirabilis is the pathogen most commonly associated with encrustation (Morris and Stickler, 1998). Attempts made to reduce the medical implant-related infections include systemic antibiotic prophylaxis as well as local administration of antimicrobial agents. The severity of device-related infections ranges between relatively mild infections to life-threatening conditions, with consequences of health risks for the patients as well as higher costs because of replacements of infected implants and prolonged hospitalizations.

8.2. Clinical potential

Efforts to develop medical devices with antibacterial properties have been ongoing for a long time. Most polymeric materials commonly used for medical devices such as catheters are easily colonized by bacteria. Therefore, efforts have been exerted to add antibacterial surface coatings onto the devices. These coatings may release antibacterial compounds, have antibacterials covalently bound to them or in themselves be resistant to bacterial colonization. Central venous catheters (CVCs) and urinary catheters with antibacterial coating are on the market (Hockenhull et al., 2009). The same strategy is under investigation for implants for orthopedic joint replacements most commonly made of titanium, stainless steel or other metal alloys. These materials show excellent biocompatibility as well as high-quality mechanical properties, but are not resistant to bacterial colonization. The most common antibacterial coatings in use today are those containing silver. Silver is known for its potent broad-spectrum antibacterial activity toward both Gram-positives and Gram-negatives, which is believed to be attributed to the release of silver ions (Monteiro et al., 2009). Coatings releasing antibiotics are also available. Concerns regarding these kinds of devices include silver toxicity as well as resistance development (Monteiro et al., 2009; Hamill et al., 2007).

8.3. Ongoing research

Within the field of urology, research on antibacterial devices mainly focuses on urinary catheters and ureteral stents. Several catheters with antibacterial coating are available, including variants with silver alloys and antibiotics (Schumm and Lam, 2008). Numerous clinical trials have been undertaken to evaluate the efficacy of these coatings. Recently, a meta-analysis of all these trials was performed (Schumm and Lam, 2008) showing that silver alloy catheters reduced the incidence of asymptomatic bacteriuria during short-term urinary catheterization. Catheters coated with minocycline/rifampicin or nitrofurazone reduced the incidence after a week, but when measured later the reduction was not significant (Schumm and Lam, 2008). The clinical implications of these results have been questioned, however (Stickler, 2008). Novel, investigational antibacterial coatings are under development. The antiseptic triclosan has shown good efficacy in several in vitro and in vivo studies (Chew et al., 2006; Cadieux et al., 2006). A triclosan-eluting stent is currently being evaluated in a Phase II clinical trial (Cadieux et al., 2009). Gendine-coated catheters have also been investigated in animal studies with promising results (Hachem et al., 2009). Several other antibacterials have shown efficacy in animal models with catheters, including chloroxylenol/thymol (Mansouri and Darouiche, 2008) and chlorhexidine/protamine sulphate (Darouiche et al., 2008). An innovative strategy that has been tested in clinical trials is “bacterial interference” (Trautner et al., 2007; Prasad et al., 2009). The catheters are precoated with an avirulent E. coli strain before insertion, potentially preventing adherence of virulent strains. Just as for urinary catheters, CVCs with antibacterial properties are commercially available, including variants impregnated with silver, silver/platinum/carbon, chlorhexidine/silver sulphadiazine, minocycline/rifampicin and heparin (Hockenhull et al., 2009). Their usefulness has been debated, but recent meta-analyses concluded that antibacterial coatings do have a beneficial effect on the rate of device-derived infections, especially minocycline/rifampicin (Hockenhull et al., 2009; Wang et al., 2010). Other alternative coatings for CVCs that have been investigated include N-acetylcysteine (Mansouri and Darouiche, 2007), triclosan/Dispersan® (Darouiche et al., 2009) and gendeine (Hanna et al., 2006). A novel, 5-fluorouracil-coated variant has recently completed Phase III clinical trials with encouraging results (Walz et al., 2010). For permanent implants based on metal alloys, coating strategies are also under investigation. Antibiotics such as gentamicin have traditionally been used in bone cement for prophylactic local delivery.
Presently, cementless bone implants are more routinely used, increasing the need for other antibacterial strategies. The task is somewhat more challenging than for non-permanent devices because osseointegration has to be taken into account. Calcium phosphate-based coatings have shown good biocompatibility and may be used as carriers for antibacterials (Radin et al., 1997). Promising in vivo data have been reported for calcium hydroxyapatite in combination with tobramycin (Moojen et al., 2009) and gentamicin (Alt et al., 2006). Other strategies include polymerization of vancomycin onto the implant (Lawson et al., 2007), direct spraying of the implant with antibiotics in methanol (Aykut et al., 2010; Darouiche et al., 2007), as well as using poly(D,L-lactide) (PDLLA) (Kalickie et al., 2006; Vester et al., 2010) or sol-gel as carriers. A recent publication describes the successful use of silver-coated megaprostheses in a clinical trial of patients with bone sarcoma (Hardes et al., 2010). Silver coating resulted in a reduction in infections as well as fewer amputations (Hardes et al., 2010).

Finally, the concepts of the other fields of this report have also been applied to prevent bacterial colonization of medical devices. This includes directly targeting biofilm formation on biomaterials using quorum-sensing inhibitors (Cirioni et al., 2007; Lovetri and Madhyastra, 2010, Anguita-Alonso et al., 2007, Cirioni et al., 2006; Christensen et al., 2007), as well as attempting to eradicate the bacteria using bacteriophages (Fu et al., 2010; Curtin and Donlan, 2006; Carson et al., 2010) or AMPs (Trautner et al., 2005; Minardi et al., 2007).

9. Discussion

Several publications have reported that the big pharmaceutical companies are cutting down on research aimed at finding new therapeutics for the treatment of bacterial infections, citing a less favorable economic incentive compared with medications for chronic medical conditions (Boucher et al., 2009). Novel strategies, however, are under investigation, both within the academic community and in biotech/pharmaceutical companies. Many of the fields examined in this report have not yet generated products approved by the EMA or the FDA, but candidates are in clinical trials. Most of these candidates have narrow specificity spectra and would probably not have been considered during the “golden age” of antibiotics. Yet, in times of emerging resistance, all additional antibacterial drugs are welcome. The more diverse our arsenal against bacterial pathogens becomes, the better will be the range of tailored treatments that can be offered. Broad-spectrum antibiotics are not always the best choice, especially not when considering the commensal flora and the risk for opportunistic infections. However, to take advantage fully of the alternative antibiotics under development, new diagnostics are needed that are more precise and faster than those in use today. The importance of better diagnostics for these novel antibacterial approaches to become successful cannot be stressed enough in today’s complex medical environment.

Anti-virulence approaches, phage therapy and therapeutic antibodies are fields that will yield drugs with high specificity and hence narrow spectra. They are all still awaiting their first drug approved for market authorization. To this date, only one antivirulence non-antibody candidate, Synsorb-PK, has entered clinical trials (Trachtmann et al., 2003). In theory, antivirulence strategies are tempting both because of the specificity of the resulting drugs and because of the low selective pressure for resistance development. These drugs, however, are at least a decade away and it is questionable whether they will ever be used as first-line drugs for life-threatening conditions (e.g. sepsis) when time is limited. Because of their narrow specificity, the same holds for therapeutic antibodies though these are a bit further in development. Although the regulatory authorities have turned down several candidates, it is still a promising field. Antibody-based drugs are common within oncology and inflammatory disease and recently the first virus-specific antibody, palivizumab, for treatment of RSV infection, was approved. With several antibacterial antibodies in late-stage clinical development, it is probably just a question of time before the first one will be in the clinics.

It has to be acknowledged that phage therapy is already in clinical use, even though parts of the Western world are not aware of it. Although the available literature within this field is largely limited to case studies and uncontrolled trials, phage therapy appears to be an alternative for certain indications. For complicated wounds and ulcers caused by multidrug-resistant bacteria, phage therapy could be an option. In the EU, it has been proposed that specific sections concerning phage therapy should be included in the Advanced Therapy Medicinal Product Regulation to make it easier to get approval for clinical trials involving such therapy (Verbeken et al., 2007). It may be easier, though, to get approval for lysins than for full phage particles. Interestingly, thus far, resistance development toward lysins has not been observed. However, most lysins are only stable in vivo for less than 30 min (Loeffler et al., 2003), making stability one of the issues that has to be solved before clinical use.

Just as for therapeutic antibodies, the field of AMPS has had several candidates in late-stage clinical trials that failed to be approved for clinical use. Some of these disapprovals have been questioned and it is possible that candidates such as pexiganan would have been approved if trials had been designed differently (Moore, 2003). Most AMPS have quite broad spectra, but because of stability and toxicity problems, they are mainly evaluated for topical use. Just as bacteriophages, they have the potential to fill the niche as topical therapeutics toward complicated wounds and ulcers. Another possible application for AMPS is as decolonizers. It will be interesting to follow the development of plectasin and the improved version of it in that these candidates seem to have the potential to be used systemically. Further, AMPS with immunomodulatory properties have garnered recent interest and may find future use as anti-inflammatory agents.

Improvement of existing drugs has become the most successful way of generating novel antibiotics. It works well and several candidates of traditional classes are in late-stage clinical development, such as the cephalosporin ceftriaxone (Corey et al., 2010), the tetracycline amadacycline (PTK0796), the streptogramin NXL-103 (Politano and Sawyer, 2010) and the macrolide CEM-101 (Woosley et al., 2010). However, all these candidates have a limited lifespan in that eventually resistance will develop. This situation may be what we have to get used to, i.e. the continued development of new drugs that can replace those already in clinical practice. An alternative would be successful vaccination strategies. It will be of special interest to follow the new vaccine candidates that have been identified through genomics and proteomics. These candidates represent genuinely novel approaches and the first one to be approved may well be the beginning of a new era. Moreover, the development of medical devices more resistant to bacterial adherence may reduce the rate of infections and hence limit the need for antibiotics. Even though many of the coatings that are evaluated and in use today involve traditional antibiotics, several take advantage of different alternatives such as silver and antiseptics.

Again, most of these alternative drugs could only replace the currently used antibiotics if efficient diagnostics are developed in parallel. This strategy is also attempted by the company developing the Pseudomonas monoclonal antibody KBPA101 (www.kentabiotech.com). Still, even if these novel drugs cannot replace antibiotics, they may become a much-needed complement to the drugs in use today. The overuse of antibiotics has left us in our current predicament. We need to learn from that mistake and take better care of the drugs that are still viable.
The PubMed database was searched using the search terms “antimicrobial peptides”, “antivirusulence”, “phage therapy”, “therapeutic antibodies”, “efflux pump inhibitor”, “beta-lactamase inhibitor”, “biomaterial”, “implant” and “vaccine” in combination with the terms “novel” or “antibacterial”, or both. For the vaccine field, “vaccine” was also combined with the names of bacterial species considered especially important. Further searches on PubMed were performed based on the authors/companies/substances that were received in the first round. Only publications written in English and published in the past ten years were included, unless an earlier publication was regarded as particularly interesting. Company web pages and ClinicalTrials.gov were subsequently searched for updates on the developmental stages.

Acknowledgements

I would like to express my sincere gratitude to the panel of advisers, Charlotte Edlund, Vincent Fieschi, Bengt Gärdfeld, Victor Nizet and John Turnidge, who critically read parts or all of the manuscript. Their comments have greatly improved the quality of the report. This work is based on the previous report “An Overview of Ongoing Research Aimed at Finding Novel Antibacterial Drugs”, which was produced in preparation for the EU meeting “Conference Innovative Incentives for Effective Antibacterials” (held in Stockholm, September 17, 2009) on assignment of the Swedish Government. We gratefully acknowledge the financial support of VINNOVA and ReAct.

References


